

Breeding effects on the root-associated microbiome of *Zea mays* L. are linked to plant-induced variation in soil water potentials

Nicolas Tyborski ^{1,*}, Tina Koehler ², Franziska A. Steiner ³, Shu-Yin Tung ^{4,5}, Andreas J. Wild ⁶, Johanna Pausch ⁶,
Tillmann Lueders ¹

¹Ecological Microbiology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, DE-95448 Bayreuth, Bavaria, Germany

²Root-Soil Interaction, TUM School of Life Sciences, Technical University of Munich, DE-85354 Freising, Bavaria, Germany

³Soil Science, TUM School of Life Sciences, Technical University of Munich, Freising, Bavaria, Germany

⁴Institute for Agroecology and Organic Farming, Bavarian State Research Centre for Agriculture (LfL), DE-85354 Freising, Bavaria, Germany

⁵TUM School of Life Sciences, Technical University of Munich, DE-85354 Freising, Bavaria, Germany

⁶Agroecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, DE-95447 Bayreuth, Bavaria, Germany

*Corresponding author. Ecological Microbiology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Dr.-Hans-Frisch-Str. 1-3, DE-95448 Bayreuth, Bavaria, Germany.
E-mail: nicolas.tyborski@uni-bayreuth.de

Abstract

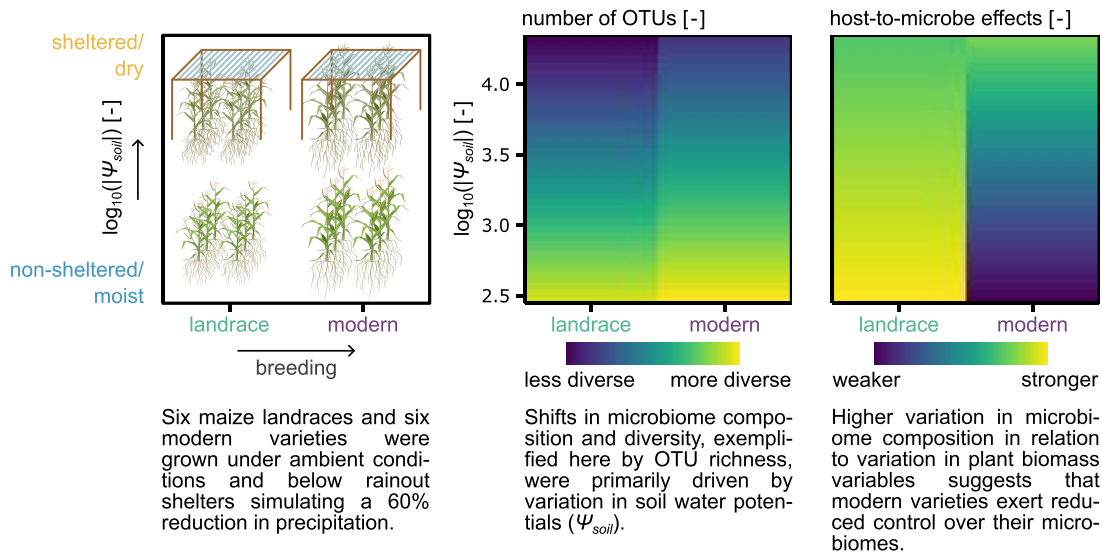
Modern crop varieties may exert reduced influence on their microbiome compared to their progenitors, as plant-microbe interactions were not targeted during breeding. Moreover, formerly beneficial microbiome functions might no longer be relevant in modern agricultural ecosystems. We hypothesised that such patterns could become particularly evident under drought, since drought-tolerance has not been a primary breeding target. To test this, we grew six maize landraces (released before 1945) and six modern varieties (released from 2010 onwards) in a field under ambient and 60% reduced precipitation. The experiment was repeated over two years, differing in amounts and temporal distributions of precipitation. We assessed the composition of root-associated prokaryotic communities during grain filling by 16S rRNA gene metabarcoding. Intra-variety dispersion in microbiome composition relative to plant biomass-based dispersion was higher in modern varieties, suggesting breeding may have affected plant control over microbiomes. Besides that, shifts in microbiome composition between landraces and modern varieties were driven mainly by the plants' impact on soil water potentials. Consequently, the taxa that increased in relative abundance during soil drying, mainly *Actinomycetota*, were similar between landraces and modern varieties. Exploring microbiome-mediated alleviation of drought effects, therefore, appears promising also for applications in modern agricultural ecosystems. Specifically, filamentous *Streptomyces* spp. potentially contributed to soil aggregate stability, which should be further investigated in the context of drought mitigation. The reduced plant control over microbiome composition of modern varieties suggested by dispersion analysis likely has functional implications beyond microbiome adaptation to drought and should be considered in future assessments of breeding.

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Graphical Abstract



Keywords *Zea mays* L., root microbiome, plant-microbe interactions, plant breeding, landraces, native soil microbiome, drought stress, microbiome dispersion, *Streptomyces*, soil aggregate stability

Introduction

Plants possess traits that enable them to exert control over their microbiomes. In evolutionary theory, these traits are referred to as “host-to-microbe effects,” whereas the traits of microbiomes influencing plants are referred to as “microbe-to-host effects” [1]. Wild plants are under strong natural selection to exert host-to-microbe effects that modulate microbe-to-host effects in a plant-beneficial way [2]. Since their existence was not yet known, host-to-microbe effects have not been targets of intentional artificial selection in past crop breeding programs [2, 3]. However, today it is evident that plant-associated microbiomes are pivotal for plant performance and health, e.g. by enhancing nutrient availability, suppressing pathogens, or improving resistance to abiotic stress [4, 5]. It has been shown that crop varieties can diverge in the composition of their associated microbiomes [6–9]. Further, recent advances in linking microbial and plant genomic information have identified heritable plant traits influencing the interactions with functionally relevant microbiome members [10–14]. Experiments with maize knockout mutants have provided direct evidence for the effects of specific compounds exuded by roots on microbiome composition [15, 16]. Thus, considering host-to-microbe effects in breeding appears promising as a means to enhance the performance and health of future crop varieties [17–20]. However, to evaluate the potential of incorporating host-to-microbe effects into breeding, it is vital to understand how past breeding has affected the capacity of current varieties to interact with their microbiomes. Despite many recent studies addressing this topic [21–26], a systematic understanding of the influence of breeding on host-to-microbe effects in interaction with environmental factors is still lacking [27].

A central hypothesis posits that the ability of plants to shape their microbiome deteriorated with breeding [2, 28–30]. Historically, breeding mainly focused on yield optimisation and above-ground traits, while root traits have only recently gained more attention [31].

Generally, plant traits can be lost if the individuals selected during breeding lacked them by chance [32] or if their loss had no immediate negative impact on the traits under selection [30]. Accordingly, breeding commonly reduces genetic diversity, as reported for many crops [33]. Symbiosis traits may be especially prone to loss because changes in agricultural practice have probably relieved selective pressures on many of them [28, 32]. For example, the intense use of mineral fertilisers in modern agricultural practice may have rendered microbial nutrient provisioning insignificant, as highlighted by the evolution of less cooperative rhizobia under high nitrogen input in a long-term field experiment [34]. Further, pesticide use may have diminished the dependence of plants on disease-suppressing microbes [30]. The modulation of the microbiome by root exudation is costly in terms of the plant’s resource (e.g. carbon) investment [35]. Accordingly, selective pressures towards reduced investments into root exudation should exist wherever plants become less dependent on microbe-to-host effects. Indeed, substantial differences in metabolite compositions of root exudates were found, comparing modern wheat and its progenitors [36].

Alternative to the common hypothesis predicting reduced host-to-microbe effects, unintentional indirect selection could have resulted in persisting beneficial host-to-microbe effects during breeding if microbe-to-host effects contributed significantly to the expression of the plant traits under artificial selection. Hence, specific plant-microbe interactions may have been differentially affected by breeding. This might explain inconsistencies in previous studies investigating effects of breeding on microbiome composition. E.g. clear shifts in microbiome composition and functional gene abundance corresponding to the year of variety release have been reported for inbred lines of maize released between 1949 and 1986 [24]. In contrast, unidirectional shifts in microbiome composition over time were not detectable for maize varieties released between 1939 and 2011, while the microbiome of

varieties from the 1960s and 70s differed from that of older and more recent varieties [21].

Modern agricultural fields, where crops are predominantly grown in monoculture, are characterised by relatively uniform spatial and temporal conditions with regard to, e.g. soil structure, nutrient availability, and below-ground biodiversity [37, 38]. Since breeding has optimised modern varieties for such uniform environments, their plasticity to respond specifically to extreme conditions is likely reduced [39, 40]. The notion that genomic regions contributing to phenotypic plasticity in response to environmental change were not under selection during breeding [41] suggests that trait plasticity may be rather costly in modern agricultural environments [39]. Accordingly, wild progenitors and landraces of multiple crop species have been reported to show higher plasticity than modern varieties in phenotypic traits and responses of associated microorganisms to environmental stressors, including drought, phosphorus deficiency, and pathogens [42–45]. It seems conceivable that plasticity in host-to-microbe effects in response to drought has been similarly affected. However, to our knowledge, this issue has not been specifically addressed.

Here, we compared the root-associated prokaryotic microbiomes of European landraces and modern varieties of maize (*Z. mays* L.) and their responses to reduced precipitation, as an important abiotic stressor. European landraces of maize have descended from flint maize introduced from the Americas in the 15th and 16th centuries, and much of their genetic material was lost with the introduction of northern American dent maize in the 1960s [46]. Today, landraces are suggested as a source of genetic diversity that could be useful in future breeding efforts [29, 47]. Longer and more intense periods of drought are expected in the course of anthropogenic climate change, and the development of means to mitigate adverse consequences on agriculture is, therefore, fundamental [48]. It has been shown that drought-adapted microbiomes [49–51] and specific prokaryotic taxa [52] can significantly reduce the impact of drought on plants. Therefore, targeting plant-microbe interactions is suggested as a means to enhance the drought resilience of crops [53]. We hypothesised that breeding has negatively affected host-to-microbe effects and, thus, that landraces generally exhibit stronger host-to-microbe effects and assemble more distinct prokaryotic communities compared to modern varieties. Further, we hypothesised that the drought response of prokaryotic taxa potentially exhibiting beneficial microbe-to-host effects would be more apparent in landraces than in modern varieties.

Materials and methods

Experimental setup

Field experiments were conducted with six central European landraces and six modern varieties (including two open-pollinated population varieties and four hybrid varieties) of *Z. mays* L. (Supplementary Table 1). Varieties represented a spectrum of responses to soil drying, assessed as the soil water potential (ψ_{soil}) at which the plants reduced transpiration in a previous greenhouse experiment [54]. The experiment was conducted in 2021 and 2022 at a field site near Pocking, Germany (lat. 48.382261, long. 13.263678, Supplementary Fig. 1). The cumulative precipitation was higher in 2021, particularly during the reproductive phase (Supplementary Fig. 2A), leading to differences in ψ_{soil} and its temporal dynamics (Supplementary Fig. 2B and C; Supplementary Tables 2

and 3). Half of the plots were covered by transparent rainout shelters, reducing precipitation by 60% (see [55] for details on the shelters and more information on the field). Replicate plots were arranged in completely randomised blocks, resulting in three (in 2021) and four (in 2022) replications for each combination of the factors variety and precipitation treatment, and yielding a total of 168 analysed samples (Supplementary Fig. 1, Supplementary Table 4). Plants were sown on 31 May 2021 and 21 April 2022, and rainout shelters were installed on 03 July 2021 and 18 May 2022. Measurements of ψ_{soil} at 30 cm depth were recorded at plot centres using TEROS21-sensors (METER Environment, Munich, Germany) for a subset of the plots.

Sampling

Plots were sampled in random order from 31 August 2021 until 05 September 2021 and from 09 August 2022 until 14 August 2022. Bulk density and gravimetric soil water contents were determined from 100 cm³ soil cores taken at 5 and 15 cm depth at the location of each sampled plant. These measures were used to derive ψ_{soil} by inverting a soil-water retention curve (Supplementary Note 1, Supplementary Figs 2C, D, and 3). Roots for metabarcoding were sampled as described previously [55]. In short, the root system was excavated, loosely adhering soil was removed by manual shaking, and secondary order lateral roots of crown roots were taken from 10 to 20 cm below the root base. Roots from three plants per plot and three locations in the root system per plant were pooled. Samples were stored at –80°C until DNA extraction. Additionally, rhizosheath (soil adhering to the roots after shaking) was collected manually and analysed for aggregate size distributions by immersed wet sieving [56]. Plant heights were measured, and the biomass of roots, shoots, and ears was determined after drying at 105°C for 24 h.

16S rRNA gene amplicon sequencing

DNA was extracted from roots after removing adhering soil by washing with NaCl solution (0.3 wt.%). Thus, our study included rhizoplane and endosphere, where we expected the strongest plant influence on the microbiome [55]. Disruption of samples by bead-beating, DNA extraction, and amplification of the V4 region of the 16S rRNA gene were performed as before [55] (primers: 515f, 5'-GTGTCAGCMGCGCGGTAA-3' [57] and 806RB, 5'-GACTACNVGGGTWTCTAAT-3' [58]) but using NEBNext Ultra II Q5 Master Mix (M0544, New England Biolabs, Ipswich, USA). Two libraries were prepared and sequenced on an Illumina iSeq-100 instrument (Illumina, San Diego, USA) in single-end mode with 293 cycles at the KeyLab Genomics and Bioinformatics (University of Bayreuth, Germany). Reads were processed as previously [55]. Briefly, raw read quality was assessed using fastqc (version 0.11.9) [59] and MultiQC (version 1.12) [60]. Forward primers were trimmed using Cutadapt (version 3.7) [61] with default settings, discarding reads where no primer was found. Unless otherwise stated, all following read-processing steps were done with USEARCH (version 11.0.667) [62]. Reads were truncated to 250 nucleotides to remove low-quality 3'-ends (Supplementary Fig. 4). Afterwards, low-quality reads were removed using fastq_filter (with fastq_maxee = 0.5). Dereplication was done with fastx_uniques, followed by 97% OTU clustering with cluster_otus (minsize = 2). The use of OTUs, rather than sequence-variant-level approaches, better prevented inflated diversity estimates arising from small sequencing-run-specific differences in error profiles [55].

DADA2 version 1.24.0 [63] with the commands `assignTaxonomy` and `addSpecies` was used for taxonomy assignment with version 138.1 of the SILVA rRNA reference database [64]. An OTU matrix was generated with the `USEARCH` command `otutab`. The following steps were done with `phyloseq` (version 1.40.0) [65] in R (version 4.2.3). OTUs assigned to mitochondria (order *Rickettsiales*) and chloroplast sequences were removed, retaining only reads from bacteria and archaea. Read counts from negative controls were subtracted from sample read counts. Following recent re-evaluations of normalisation strategies, suggesting rarefaction as the most robust approach for accounting for differences in sequencing depth [66, 67], we rarefied the number of observations in all samples to the number of observations in the sample with the lowest read count after all filtering steps (8209 reads).

Statistical analysis

For univariate comparisons including random factors, we used linear mixed models (LMMs) (`lmerTest`, version 3.1.3) in R (version 4.2.3) or linear models (LMs) if including random factors resulted in overfitting. Model fits were checked using diagnostic plots generated with `ggResidpanel` (version 0.3.0.9000). If the main test indicated significant effects for a term, we used `marginalEffects` (version 0.20.1) for specific comparisons among levels of involved factors. For multivariate comparisons on multiple plant variables, we normalised the variables (z-transformation) and calculated Euclidean distances. For multivariate comparisons on microbial relative abundance data, we applied square root transformations to reduce the influence of highly abundant taxa [68, 69] and calculated Bray-Curtis dissimilarities. We then applied PERMANOVA [70] (sums of squares type I, permutation of residuals under a reduced model) in `PRIMER` (version 7.0.23). To assess the effect of Ψ_{soil} , we included it as a covariate (see [Supplementary Note 2](#)). Since shifts in location corresponding to multiple experimental factors would overlap in 2D standard nMDS, we calculated bootstrap averages in `PRIMER` per combination of the factors year, precipitation treatment, and breeding era, and visualised microbiome similarity by MDS. To measure the average difference in microbiome composition between groups, we calculated distances among centroids in the space of the Bray-Curtis dissimilarity.

To test the distinctiveness of the categorisation of maize varieties by breeding era, we calculated averages per variety (across years and precipitation treatments) and performed hierarchical clustering on microbiome (Bray-Curtis dissimilarities) and plant biomass (Euclidean distances) data with `pvcust` (version 2.2.0) in R. To estimate confidences of dendrogram nodes, we calculated “approximately unbiased” *P*-values by bootstrapping.

To estimate the strength of host-to-microbe effects, we assumed they act as an unidirectional, deterministic selective force on microbiome composition, consistent with evolutionary theory [1] ([Supplementary Note 3](#)). This implies that stronger host-to-microbe effects should result in more similar microbiomes among individuals within a variety (lower within-variety variation). To compare within-variety variation in microbiome composition, we calculated the mean multivariate dispersion [71] (D_{mic}) among individuals within each variety based on Bray-Curtis dissimilarity for each combination of breeding era and precipitation treatments, using `betadisper` from `vegan` (version 2.6.4) ([Supplementary Fig. 5](#)). Landraces generally have higher genetic and phenotypic heterogeneity, which may influence D_{mic} . We accounted for this by first calculating multivariate dispersion based on Euclidean distances on z-transformed measures

of plant biomass (root, shoot, and cob) (D_{plant} , [Supplementary Fig. 5](#)). To evaluate the relative strength of host-to-microbe effects, we then derived a normalised measure of microbiome dispersion (D_{norm}) as

$$D_{norm} = \frac{D_{mic}}{D_{plant}}$$

where higher values indicate higher relative microbiome dispersion and therefore weaker host-to-microbe effects.

To identify taxa with differential relative abundance between treatments, we used `ANCOMBC` (version 2.4.0) [72]. We ran tests per year and breeding era to compare relative abundance changes in response to the precipitation treatment. Since `PERMANOVA` had indicated a significant contribution of sequencing run and block, we included those factors as random effects if this did not result in overfitting the underlying linear mixed effects model.

Results

Modern varieties grew larger and tended to have higher biomass than landraces ([Supplementary Fig. 6](#), [Supplementary Table 5](#)). Under the overall drier conditions in 2022, shoot biomass tended to decrease while root biomass increased under sheltered conditions, resulting in an increased root-to-shoot ratio. Statistically significant differences between landraces and modern varieties in their response to the sheltered conditions were not detected for these plant parameters.

Amplicon sequencing of 168 samples yielded 7 090 803 reads with an average read count of 40 088 (SD $\pm$ 9397) per sample. We identified 4600 root-associated prokaryotic OTUs across all samples, with members of the *Actinomycetota*, *Pseudomonadota*, and *Bacteroidota* dominating microbiomes ([Supplementary Fig. 7A](#) and [B](#)). *Streptomyces*, *Lechevalieria*, and *Sphingobium* were among the most prevalent genera ([Supplementary Fig. 7C](#) and [D](#)).

Effects of year, precipitation treatment, and breeding era on microbiome composition

Applying hierarchical clustering, landraces and modern varieties were clearly distinguishable (100% bootstrap-support) based on plant biomass variables ([Fig. 1A](#)) and prokaryotic community composition ([Fig. 1B](#)). One exception was the landrace “Gelber Badischer Landmais” (GB), which clustered closer to the modern varieties. We did not find a systematic differentiation between population and hybrid varieties within the modern varieties.

Significant differences in microbiome composition corresponding to all factors included in our experiment were apparent ([Fig. 1C](#), [Table 1](#)). Year had the strongest effect, followed by precipitation treatment and breeding era. In both years, microbiomes were significantly distinct between precipitation treatments and breeding eras ([Table 1](#)). Significant differences in microbiome composition between breeding eras were especially detectable for the sheltered group ([Table 1](#)). The microbiome response to the precipitation treatment and the effect of the breeding era differed between years, as indicated by significant interaction terms ([Table 1](#)). Considering distances among centroids, the differences in microbiome composition between the precipitation treatments were larger for landraces than for modern varieties in 2021 ([Fig. 1C](#), [Supplementary Table 6](#)), mirroring differences in Ψ_{soil} of the upper soil layer (5–15 cm) at sampling between these groups ([Fig. 2A](#)).

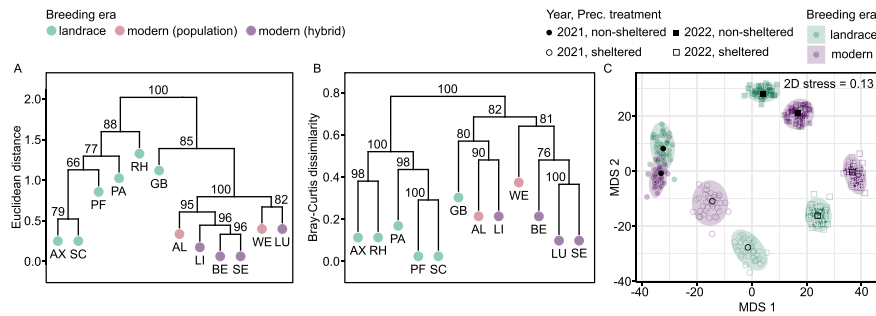


Figure 1 Distinctiveness of landraces and modern varieties. **(A)** Dendrogram visualising the similarity of the maize varieties in our experiment, determined by hierarchical clustering based on Euclidean distances calculated on log-transformed plant biomass variables (root, shoot, and cob dry weights). Numbers on nodes indicate the probability (given in %) that this node correctly represents the actual similarity of the joint branches. The two-letter codes correspond to the variety names, as listed in [Supplementary Table 1](#). **(B)** Dendrogram visualising the similarity of varieties determined by hierarchical clustering based on Bray–Curtis dissimilarity calculated on microbial relative abundance. **(C)** Similarity of microbiomes displayed for group averages. Group averages were calculated on Bray–Curtis dissimilarities between samples determined after rarefaction and square root transformation of relative OTU abundances. Bootstrapping (38 bootstraps per group) was used to visualise the uncertainty of the group averages. The averages (black dots) and bootstrapped values (coloured dots) were visualised by metric multidimensional scaling. Ellipses represent the 95% confidence regions for the bootstrapped values.

Table 1 PERMANOVA on Bray-Curtis dissimilarities calculated on relative OTU abundance data after square root transformation. We tested the full experimental design, including all factors except for “sequencing run.” The factors “sequencing run” and “variety” are confounded and can thus not be tested simultaneously. Terms with $P > .25$, despite a large number of possible unique permutations, were pooled. Pairwise comparisons were performed for factors involved in significant interaction terms in the main test. Here, the design including “batch” and omitting “variety” was used. When the number of unique permutations was low, we considered the P -values generated by Monte-Carlo testing (P(MC)) more informative. Significant ($P < .05$) differences between levels are shown in bold.

Main test:

Term	df	SS	MS	pseudo-F	P(perm.)	unique perm.	P(MC)	$\sqrt{\text{var}}$
Ear	1	31 444	31444.0	13.9350	0.0331	210	0.0001	18.9270
Prec. treatment	1	14 785	14785.0	2.9341	0.5026	6	0.0003	10.9460
Breeding era	1	5421.8	5421.8	1.8494	0.3387	6	0.0089	5.5331
Block (year)	5	11 280	2255.9	1.6364	0.0001	9593	0.0001	6.0770
Variety (breeding era)	10	17 374	1737.4	1.1978	0.0348	9726	0.0178	4.5966
Year $\times$ prec. treatment	1	5039	5039.0	3.6552	0.0001	9782	0.0001	9.4871
Year $\times$ breeding era	1	2931.7	2931.7	2.1266	0.0002	9803	0.0003	6.1797
Prec. treatment $\times$ breeding era	1	3181.7	3181.7	2.3080	0.0001	9767	0.0002	6.5766
Year $\times$ variety (breeding era)	10	14 505	1450.5	1.0522	0.1407	9421	0.1813	3.2555
Pooled	135	1.86E+05	1378.6				residual: 37.1290	
Total	166	2.93E+05						

Pairwise comparisons of factors involved in significant interaction terms:

Factor	Level	Comparison	t	P(perm.)	unique perm.	P(MC)
Year	2021	Non-sheltered, sheltered	2.5441	0.0001	9831	0.0001
		Non-sheltered, sheltered	2.8327	0.0001	9850	0.0001
	2022	Landrace, modern	1.5391	0.0001	9819	0.0009
		Landrace, modern	1.9753	0.0001	9822	0.0001
	Non-sheltered	Landrace, modern	1.2385	0.3314	6	0.0928
	Sheltered	Landrace, modern	1.5326	0.2520	3	0.0141

Community richness (number of OTUs) and evenness (Simpson index) were substantially reduced under drier conditions (Fig. 2B and C, [Supplementary Fig. 8](#), [Supplementary Table 7](#)). Differences in alpha diversity between landraces and modern varieties largely mirrored differences in ψ_{soil} (Fig. 2A). The decrease of community evenness under drier conditions was slightly more pronounced for modern varieties than for landraces, likely driven by the observations under the driest conditions (LM, $\psi_{\text{soil}} \times$ breeding era, $\beta = -0.05$,

95% CI: -0.11 – 0.00 , $P = .048$, [Supplementary Fig. 8C](#), [Supplementary Table 7](#)).

Influence of breeding on dispersion in microbiome composition and plant-biomass

Phenotypic dispersion based on plant-biomass parameters (D_{plant}) was generally higher for landraces than modern varieties, particularly

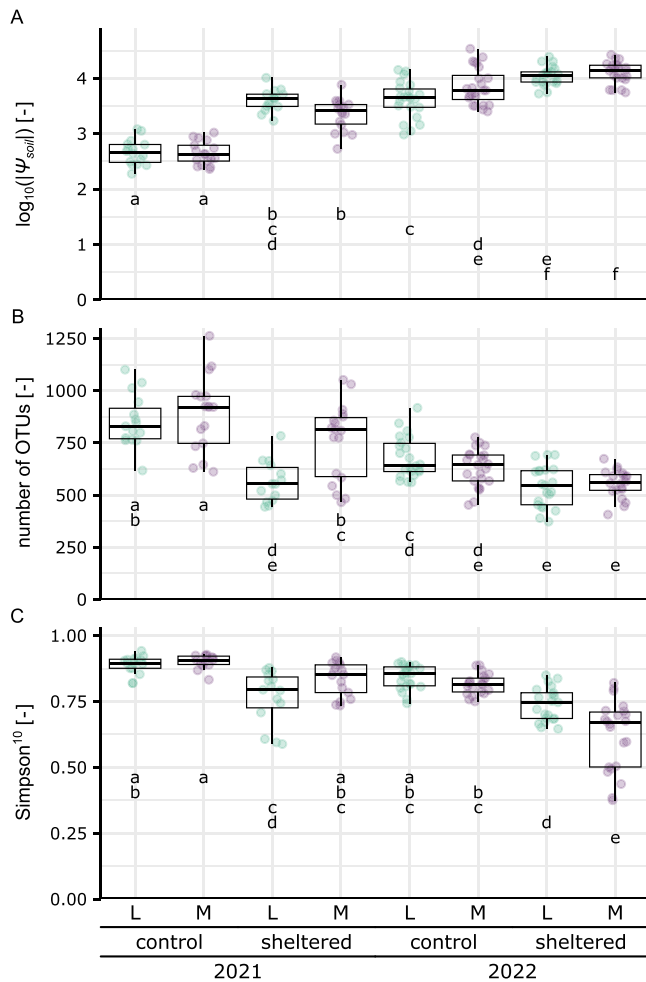


Figure 2 Ψ_{soil} and alpha-diversity metrics shown per year, precipitation treatment, and breeding era: landrace – L, modern – M. Differences between the groups formed by these factors were analysed by fitting a linear model and comparing estimated marginal means using the multcomp package (version 1.4.28) in R. Significant differences ($P < .05$) are indicated by the compact letter display where groups not sharing a letter are significantly different from one another. (A) Ψ_{soil} at sampling. Soil water potentials, measured in hPa, were expressed by the decadic logarithm of their absolute value: $\log_{10}(|\Psi_{soil}|)$. Higher values for $\log_{10}(|\Psi_{soil}|)$ correspond to less plant-available water. (B) Number of OTUs (richness) observed per sample. (C) Simpson index, measuring community evenness. Note that values were transformed by exponentiation (base 10) to enhance the visualisation of small variations.

under moist conditions (LM, $\Psi_{soil} \times$ breeding era, $\beta = 0.28$, 95% CI: 0.01–0.54, $P = .041$, [Supplementary Fig. 9A](#), [Supplementary Table 8](#)). However, the higher D_{plant} of landraces did not translate into higher microbiome dispersion (D_{mic}) ([Supplementary Fig. 9B](#), [Supplementary Table 8](#)). The relationship of D_{plant} and D_{mic} was contrasting between the varieties from the two breeding eras (LM, $\Psi_{soil} \times$ breeding era, $\beta = 0.07$, 95% CI: 0.03–0.11, $P = .001$, [Supplementary Fig. 9C](#), [Supplementary Table 9](#)). D_{mic} appeared independent of D_{plant} for landraces, while for modern varieties, D_{mic} significantly increased with D_{plant} (marginal effects, estimate = 0.057, 95% CI: 0.028–0.085, $P < .001$, [Supplementary Table 10](#)).

To further investigate the relative strength of host-to-microbe effects, accounting for the influence of plant phenotypic heterogeneity on D_{mic} , we calculated D_{norm} . D_{norm} was significantly higher in modern

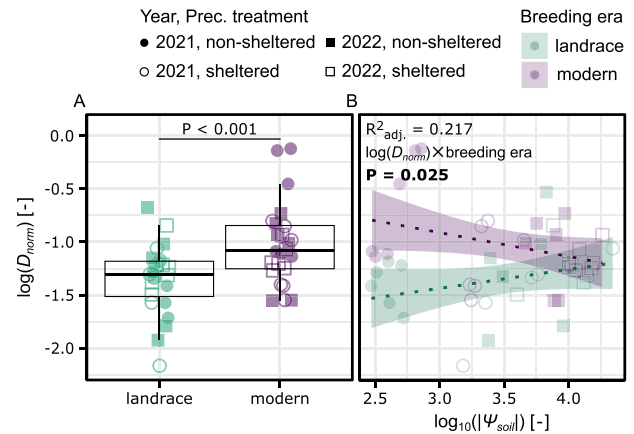


Figure 3 Within-variety dispersion in microbiome composition normalised to within-variety dispersion in plant traits (D_{norm}) was used as a proxy for host-to-microbe effects. D_{norm} was log-transformed for statistical analysis and visualisations to account for the skewed data distribution, which resulted in negative values since the original values of D_{norm} ranged from 0.11 to 0.89. Still, higher (less negative) values refer to higher D_{mic} per D_{plant} , indicative of weaker host-to-microbe effects. (A) D_{norm} shown for landraces and modern varieties. The P -value displayed refers to the effect of breeding era and was obtained by fitting a linear model to the observed data. (B) Relationship of D_{norm} and Ψ_{soil} at sampling. Higher values of $\log_{10}(|\Psi_{soil}|)$ correspond to lower amounts of plant-available water. The regression lines, displayed separately for the landraces and modern varieties, represent predictions from a linear model fitted to the observed data ([Supplementary Table 11](#)). Shaded areas around the regression lines denote 95% confidence intervals for the model predictions. Annotated are the adjusted R^2 of the model and the P -value for the interaction between Ψ_{soil} and breeding era. While D_{norm} and Ψ_{soil} did not correlate significantly ([Supplementary Table 12](#)), the significant interaction term indicates contrasting relationships between D_{norm} and Ψ_{soil} for landraces and modern varieties.

varieties (LM, breeding era, $\beta = 0.72$, 95% CI: 0.32–1.13, $P = .001$, [Fig. 3A](#)), meaning that dispersion in microbiome composition was relatively greater when accounting for the lower phenotypic dispersion of these varieties. A significant interaction (LM, $\Psi_{soil} \times$ breeding era, $\beta = -0.40$, 95% CI: -0.75 – -0.05 , $P = .025$) indicated contrasting responses of landraces and modern varieties to Ψ_{soil} ([Fig. 3B](#), [Supplementary Table 11](#)), where D_{norm} tended to decrease under drier conditions for the modern varieties ([Supplementary Table 12](#)). Differences in D_{norm} between breeding eras converged in the drier range ([Fig. 3B](#)).

Relative abundance shifts in response to drier conditions

Relative abundance shifts corresponding to Ψ_{soil} affected most phyla and were consistent between years ([Fig. 4](#), [Supplementary Fig. 7B](#), [Supplementary Table 13](#)). Reflecting the general reduction of alpha diversity, most phyla showed decreased relative abundance under drier conditions. *Actinomycetota* were the only phylum showing a significant relative abundance gain under drier conditions across all varieties (LM, Ψ_{soil} , $\beta = 0.15$, 95% CI: -0.113 – 0.187 , $P < .001$, [Fig. 4](#), [Supplementary Table 13](#)). In addition, we observed a significant difference in the drying response of *Bacillota* associated with landraces compared to modern varieties (LM, $\Psi_{soil} \times$ breeding era, $\beta = 0.01$, 95% CI: -0.003 – 0.011 , $P < .001$, [Fig. 4](#)). The reduction of relative abundances observed for *Pseudomonadota* under drier conditions was

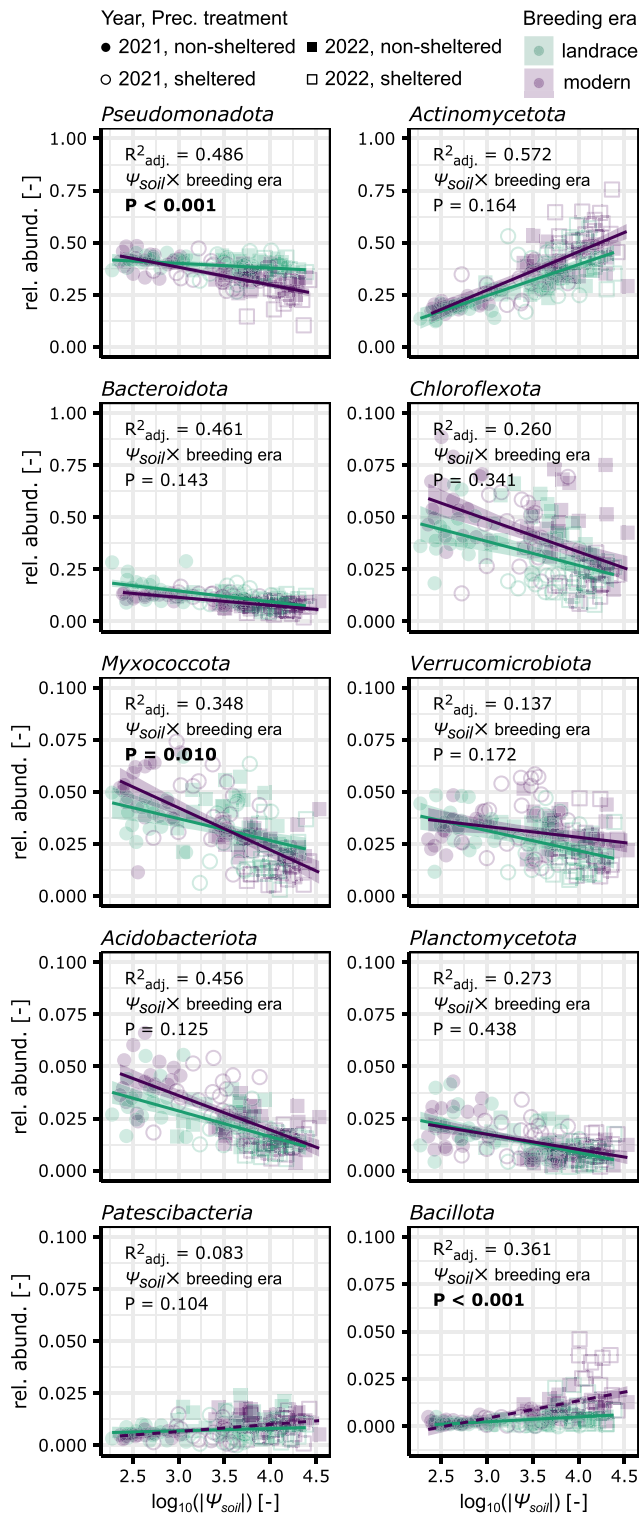


Figure 4 Relationships between relative abundances and ψ_{soil} shown for the ten most abundant phyla. The regression lines represent predictions from linear mixed-effects models fitted to the observed data (Supplementary Table 13). Significant ($P < .05$) correlations are displayed as solid lines. Shaded areas around the regression lines denote 95% confidence intervals for the model predictions. The adjusted R^2 for each model and the P -values for the interaction term, including ψ_{soil} and breeding era, are annotated. Note that the y-axis was restricted to a range of 0 to 0.1 for phyla other than the three most abundant (*Pseudomonadota*, *Actinomycetota*, and *Bacteroidota*) to enhance the visibility of variations. See Supplementary Table 13 for detailed statistical results.

stronger for modern varieties than for landraces (LM, $\psi_{soil} \times$ breeding era, $\beta = -0.06$, 95% CI: -0.091 – 0.035 , $P < .001$, Fig. 4). Also, at the genus level, most taxa that gained relative abundance in the sheltered group were *Actinomycetota* (Fig. 5). The genera with the strongest relative abundance gains were *Glycomyces*, *Pseudonocardia*, and *Nocardia* (Fig. 5A). Also, *Streptomyces* spp., which showed high relative abundances under all conditions (Fig. 5B), further increased relative abundance in the dry range. Analysis of taxa that showed relative abundance shifts under the sheltered treatment showed that most of these taxa also became more abundant with increasing proximity to the root in a previous study [55] using plants from the same experiment (Supplementary Fig. 10).

Association of filamentous taxa with soil aggregate size under drier conditions

Soil aggregate sizes were generally smaller under drier conditions (Supplementary Fig. 11). We investigated whether the relationship between aggregate size and ψ_{soil} differed with relative abundances of three groups of microbial taxa and compared this for landraces and modern varieties. These groups were (i) all taxa with higher relative abundance in the sheltered group, (ii) all taxa with typically filamentous morphologies, and (iii) the most abundant OTU with increased relative abundance in the sheltered group (*Streptomyces* spp.). We did not find significant changes in the relationship of aggregate size and ψ_{soil} with variation in the relative abundance of the first two groups of taxa. However, in 2021, higher relative abundances of *Streptomyces* spp. were associated with lesser reduction of aggregate sizes under drier conditions in modern varieties (marginal effects, estimate = 561.31, std. err. = 263.51, $P = .033$, Fig. 6, Supplementary Tables 14 and 15). In 2022, the overall drier conditions prior to sampling resulted in reduced variation in ψ_{soil} and aggregate sizes (Supplementary Fig. 11). Under these conditions, we found no association of the relative abundance of *Streptomyces* spp. with the relationship between aggregate sizes and ψ_{soil} .

Discussion

Studies comparing maize varieties bred over the last century have reported varying influences of breeding on plant-associated microbiomes [21, 24]. Here, we demonstrate breeding effects on the root-associated prokaryotic communities of European landraces and modern maize varieties. This was highlighted by the distinct clustering of landraces and modern varieties both based on plant biomass (Fig. 1A) and microbiome data (Fig. 1B), and is further underscored by the observation that the landrace “Gelber Badischer Landmais”, historically used to breed modern varieties [73], clustered with the modern varieties. Our results show that the impact of breeding on root-associated microbiomes is strongly connected to presumably plant-induced differences in available soil water between landraces and modern varieties.

Plant impact on ψ_{soil} as a main driver of root-associated microbiomes

We observed that reductions of prokaryotic alpha diversity (Fig. 2B and C) and shifts in microbiome composition (Figs 1B and C, 4, and 5)

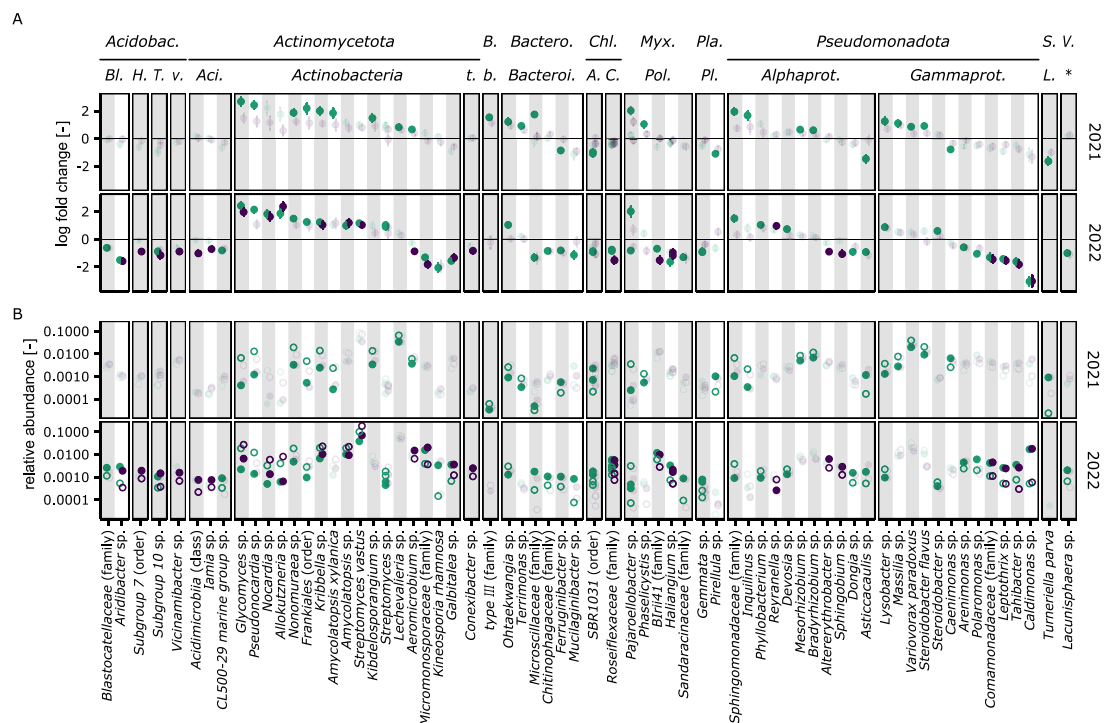


Figure 5 Relative abundance shifts of individual OTUs under sheltered versus non-sheltered conditions. Taxonomic classifications are shown at the lowest taxonomic rank with bootstrap support (minBoot = 50) from the DADA2 assign Taxonomy command. **(A)** Significant shifts in relative abundance ($P < .05$) were identified using ANCOM-BC2. The magnitudes of the relative abundance shifts (log fold change) are displayed separately for both years, landraces, and modern varieties. All taxa with a significant abundance shift in at least one group are shown. Observations not meeting the significance threshold are indicated by dots with reduced saturation. Taxa are grouped at the phylum and class levels and sorted on the x-axis according to the magnitude of their relative abundance shifts in decreasing order. Error bars represent standard errors. **(B)** Mean relative abundances per group shown for all taxa with significant relative abundance shifts in any group. Relative abundances for non-significant comparisons are indicated by dots with reduced saturation. Note that the y-axis is presented on a logarithmic scale to visualise the wide range of relative abundance values.

aligned closely with the precipitation treatment and Ψ_{soil} at sampling. This was evident, despite substantial differences in precipitation dynamics between years (Supplementary Fig. 2A). Strikingly, also shifts in microbiome composition associated with landraces and modern varieties (Fig. 1C) largely mirrored differences in Ψ_{soil} between these groups. This was especially apparent in 2021, when the upper soil layer (5–15 cm) tended to be drier for landraces than for modern varieties in the sheltered group (Fig. 2A). The drier conditions in these plots were accompanied by more pronounced relative abundance increases of microbial taxa apparently adapted to drier conditions (Fig. 5A).

In 2022, when plots with modern varieties were equally dry or even drier compared to plots with landraces, microbiome responses to the precipitation treatment were similar across breeding eras (Fig. 5A). This contradicts the hypothesis that microbiomes of modern varieties may show reduced plasticity in their drought response. Instead, our results suggest that shifts in the composition of root-associated microbiomes between landraces and modern varieties were mainly adaptations to plant-induced differences in soil moisture, and thus only indirectly plant-controlled. Specifically, the drier conditions in plots with landraces in 2021 may have resulted from their smaller size and lower above-ground biomass (Supplementary Fig. 6), causing reduced soil shading and higher direct evaporation. Alternatively, landraces and modern varieties may have preferentially extracted water from different soil layers under relatively moist conditions, as suggested by differences in Ψ_{soil} at 30 cm depth under non-sheltered conditions in 2021 (Supplementary Fig. 2B). Besides direct adaptations

to variation in soil moisture, microbiomes likely responded to differences in root anatomical traits [74, 75] and shifts in exudation patterns [76, 77] which may constitute plant adaptations to the drier conditions potentially affected by breeding.

The strong influence of soil moisture on microbiomes is well recognised [78, 79]. Still, to our knowledge, this has not been regarded in experiments assessing the effects of plant breeding on root-associated microbiomes. Our observations highlight that potential differences in the impact of plants on soil water availability must undoubtedly be considered as a confounding factor in such studies. Interestingly, it has been shown that the positive impact of drought-adapted microbiomes on plant growth can be uncoupled from whether the drought adaptation occurred in the presence of a plant or not [80]. Thus, it is important to note that although the relative abundance shifts in drying soil were likely driven primarily by indirect plant influences, they may still confer beneficial effects on the plant.

Variation in microbiomes decoupled from plant phenotypic variation in landraces

Our results show that, besides the noticeable influence of plants on drought-responsive taxa through shifts in Ψ_{soil} , landraces and modern varieties differed in how within-variety variation in microbiome composition related to within-variety variation in plant traits, indicating stronger host-to-microbe effects in landraces. In modern varieties, microbiome dispersion clearly reflected increasing dispersion in plant traits, whereas in landraces, it remained relatively low even

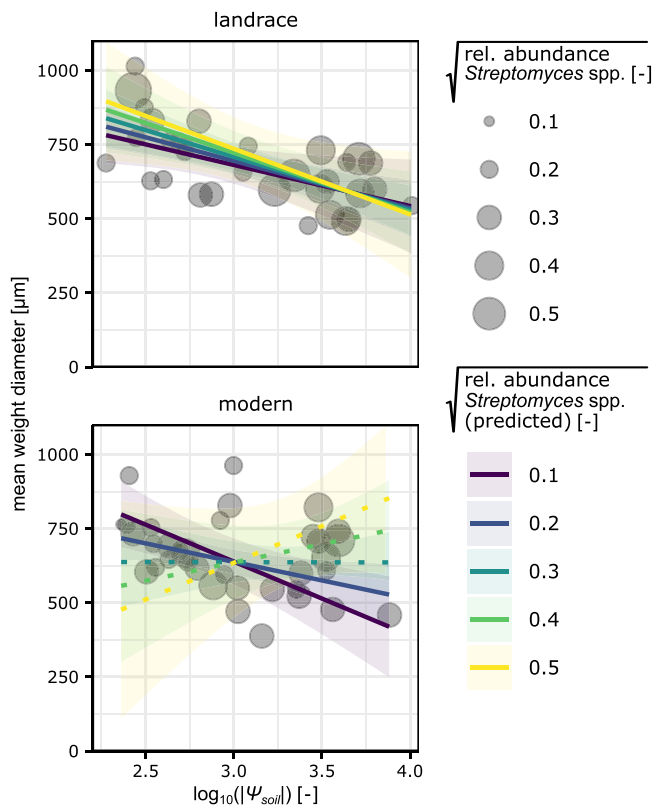


Figure 6 Relationship between soil aggregate mean weight diameter, Ψ_{soil} , and the relative abundance of OTU3 (classified as *Streptomyces* spp.) in 2021, shown separately for landraces and modern varieties. Note that the relative abundance values were transformed by taking the square root to improve visualisation across a wide range of values. The regression lines are displayed exemplarily for different relative abundances of *Streptomyces* spp. and represent predictions from a linear model fitted to the observed data (Supplementary Table 14). Significant ($P < .05$) correlations between Ψ_{soil} and the soil aggregate mean weight diameter are displayed as solid lines. Shaded areas around the regression lines denote 95% confidence intervals for the model predictions.

when plant trait dispersion was high (Supplementary Fig. 9C). This pattern suggests that, in landraces, host-to-microbe effects (e.g. root exudation and immune functioning) acted consistently and largely independently of variation in biomass traits. In contrast, microbiome composition in modern varieties appeared to reflect responses to variation in biomass, an effect likely not resulting from processes actively mediated by plants. Consequently, dispersion values obtained for landraces were relatively lower (Fig. 3A) after accounting for the overall higher plant phenotypic dispersion of this group (Supplementary Fig. 9A), suggesting that host-to-microbe effects conserved among landrace individuals lead to more similar microbiomes within varieties compared to the modern group. Notably, this pattern was most pronounced under moist conditions (Fig. 3B), which may reflect an increasing influence of abiotic drivers on microbiome dispersion when soil becomes drier. We acknowledge that the biomass parameters used in our study only serve as proxies for estimating plant phenotypic variation. An extended analysis incorporating traits directly influencing microbiome composition, such as root exudate profiles and root anatomical traits, would be an interesting objective for future research. Still, our results showcase that the relationship between microbiome and plant phenotypic dispersion was affected

by breeding and can provide a helpful measure for approaching host-to-microbe effects. A clear increase in microbiome dispersion among maize varieties with year of variety release has previously been observed based on prokaryotic and fungal community data, as well as microbial functional gene abundances [24], which may also indicate reduced host control over microbiomes. Thus, we suggest that assessments of breeding effects extend beyond identifying specific taxa preferentially associated with historical or recent varieties, but also routinely incorporate measures of variation in microbiome composition.

Filamentous *Actinomycetota* as a relevant group in drying soil

Despite differences in the magnitude of the abundance shifts, the taxa affected by the precipitation treatment and variation in Ψ_{soil} were broadly consistent, comparing years and varieties from both breeding eras (Fig. 4 and 5). Strikingly, the majority of the OTUs in our study that gained relative abundance under sheltered conditions, such as *Glycomyces* spp., *Lechevalieria* spp., *Sphingomonadaceae*, and *Streptomyces* spp., were shown to also gain relative abundance with increasing proximity toward the root in a previous study (Supplementary Fig. 10, [55]). Thus, we assume these taxa are better adapted to the root-associated microenvironment than others. The gradient towards the root can constitute a gradient in water availability, with drier conditions near the root surface caused by local water uptake [81] (However, mucilage exudation can invert this pattern, even in dry soil [82]). Therefore, relative abundance shifts toward the root might again be a consequence of the root's influence on soil water. Additionally, plants may actively promote taxa gaining relative abundance toward the root and in drying soils. Resolving the relative importance of water availability and active plant effects in explaining shifts in microbiome composition should be regarded in future work, e.g. by measuring and modifying root exudation [83] or by simulating moisture gradients around the root [84]. Below, we discuss whether it could be beneficial for plants to promote the observed taxa based on previous knowledge of their traits.

Actinomycetota constituted the phylum with the most pronounced relative abundance gain under drier conditions in our experiment (Fig. 4, Supplementary Fig. 7B) and are commonly associated with drought effects on rhizosphere and root-associated microbiomes [85–87]. Previous work showed that *Actinomycetota* not only persist but maintain growth under drought [88]. Further, it has been experimentally demonstrated that certain *Actinomycetota* alleviate effects of drought [89, 90] and salt stress [91] on plants. Strikingly, wheat grown from seeds coated with different *Streptomyces* strains showed a substantial increase in biomass in a drought-exposed field [92]. However, the underlying mechanisms remain poorly understood and are hypothesised to include pathogen suppression through antibiotic production and the activation of plant enzymes protecting from adverse drought effects [93]. Also, the influence of *Streptomyces* strains on plants appears to be context-dependent. For instance, the relative abundance of *Streptomycetaceae* has been negatively associated with maize shoot biomass under well-watered conditions but not under drought [87].

One trait typical of *Streptomyces* and other *Actinomycetota* is filamentous growth [94]. Bacteria forming hyphae-like structures span air-filled gaps, exhibit stronger surface attachment, and show higher motility under water limitation than other morphologies [95]. These are advantageous traits when microhabitats become disconnected in

drying soils [96]. In an experiment with filamentous *Streptomyces* and motile *Bacillus*, *Streptomyces* gained relative abundance in soils with larger pore sizes under dry conditions [97]. Thus, from a microbial perspective, filamentous growth is certainly beneficial in drying soils. It further seems conceivable that, similarly to the exopolysaccharides produced by *Bacillus* [98], filamentous structures could contribute to sustaining hydraulic connectivity in drying soil. Our observation of filamentous taxa occurring directly at the root may indicate a role in maintaining hydraulic connectivity at the root-soil interface. Future work should investigate whether the filamentous structures contribute to the beneficial effects that specific *Actinomyces* have on plants and, thus, if expressing host-to-microbe effects promoting this group could be advantageous for plants in terms of drought tolerance.

Streptomyces associated with soil aggregate size in the moister year

Under the comparatively moist conditions in 2021, we found that the relative abundance of an OTU classified as *Streptomyces* spp. interacted with the reduction of soil aggregate size in the rhizosphere, typically observed for drying soil [99]. The reduction of the aggregate sizes tended to be stronger when the relative abundance of this OTU was low (Fig. 6). Strikingly, we observed this interaction only for modern varieties but not for landraces. This difference may result from the generally moister soil in the modern group in 2021 (Fig. 2A), indicating that the relationship between aggregate size and the abundance of *Streptomyces* spp. only occurs under moderate reductions of available water. This aligns with the complete absence of correlations between the abundance of *Streptomyces* spp. and aggregate size under the overall drier conditions in 2022 (not presented). Together, this suggests that the association between the relative abundance of *Streptomyces* spp. and aggregate size is likely mainly relevant in moderately dry soils. We could not find significant associations with aggregate size for other groups of filamentous taxa. While *Streptomyces* constituted the genus with the highest relative abundance among those increasing in relative abundance under drier conditions, we also identified genera with a much stronger relative abundance gain, such as *Glycomyces*. A substantial increase in the abundance of endophytic *Glycomyces* spp. under drought has previously been reported for barley [100]. However, still little is known about the functional potential and effects of this genus on plants.

Conclusions

We demonstrated that European landraces and modern varieties of maize differed in the composition of their root-associated prokaryotic microbiomes. Our observations show that shifts in microbiome composition between landraces and modern varieties were likely, primarily, a passive consequence conveyed by differences in the effects of plants on water availability. This mechanism should, therefore, always be considered when assessing the impact of plant breeding on microbiomes. Future research should quantitatively evaluate the relative contribution of plant-induced changes in water availability compared to more direct mechanisms, such as modulation of root exudation, in shaping microbiome composition. Further, the reduced host-to-microbe effects of modern varieties indicated by dispersion analysis emphasise the need to consider the impact of breeding on the extent of plant control over microbiome composition at the community level rather than focusing on changes in the abundance of individual taxa.

While reduced host-to-microbe effects may have impaired microbiome functions unrelated to mitigating drought effects, we assume that, as soil water availability appears to be the underlying major driver, breeding did not negatively affect potentially beneficial microbiome functions under drier conditions. We believe it is of fundamental importance to further elaborate a systematic understanding of the mechanisms by which filamentous *Actinomyces* may contribute to alleviating adverse drought effects. Our observation that the relative abundance of *Streptomyces* spp. associated with modern varieties was linked to soil aggregate stability provides an interesting starting point for this.

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Supplementary material

Supplementary material is available at ISME Communications online.

Conflicts of interest

The authors declare no conflicts of interest.

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Data availability

Raw sequence data were deposited at the European Nucleotide Archive under study accession number [PRJEB87603] (sample accession numbers [ERS23861137] – [ERS23861315]).

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