PLANT DOMESTICATION DOES NOT REDUCE DIVERSITY IN ROOT MICROBIOMES

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SUMMARY

- Domestication has profoundly shaped the genetic makeup of numerous plant and animal species. While the effects of plant domestication at the genetic and phenotypic levels are well-documented, its impact on plant microbiome remains less understood.
- Two primary hypotheses have been proposed: 1) the reduction in microbial diversity resulting from the domestication process, and 2) the diminished ability of host plants to control their microbiomes.
- We conducted a meta-analysis of multiple crops, comparing the root microbiomes of domesticated plants and their wild relatives. Our results indicate that the effects of domestication are species-specific and context-dependent, with most domesticated plants exhibiting increased microbial diversity and more structured communities.
- Overall, this study provides evidence that plant domestication does not lead to a uniform reduction in microbial diversity or a consistently diminished ability of plants to influence their microbiomes.
- Based on these findings, we discuss new perspectives and the need for future studies incorporating native soils and host genetic variation in such experiments, analyzing diversity and microbiome function, and considering how root morphology might affect microbiome recruitment.

INTRODUCTION

Domestication, the artificial selection of phenotypic traits useful to humans, has profoundly shaped human civilization and the genetic makeup of numerous plant and animal species (Purugganan & Fuller, 2009). The consequences of domestication at the genetic and phenotypic level are well-documented (Meyer *et al.*, 2012). Typically, domestication events involve propagating only selected plant seeds, leading to genetic bottlenecks and, thus, a reduction in genetic diversity across the genome (Doebley *et al.*, 2006). Phenotypically, convergent changes in certain traits, known as 'domestication syndrome', have been observed across diverse domesticated species (Alam & Purugganan, 2024). In plants, this syndrome is characterized by enlarged fruit sizes, increased apical dominance, and the loss of seed dispersal mechanisms and dormancy, among other traits (Doebley *et al.*, 2006). Many of these traits are the target of selection, but many others are not. Those non-target traits can evolve through correlations (genetic linkages or pleiotropy) with the target traits (e.g. reduction of seed shattering, uniform flowering, and ripening) and can greatly influence the ecology of the plant (Purugganan & Fuller, 2009; Meyer *et al.*, 2012). The extent to which domestication-induced phenotypic changes affect ecological interactions remains an open question.

It is often proposed that domestication leads to reduced investment in other ecological interactions. In the case of plant-herbivore interactions, a commonly proposed hypothesis suggests that domestication has modified plant traits in ways that decrease resistance to herbivores (Benrey *et al.*, 1998). Nonetheless, a meta-analysis (Whitehead *et al.*, 2017) conducted in 2017 assessed the overall impact of crop domestication on plant-herbivore interactions and found that, while domestication appears to have a generally negative effect on herbivore resistance, it did not significantly alter plant defense traits. Similarly, a recent study (Glasser *et al.*, 2023) exploring the consequences of domestication on plant-pollinator interactions in members of the *Cucurbita* genus revealed that domestication in this group may have promoted greater resource investment in floral traits. This increased investment likely enhances attractiveness to pollinators, potentially increasing reproductive success.

Roots are an important locus for the evolution of non-target traits and species interactions. Root phenotypes have often significantly diverged from their wild relatives due to adaptation to the agricultural environment or as non-target modifications associated with selected traits above ground. Domesticated plants have a different root system architecture, altered root exudate compositions, and significant changes in secondary metabolites across a wide range of species

(Meyer *et al.*, 2012; Singh & van der Knaap, 2022) which necessarily has an impact in plant interactions with other organisms, including symbiotic microbial communities associated with plant roots (the root or rhizosphere microbiome).

Plants rely on their microbiomes for several functions, including nutrient acquisition, protection against pathogens, and tolerance to biotic stress (Pérez-Jaramillo *et al.*, 2018). Often, the root microbiome promotes plant health and growth, contributing to plant development (Singh *et al.*, 2020). Given the pivotal importance of root microbiomes for plants and considering the known effects of plant domestication on root systems, in recent years, efforts have been undertaken to decipher the impact of plant domestication on the diversity and assembly of these microbial communities. Despite these efforts, it is still unclear if, or to what extent, plant domestication has modified the ability of the plants to interact with the root microbiome.

Two main hypotheses have been proposed regarding the consequences of domestication: 1) the reduction in microbial diversity resulting from the domestication process and 2) the diminished ability of host plants to shape the assembly of their microbiomes. These hypotheses arise from the idea that the genetic bottlenecks accompanying domestication may lead to the loss of alleles crucial for plant-microbiome interactions, that alterations in root exudates metabolic profiles might further influence microbiome assembly in domesticated plants (Gutierrez & Grillo, 2022), and because of a potential reduction in the benefits of mutualism for domesticated plants. After growing in environments with important nutrient inputs, domesticated plants could reduce the investment in symbiosis, leading to the evolution of reduced symbiont cooperation (Porter & Sachs, 2020). To evaluate these hypotheses, we conducted a meta-analysis comparing studies inoculating domesticated plants and their wild relatives with the same bulk soil in common garden conditions. We found that plant domestication does not result in a uniform reduction in microbial diversity or a consistently diminished ability of host plants to influence their microbiomes. The effects of domestication are species-specific and context-dependent, with some domesticated plants showing increased microbial diversity and structured communities, while others do not. Despite this variation, we found consistent losses and enrichments of microbial taxa in domesticated crops relative to their wild progenitors.

MATERIALS AND METHODS

Data collection

To investigate the impact of the domestication process on the diversity and composition of the root microbiome across various crop species, we collected 16S rRNA sequence data from common garden experiments reported in scientific publications. We conducted a comprehensive search in the Scopus and Google Scholar databases using the Boolean search string: Wild [AND] domesticated [AND] microbiome [OR] rhizosphere [AND] crop. Our search centered on contemporary crops with well-identified crop wild relatives, including but not limited to maize, potato, tomato, squash, sorghum, bean, coffee, barley, soybean, cotton, and sunflower. Eligibility for inclusion in our meta-analysis was based on the following selection criteria: 1) the experiments must have been conducted under controlled environmental conditions typical of common garden studies, 2) wild and domesticated variants of the same crop species must have been cultivated concurrently, and inoculated with the same soil, and 3) sequencing must have been performed using Illumina platforms. We excluded studies that assessed the impact of extreme environmental stressors, such as herbivory, but we incorporated those that utilized different soil types (e.g., forest or agricultural soils) as inoculum. Applying these criteria, we narrowed down the pool to 15 publications, representing eight crops: barley, soybean, cotton, tomato, potato, sunflower, maize, and bean. A detailed overview of the number of crop accessions, experimental replicates, and analyzed publications per crop is available in Table S1.

Additionally, due to the large number of papers we could not include in our meta-analysis because of missing raw sequences or metadata, we selected a subset of studies that met our inclusion criteria and reported diversity data and/or ASV tables. A detailed summary of the crop accessions, experimental replicates, and publications analyzed in this secondary dataset is provided in Table S2.

With these datasets, we investigated two primary hypotheses regarding the impact of plant domestication on root microbiomes: 1) the reduction in microbial diversity resulting from the domestication process, and 2) the diminished ability of host plants to control their microbiomes. Since this data did not experimentally assess plant “control”, hereafter we refer as “host influence” to the contribution of plants in shaping root microbiome assemblies. Our analysis focused on the comparison between wild and domesticated categories. To mitigate the potential

154 bias introduced by merging amplicon sequence data from disparate studies, our comparative
155 analyses were restricted to within-study wild and domesticated pairs (W-D pairs). We defined
156 W-D pairs as plants cultivated concurrently and inoculated with the same soil. Typically, each
157 paper contributed a single W-D pair to our analysis. However, in instances where a study
158 investigated more than one soil type, the number of W-D pairs was equivalent to the number of
159 different soil treatments applied.
160
161 *Data processing*
162
163 16S rRNA raw sequences and metadata were downloaded from public repositories, including
164 NCBI, MG-Rast, and Github, and were analyzed with the Quantitative Insights Into Microbial
165 Ecology (QIIME2) platform (Bolyen *et al.*, 2019) version 2023.5. For each dataset corresponding
166 to a specific publication, paired-end sequences were analyzed independently. Sequence
167 denoising and quality filtering, including primers and adapters trimming, were performed with the
168 DADA2 plug-in (Callahan *et al.*, 2016). Given that all selected studies utilized primers targeting
169 the V4 16S rRNA gene region (e.g., V4, V3-V4, V4-V5), we standardized the analysis by
170 extracting the V4 segment using the 515F–806R primer pairs (Caporaso *et al.*, 2011). This
171 approach minimized discrepancies arising from taxonomic assignments across different regions
172 of the 16S rRNA gene. The length of the V4 region sequences was standardized to a range
173 between 120 and 150 nucleotides, based on sequence quality and primer positioning. This
174 criterion was consistently applied across all datasets. Taxonomic classification of the Amplicon
175 Sequence Variants (ASVs) for each dataset was conducted using the "sklearn-base" classifier
176 within QIIME2, referencing the *Greengenes* 2 database (McDonald *et al.*, 2024) with a 99%
177 sequence identity threshold. Mitochondrial, chloroplast, and ASVs that were not assignable at the
178 kingdom level were excluded from the analysis. Comprehensive details on the total number of
179 reads (before and after processing) and the count of ASVs for each study have been compiled in
180 Table S3.
181
182 *Data Analysis*
183
184 Data analysis was performed on R software (v.4.3.0) using custom scripts and employing the
185 following packages: Phyloseq (v. 1.46.0) (McMurdie & Holmes, 2013), ggplot2 (v. 3.5.1) (Valero-
186 Mora, 2010), metafor (v. 4.2-0) (Viechtbauer, 2025), vegan (v.2.6-4) (Oksanen, 2017), DESEq2
187 (v.1.40.2) (Love *et al.*, 2014), Source Tracker 2 (Knights *et al.*, 2011), and breakaway (v.4.8.4)

188 (Willis & Bunge, 2015). Prior to amplicon analysis, each of the 15 datasets, corresponding to the
189 individual studies included in our meta-analysis, was rarified to a standardized depth of 15,000
190 reads per sample to ensure comparability across datasets. Specifics on the number of samples
191 and ASVs retained after rarification are available in Table S3. As described previously, all
192 analyses were performed using W-D pairs (pairs of wild and domesticated plants from the same
193 study, species, and treatment).
194
195 **Alpha diversity analyses:** To assess the effect of plant domestication on the diversity of the
196 microbiome we calculated Chao1 and Shannon alpha diversity indexes using the *estimate*
197 *richness* function in the phyloseq package. In addition, we estimated species richness using
198 frequency ratios with the breakaway package. This method applies statistical modeling to predict
199 the number of unobserved taxa, providing flexible modeling of rare species. To test for differences
200 in these indexes we applied the Log Ratio of Means (LogRoM) analysis which compensates for
201 the non-independence of the data by calculating the effect size and variance for each study
202 individually (Friedrich *et al.*, 2008). We implemented this test with the *metafor* package, using W-
203 D pairs from all crops and the diversity of the wild plants as reference.
204
205 Also, we calculated Rank Abundances Distributions (RADs) for all samples using vegan package,
206 following the methods reported in (Dal Bello *et al.*, 2021). From the RAD data, we computed log-
207 linear models to fit linear regressions and compare the slopes between wild and domesticated
208 microbiomes. Absolute slope values were plotted and contrasted; in general, more even
209 communities display smaller slopes.
210
211 Additionally, from our secondary dataset (see *Data collection*), we obtained Chao1 and Shannon
212 diversity data either from supplementary information provided in the papers or by downloading
213 ASV tables and using the *estimate richness* function in the phyloseq package. Using these data,
214 we constructed separated databases to test for differences in wild and domesticated microbiomes
215 using the LogRM analysis as previously described.
216
217 **Beta diversity analyses:** To explore potential differences in beta diversity between plant
218 categories and crops, we calculated the Bray-Curtis distance for every W-D pair in the dataset.
219 Dissimilarity matrices were analyzed with Permutational Multivariate Analysis of Variance
220 (PERMANOVA) using the *Adonis* function in the vegan package. From the PERMANOVA result,
221 we extracted the R^2 values to represent the proportion of variation (in terms of beta diversity)

222 explained by category for all W-D pairs and all crops. A dot plot was constructed with this
223 information using ggplot2.
224
225 Furthermore, from the dissimilarity matrices we calculated, for all crops, the mean dissimilarity to
226 the bulk of both wild and domesticated plants, and the mean distance to centroid between groups
227 using the *betadisper* function in the vegan package. We plotted both metrics for all W-D pairs in
228 the different crops using ggplot2. Finally, a Bayesian microbial source tracker was performed with
229 the SourceTracker2 program to calculate the contribution of the bulk soil microbial community to
230 the final root microbiome. All samples were rarefied to a depth of 15,000 reads per sample before
231 the analysis.
232
233 **Differentially enriched microbes between categories:** We identified differentially enriched
234 microbes between W-D pairs across all crops using the DESeq2 test, implemented in the DESeq2
235 package. This test was applied exclusively to W-D pairs (excluding bulk soil sequences), with a
236 P-value cutoff of <0.01 and a False Discovery Rate (FDR) correction. Subsequently, we merged
237 the results from all W-D comparisons with differentially abundant features at the family level and
238 selected only features with a log2Fold change of ≤-5 or ≥5, or those present in two or more crops.
239 The results were visualized by crop using forest plots constructed in ggplot2. Additionally, we
240 plotted the individual abundances of microbial families enriched in the same category across
241 multiple crop species (Fig. S6).
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253 **RESULTS**

254

255 We conducted a systematic meta-analysis to assess the influence of plant domestication on root
256 microbiomes across different plant species. After applying our inclusion criteria and filters of data
257 quality, 15 independent studies were retained to perform the full analyses. These studies
258 encompass eight different crop species and included 947 individual plants belonging to maize (n
259 = 128), wheat (n= 172), barley (n= 55), sunflower (n= 127), tomato (n= 169), cotton (n= 31), bean
260 (n= 52), and soybean (n= 160) (Table S1). Except for soybean, and sunflower, all crops were
261 represented by more than one accession of wild and domesticated plants (Table S1).
262 Additionally, we included diversity metrics from studies that met our inclusion criteria but were
263 excluded from the full analyses due to missing data. This secondary dataset included eight
264 independent studies from six different crop species, including 758 individual plants belonging to:
265 tomato (n= 28), potato (n= 210), maize (n= 264), sorghum (n= 100), rice (n= 115), and agave (n=
266 41) (Table S2). All crops in this dataset were represented by more than one accession of wild and
267 domesticated plants, increasing the host genetic diversity analyzed in this study.
268 In total, we analyzed 40 different W-D pairs (pairs of wild and domesticated plants from the same
269 study, species, and treatment). This included 23 W-D pairs from the main dataset, used for the
270 full analyses, and 17 W-D pairs from the secondary dataset, used exclusively for diversity
271 comparisons.

272

273 ***Domesticated plants show increased microbiome diversity***

274

275 To test for differences in microbial diversity between wild and domesticated plants we performed
276 multiple diversity, richness, and evenness analyses. Contrary to the common expectation that
277 domestication reduces microbiome diversity, we found, on average, domesticated plants
278 exhibited slightly higher diversity in terms of Chao1 index and species richness calculated using
279 frequency ratios. This pattern was consistent across both the main dataset that included the raw
280 sequences (LogRoM: 0.01, 95% CI: -0.06-0.7, $p < 0.000$, Fig. 1 - Chao1; LogRoM: 0.01, 95% CI:
281 -0.01-0.8, $p < 0.000$, Fig. S1 - Frequency ratios) and the secondary dataset (LogRoM: 0.04, 95%
282 CI: -0.08-0.17, $p < 0.000$, Fig. S3b - Chao1 index). Despite these differences in estimated
283 richness, wild and domesticated species tended to have similar diversity using the Shannon index
284 (main dataset: LogRoM: -0.01, 95% CI: -0.01-0.0, $p= 0.1$, Fig. S2; secondary dataset: LogRoM:
285 0.00, 95% CI: -0.02-0.02, $p= 0.1$ (without agave data - figure not shown)). The main exception to

286 this trend was the comparison using the Shannon index for Agave samples in the secondary
287 dataset, where we found higher diversity in wild plants, influencing the overall model toward
288 greater diversity in wild plants (LogRoM: -0.01, 95% CI: -0.02-0.01, $p < 0.00$, Fig. S3a).
289
290 Most pairwise comparisons (pairs of wild and domesticated plants from the same study, species,
291 and treatment) showed differences in diversity, but the effects were highly variable across species
292 (Fig.1). Among the 23 Wild-Domesticated (W-D) pairs evaluated, only three (13%) exhibited no
293 statistical difference. Of the 19 (82%) pairs that showed statistically significant differences, 12
294 (63%) displayed increased diversity in domesticated plants, whereas eight (42%) had higher
295 diversity in their wild counterparts. Whereas for the secondary dataset (Fig. S3b), we found that
296 77% of the W-D pairs analyzed showed significant differences, from those, 71% showed
297 increased diversity in domesticated plants, while 29% showed higher diversity in wild plants.
298 Notably, the effect of domestication appears to be species-specific and related to functional
299 groups. Crops like wheat, tomato, and cotton showed increased diversity in their domesticated
300 forms compared to their wild forms, whereas maize and legumes (beans and soybeans)
301 presented greater diversity in their wild forms. Despite these overall patterns, we observed
302 different outcomes, even within the same species. The only exception to this inconsistency was
303 observed in tomatoes, where all four comparisons consistently revealed higher diversity in
304 domesticated plants. The most variable crop species was soybean, where of the six total W-D
305 pairs analyzed, two resulted in higher diversity in domesticated versions, three in higher diversity
306 in wild plants, and one showed no statistically significant difference. Furthermore, we did not find
307 differences in microbiome evenness between wild and domesticated plants; when comparing
308 absolute slope values calculated from the RADs data, we found that all analyzed microbial
309 communities had small slopes, indicating highly even communities regardless of domestication
310 category (Fig. S4).
311
312 ***Host species effect is more important than domestication in shaping microbiome***
313 ***composition***
314
315 We then evaluated the effect of domestication on microbiome composition using PERMANOVA.
316 We found that the domestication category accounted for a relatively small fraction of the variation
317 across most of the crop species studied (Fig. 2 and Fig. S5), suggesting a potential species-
318 specific effect. For several species, including maize, wheat, barley, sunflower, tomato, and bean,
319 domestication explained approximately 10% of the observed variance in microbial community

320 composition. Notably, cotton and soybean (except for two pairs) stood out, with domestication
321 accounting for a larger portion of the variance, ranging from 12% to 25%. Additionally, we
322 leveraged experiments that used multiple soil types to inoculate plants, allowing us to test the
323 contribution of the inoculum to the final microbial community by separating wild and domesticated
324 plants independently (Table S4). Although only a small set of papers had different soil types, for
325 barley, different initial soils accounted for a minor portion of the total variation (9.8% for wild plants
326 and 10.9% for domesticated plants). In contrast, for soybeans, using soils from different origins
327 accounted for a significant percentage of the variation (~75% for wild and ~78% for domesticated
328 plants). Taken together, these results suggested that domestication could affect the degree of
329 host influence but that there might be variation across species.
330
331 ***Wild relatives do not display systematic evidence of increased host influence of their***
332 ***microbiomes***
333
334 To evaluate the extent of host influence exerted by wild and domesticated plants over their
335 microbiomes, we leveraged beta diversity metrics, comparing the microbial communities
336 associated with plants to those from bulk soils, which served as a reference for the initial microbial
337 community (Fig. 3). We hypothesized that if plants have a relatively strong influence in the
338 assembly of their microbiomes, then we would expect to find: a) significant dissimilarity between
339 plant-associated microbiomes and bulk soil microbiomes (Fig. 3a), suggesting that plants are
340 selecting specific microbial assemblages distinct from the initial soil; b) low intra-group dispersion
341 (Fig. 3b), indicating that plant replicates of the same genotype consistently select for similar
342 microbial compositions; and c) less proportion of the final community explained by the initial bulk
343 soil (Fig. 3c), implying that the host plays a decisive role in shaping its microbiome, potentially
344 steering it away from the original soil community composition.
345
346 Overall, we did not identify a consistent pattern linking host influence to the category of
347 domestication. Most comparisons were not statistically significant. Among the significant
348 comparisons, we observed greater dissimilarity to bulk in wild barley and cotton and in
349 domesticated wheat and soybean (Fig. 3a). Similarly, we found a diverse range of outcomes in
350 the intra-group dispersion analyses (Fig. 3b). Domesticated plants generally exhibited more intra-
351 group dispersion in barley and soybean, whereas we found the opposite effect (more intra-group
352 dispersion in wild varieties) in wheat, cotton, and bean. Maize, sunflower, and tomato showed no

differences. We didn't find a clear pattern in the contribution of bulk soil microbiomes to the final plant-associated communities across different levels of domestication (Fig. 3c).

We explored if some other plant attribute could better account for our results. Grouping our results by functional plant group rather than domestication status provided increased insight.
Grasses (maize, wheat, barley) consistently demonstrated a more distinct divergence from their initial bulk soil microbiomes (Fig. 3a), with less than 50% of the final community composition attributable to the bulk soil (Fig. 3c). Whereas the legume soybean presented final communities closely aligned with the initial bulk soil, reflecting less than 50% differentiation.

Some microbial taxa differentially associate with plants across species according to the domestication status

Finally, we investigated if, despite all the variation, we could identify microbial groups differentially present in wild and domesticated crops (see methods; Fig. 4). We identified a few families consistently enriched or depleted from domesticated crops (relative to their wild counterparts) across different crops. Two members of the *Rhizobiaceae* family (*Rhizobiaceae_A_500471* and *Rhizobiaceae_A_500279*) were significantly enriched in the domesticated variants of wheat, tomato, and soybean. Whereas two members of the *Burkholderiaceae* family (*Burkholderiaceae_A_592522* and *Burkholderiaceae_A_574758)* were consistently enriched in the wild counterparts of wheat, tomato, bean, and soybean. We found these *Burkholderiaceae* taxa in both the domesticated and the wild rhizosphere. In contrast, the *Rhizobiaceae* members appeared almost absent in the wild versions of wheat and tomato (minimum absolute abundance < 50 reads; Fig. S6).

DISCUSSION

Our study provides new insights into the relationship between plant domestication and microbial diversity and assembly, challenging the widely held belief that domestication always leads to a decrease in microbial diversity and impairs the plant's influence in the assembly of its microbiome. Our results indicate that while domestication does have an effect on microbial diversity and community composition, these effects vary widely across species and experimental conditions.

Contrary to the hypothesis that domestication generally reduces microbial diversity, we found a small but significant reduction of microbiome diversity in the rhizosphere of wild relatives (Fig. 1, Log Ratio of Means with Chao1 index, $p < 0.001$). In fact, for the Chao1 index as well as for the frequency ratios test, most of the significant comparisons indicated that domesticated plants exhibited higher microbial diversity than wild plants (Fig. 1 and Fig. S1). When using the Shannon diversity index, we observed fewer differences and mostly saw no change in diversity across comparisons. The hypothesis that domestication leads to a reduction in microbiome diversity is largely based on the idea that domestication reduces genetic diversity, potentially leading to the loss of alleles important for plant-microbe interactions through selective bottlenecks (Gutierrez & Grillo, 2022) and that in domesticated plants, a reduced investment in symbiosis could promote the evolution of reduced symbiont cooperation (Porter & Sachs, 2020).

It is not entirely clear why the reduction of genetic diversity at the level of populations would lead to reduced microbiome diversity at the level of individuals. Instead, we might expect a decrease in microbiome diversity across hosts (less dispersion) in domesticated varieties compared to their wild counterparts. However, our results highlight that this is only the case in some pairs –mostly wheat, barley, and cotton. The opposite is true in some other pairs, including many of the soybean comparisons (Fig. 3). One of the limitations in making clear conclusions about the impact of host diversity on the microbiome is that host diversity is rarely controlled in these studies (for example, many domesticated accessions might be compared against a single population of wild relatives).

Even the loss of alleles important for plant-microbe interactions does not have to be associated with decreased microbiome diversity. It has been proposed, for example, that a reduction in genetic diversity might lead to less efficient microbiome filtering mechanisms, allowing domesticated plants to be less selective and thus associate with a greater diversity of microbes

418 (Soldan *et al.*, 2021; Favela *et al.*, 2022; Gutierrez & Grillo, 2022). This hypothesis has gained
419 attention due to its potential implications for agriculture and resilience to climate change. The
420 variation in microbial communities explained by host genotype represents the substrate upon
421 which natural selection can act and, therefore, it is also the variation that could be manipulated
422 for agricultural improvement (Brown *et al.*, 2020). Thus, a reduction in host influence resulting
423 from domestication could lead to decreased evolutionary potential within microbial communities,
424 potentially limiting the adaptive resources of plants in response to environmental stresses (Soldan
425 *et al.*, 2021). This hypothesis of less efficient filtering mechanisms in domesticated crops is
426 consistent with our alpha (within-plant) diversity results, where we observed a small increase in
427 microbiome diversity in domesticated varieties. But, contrary to this expectation, we found little
428 support for decreased host influence in domesticated plants. In most pairs, the microbial
429 community of the rhizosphere is equally distant to the bulk soil, with soybean being the only
430 consistent example of increased distance to bulk soil in the domesticated varieties (even if most
431 are not significant) (Fig. 3).
432
433 Until recently (Doebley *et al.*, 2006), the existence of a domestication syndrome had promoted
434 the idea that most plant species that undergo domestication suffer the same phenotypic
435 consequences. Likewise, this could have also promoted the assumption that, for all crop species,
436 domestication would always negatively affect microbial diversity. In addition to finding, on
437 average, increased microbial diversity in domesticated plants, our analysis revealed significant
438 variability inside species. For many of the crops analyzed, there were instances where wild plants
439 exhibited higher microbial diversity, while in other cases, domesticated plants showed greater
440 diversity. We found this variability in different studies using the same plant accessions and even
441 within the same study when different initial soils were used, implying a significant role of genotype-
442 by-environment (GxE) interactions in shaping microbiome assembly.
443
444 It is known that microbial communities from different habitats (i.e., different initial soils) can exhibit
445 distinct responses to the same host genotype. It has been proposed that environmental
446 heterogeneity can decouple host genotypes from their microbiomes by altering genotype-microbe
447 relationships (Bradshaw, 1965; Wagner *et al.*, 2016), potentially promoting mixed results even
448 when controlling for host genotypes. Our results suggest that domestication's effects on microbial
449 diversity are not universally negative and may vary depending on the specific crop species and
450 environmental conditions involved. This observation is further supported when examining the
451 impact of domestication on microbial community composition. Although most comparisons

452 showed differences in beta diversity (Figure S5), domestication status accounted for a relatively
453 minor fraction of the microbial variation across the analyzed species (Fig. 2). Instead, much of the
454 variation, at least in studies using more than one soil type, is explained by the soil origin (Table
455 S4). This finding aligns with the results of individual studies on rice (Edwards *et al.*, 2015), poplar
456 (Bonito *et al.*, 2019), soybean (Liu *et al.*, 2019), and the legume *Medicago truncatula* (Brown *et*
457 *al.*, 2020), where soil origin is a stronger predictor of community composition than host genotype.
458 Some of these studies suggest that endophytes are more sensitive to host genotype signals, while
459 rhizosphere communities are more affected by the environment (Brown *et al.*, 2020). Future
460 studies looking at the effect of domestication should aim to separate the effects of domestication
461 on different microbial functional groups.
462
463 Despite all the variation found between crops, we detected two members of the *Rhizobiaceae*
464 family consistently enriched in domesticated wheat, tomato, and soybean, and two members of
465 the *Burkholderiaceae* family enriched in wild wheat, tomato, bean, and soybean (Fig. 4). In some
466 crop species, domesticated plants exhibit increased photosynthetic capacity compared to wild
467 relatives, which is thought to support the development of larger grains or fruits (Koester *et al.*,
468 2016; Brestic *et al.*, 2018). This greater carbon fixation could be increasing the root exudation in
469 domesticated plants, potentially attracting more rhizobia and other copiotrophs in the rhizosphere
470 (Richards, 2000). Additionally, prolonged nitrogen fertilization in agricultural systems is proposed
471 to promote the evolution of less-cooperative rhizobia in domesticated cultivars, potentially leading
472 to higher root abundance of *Rhizobiaceae* members, many of which may not contribute
473 significantly to plant nutrition (Weese *et al.*, 2015; Liu *et al.*, 2019). Particularly, rhizobia with poor
474 or null symbiotic effectiveness are more prevalent in agricultural soils and are predominantly
475 associated with cultivated crops (Sachs *et al.*, 2010). Thus, a high abundance of those microbes
476 does not necessarily indicate a higher nitrogen fixation in the rhizosphere of domesticated crops.
477
478 On the other hand, the consistent enrichment of *Burkholderiaceae* family members in the wild
479 versions of multiple species aligns with previous research on common beans and rice (Pérez-
480 Jaramillo *et al.*, 2019; Wu *et al.*, 2022). Although the mechanisms behind wild plants harboring an
481 increased abundance of those particular microbes remain unknown, it has been shown that
482 members of the *Burkholderiaceae* family are known for their ability to promote plant growth and
483 suppress soil-borne pathogens, supporting plant health overall (Estrada-De Los Santos *et al.*,
484 2001). One potential explanation is that compared to domesticated plants, wild relatives produce
485 a greater diversity of secondary metabolites, which may promote the recruitment of these

beneficial microbes (Ku *et al.*, 2020). Nonetheless, for the case of the common bean, positive associations with *Burkholderiaceae* family members were found only when using native soils (e.g. versus agricultural soils) (Pérez-Jaramillo *et al.*, 2019), suggesting potential local adaptation to microbes found within the natural distribution of such species.

CONCLUSIONS AND FUTURE DIRECTIONS

In this study, we challenged accepted hypotheses regarding the consequences of plant domestication on root microbiomes. Contrary to the expectation that domestication generally reduces microbial diversity, we found a small but significant reduction of microbiome diversity in the rhizosphere of wild relatives. Furthermore, by analyzing multiple crop species, we did not find sufficient evidence to support the hypothesis that domestication negatively affects host influence on the assembly of the microbiome. The consistent enrichment of certain bacterial families in both wild or domesticated versions of different crop species (*Rhizobiaceae* for domesticated and *Burkholderiaceae* for wild plants) could be suggesting, at least for the data analyzed in this work, that the effects of domestication may be stronger at the level of recruiting specific microbes rather than affecting the entire community. Overall, our results suggest that the impacts of domestication on the root microbiomes are not universally negative in terms of microbial diversity. Instead, they appear to be species-specific and contingent upon environmental factors.

Despite our findings, much work remains to fully understand the ecological and evolutionary consequences of plant domestication on root microbiomes. First, diversity is not everything; characterizing functional traits of microbiomes associated with wild and domesticated plants is crucial to better understanding the consequences of domestication for plant-microbe interactions. Second, it is possible that the effect of domestication would be more pronounced with close symbionts and endophytes, more work is necessary to understand how domestication can alter these interactions. Third, it is well-documented that domestication significantly impacts root system architecture, but there is limited research on how such changes affect the microbes inhabiting the roots. How changes in root size, phenotype, and physical properties of the roots impact the recruitment of microbes remains as open questions. Similarly, although exudates have been studied more than the physical structure of roots for microbiome recruitment, this is still an area where more research is needed to identify general patterns.

Critical directions for future research are to incorporate plant diversity in the design or analysis of the experiments to start uncovering the connection between genetic changes of domesticated crops and their impact on the microbiome. In particular, controlling for genetic diversity between plants is important to be able to attribute differences between groups to the process of domestication itself. Similarly, to evaluate the role of domestication on the loss of host influence, it will be important to incorporate native soils into common garden experiments. Local adaptation is important for wild populations, and relying exclusively on agricultural soils (or other soils outside the natural range of wild plants) may hinder significant microbial interactions that occur only with the specific set of microbes to which wild plants are naturally adapted.

Finally, introgression between wild and domesticated plants is a common phenomenon, especially at the centers of origin of the species (Ellstrand *et al.*, 2002). The evolutionary consequences of this have been extensively studied from a genetic perspective (Janzen *et al.*, 2019), and as a source for key agronomic traits (Le Corre *et al.*, 2020). Nonetheless, even though there is increasing evidence showing that hybridization impacts root microbiomes (Bouffaud *et al.*, 2014; Zhang *et al.*, 2023), the consequences of introgression between wild and domesticated plants remain unclear. Recently, studies on rice (Zhang *et al.*, 2023) and maize (Wagner *et al.*, 2021) have found that both root and seed microbiomes could contribute to heterosis ("hybrid vigor") in hybrids, providing evidence of the important role of microbiomes in the fitness of inbreds. Studying the potential adaptive and maladaptive consequences of microbiome introgression is a new and exciting area of study, with significant implications for plant breeding and conservation biology.

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AUTHOR CONTRIBUTIONS

AHT and MRG designed the research with input from AE. AHT performed all statistical analyses. AHT wrote the manuscript with substantial feedback from MRG and AE.

COMPETING INTERESTS

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data and R code for analyses is available on Github at:
https://github.com/AlejandraHT/Plant_domestication_microbiomes

FIGURES

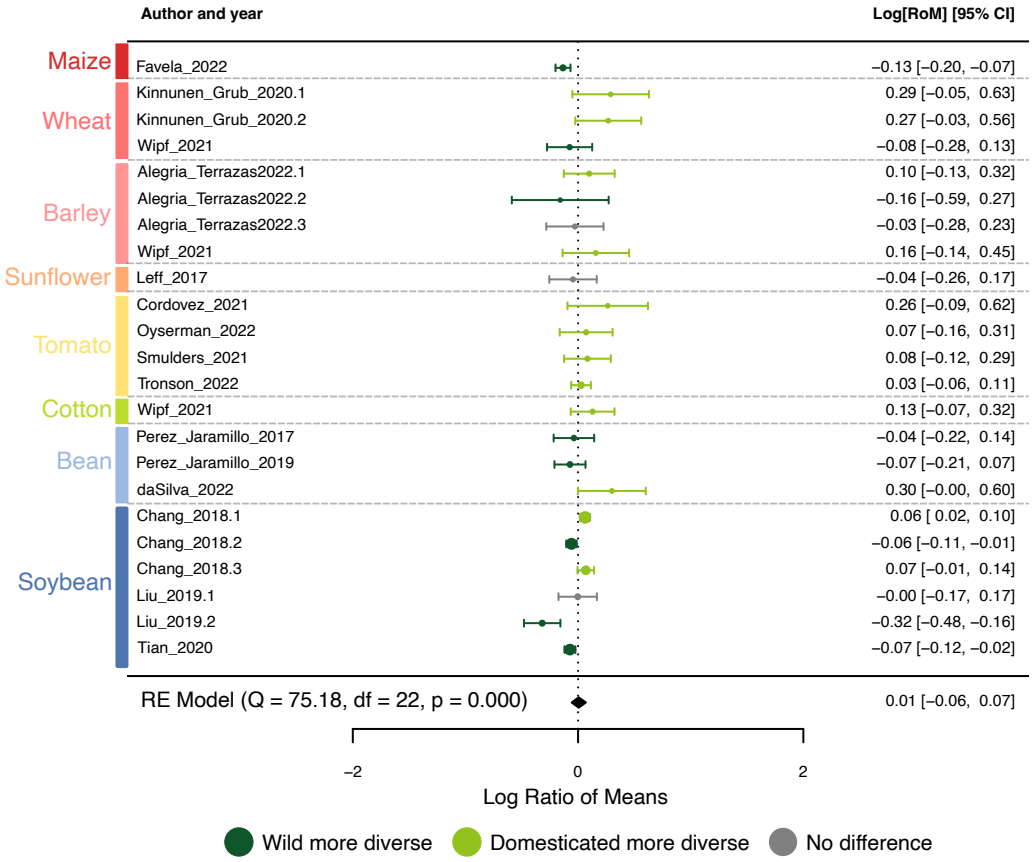

Figure 1. Effect of Domestication on Microbial Alpha Diversity Across Different Crop Species. Forest plot showing the log Ratio of Means (LogRoM) using Chao1 diversity index for all 23 Wild-Domesticated (W-D) pairs. The mean microbial diversity of wild plants was used as reference. Dashed lines denote the separation between different plant species. Bars represent the mean response ratios with 95% confidence intervals. Colors indicate the results: dark green represents higher diversity in wild plants, light green represents higher diversity in domesticated plants, and grey indicates no statistical difference.

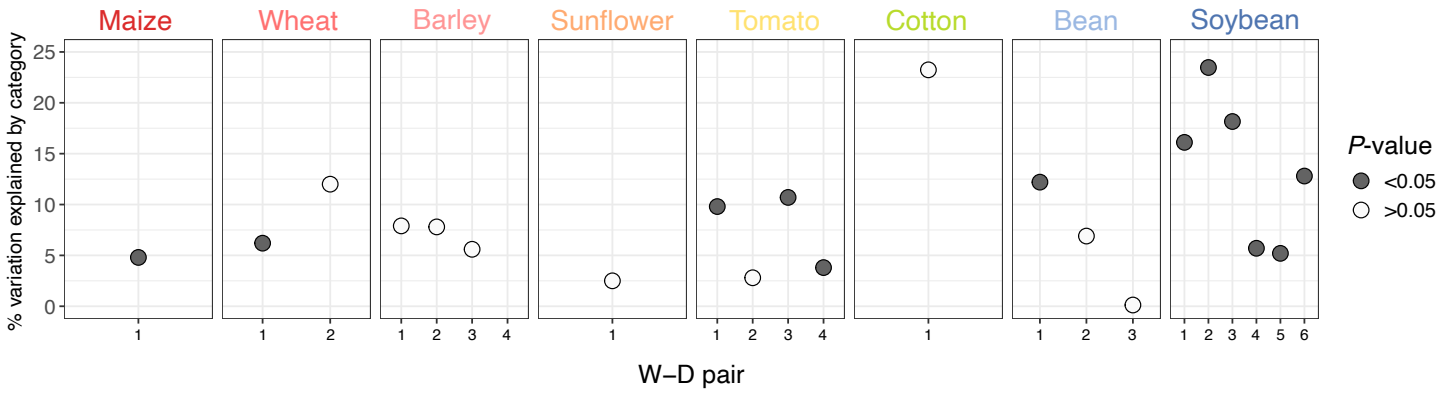

Figure 2. Effect of Domestication on Microbial Community Composition Across Different Crop Species. Dot plot showing the percentage of variation in microbial community composition explained by domestication category (wild vs. domesticated), according to the Bray-Curtis distance measure. Numbers in X axis represent the number of Wild-Domesticated pairs (pairs of wild and domesticated plants from the same study, species, and treatment) analyzed per crop.

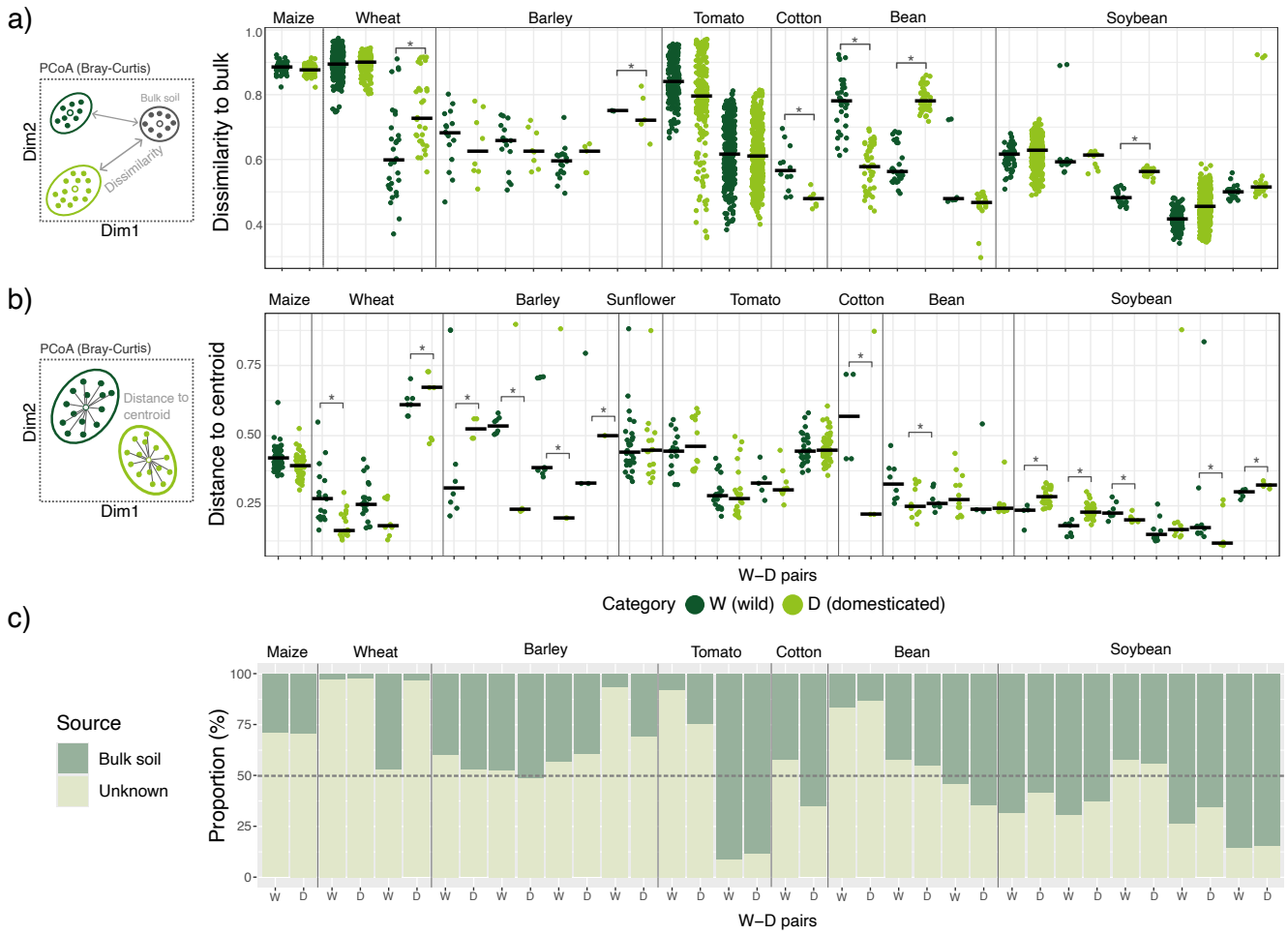

Figure 3. Impact of Domestication on Host Influence of the Microbiome

A) Bray-Curtis dissimilarity to bulk soil for wild-domesticated (W-D) pairs across different crop species. Statistically significant differences are indicated by an asterisk (Wilcoxon rank-sum test, $p < 0.05$). This analysis includes only experiments that included bulk soil sequences. **B)** Intra-group dispersion for all W-D pairs analyzed using Bray-Curtis distance. Statistically significant differences are indicated by an asterisk (Wilcoxon rank-sum test, $p < 0.05$). **C)** Source Tracker analysis estimating the contribution of bulk soil to the final microbial community of wild and domesticated plants across different species.

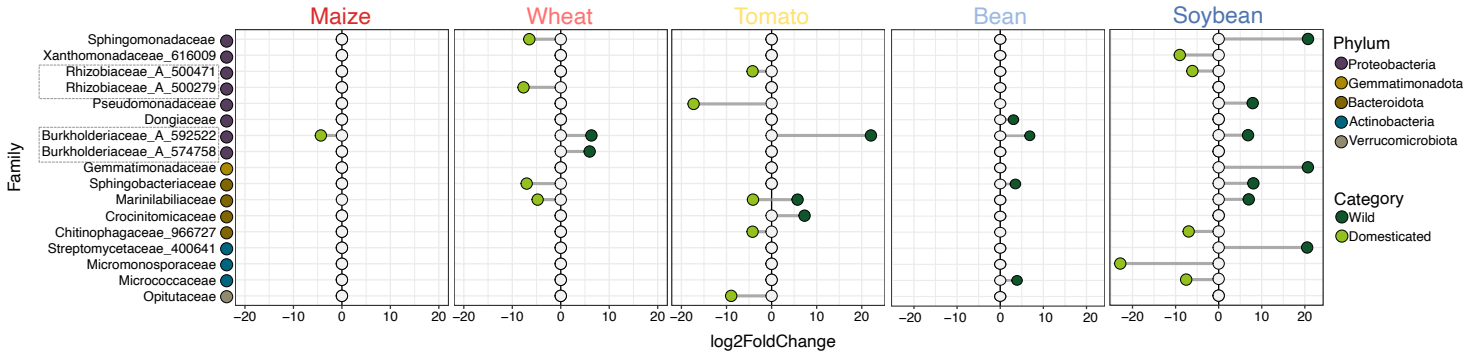

Figure 4. Differentially enriched microbial families between wild and domesticated plants across various crop species. DESeq analysis at the family level displaying microbes either enriched in two or more crop species or exhibiting a fold change greater than +5 or less than -5.

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