PLANT DOMESTICATION DOES NOT REDUCE DIVERSITY IN ROOT MICROBIOMES

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Classification: Biological Sciences - Plant Biology

Keywords: Plant domestication, root microbiome, rhizosphere

ABSTRACT

Domestication has profoundly shaped human civilization and 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. Root microbiomes play crucial roles in nutrient acquisition, pathogen defense, and biotic stress tolerance, yet the influence of domestication on their diversity and assembly is still debated. 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. To evaluate these hypotheses, 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, while others show no significant change. 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 not only diversity but also microbiome function, and considering how root morphology might affect microbiome recruitment. Finally, we highlight the need for research on the potential adaptive or maladaptive consequences that introgression between wild and domesticated plants could have from a microbiome perspective.

SIGNIFICANCE STATEMENT

Plant domestication has had profound impacts on plant traits and their genetic makeup. These traits are likely to influence microbial interactions with the plants, but the overall effect of domestication on plant microbiomes is still subject to debate. In this work, we challenge two primary hypotheses regarding the effects of domestication on root microbiomes; we compared published microbiomes of wild and domesticated plants and found that domestication does not reduce microbial diversity or the plant's contribution to their associated microbial communities. Instead, these effects vary between plant species and environmental conditions. This work provides insights into how domestication shapes plant-microbe relationships and discusses new approaches that are critical for advancing this area of research.

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 (1). The consequences of domestication at the genetic and phenotypic level are well-documented (2). Typically, domestication events involve propagating only selected plant seeds, leading to genetic bottlenecks and thus, a reduction in genetic diversity across the genome (3). Phenotypically, convergent changes in certain traits, known as 'domestication syndrome', have been observed across diverse domesticated species (4). 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 (3). 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 (1, 2). 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 (9). Nonetheless, a meta-analysis (10) 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 (11) 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.

105

106 Roots are an important case for the evolution of non-target traits and species interactions. Root
107 phenotypes have often significantly diverged from their wild relatives due to adaptation to the
108 agricultural environment or as non-target modifications associated with selected traits above
109 ground. Domesticated plants often have a different root system architecture, altered root exudate
110 compositions, and significant changes in secondary metabolites across a wide range of species
111 (2, 12), which necessarily has an impact in plant interactions including one of the most critical
112 plant interactions, the symbiotic relationship between microbial communities and plant roots (the
113 root or rhizosphere microbiome).

114

115 Plants rely on their microbiomes for several functions, including nutrient acquisition, protection
116 against pathogens, and tolerance to biotic stress (13). Often, the root microbiome promotes plant
117 health and growth, contributing to plant development (14). Given the pivotal importance of root
118 microbiomes for plants and considering the known effects of plant domestication on root systems,
119 in recent years efforts have been undertaken to decipher the impact of plant domestication on the
120 diversity and assembly of these microbial communities. Despite these efforts, it is still unclear if,
121 or to what extent, plant domestication has modified the ability of the plants to interact with the root
122 microbiome.

123

124 Two main hypotheses have been proposed regarding the consequences of domestication: 1) the
125 reduction in microbial diversity resulting from the domestication process, and 2) the diminished
126 ability of host plants to shape the assembly of their microbiomes. These hypotheses arise from
127 the idea that the genetic bottlenecks accompanying domestication may lead to the loss of alleles
128 crucial for plant-microbiome interactions and that alterations in root exudates metabolic profiles
129 might further influence microbiome assembly in domesticated plants (15), and because of a
130 potential reduction in the benefits of mutualism for domesticated plants. Specifically, domesticated

plants, after growing in environments with important nutrient inputs, could potentially reduce the investment in symbiosis, leading to the evolution of reduced symbiont cooperation (16). 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.

157 **RESULTS**

158

159 We conducted a systematic meta-analysis to assess the influence of plant domestication on root
160 microbiomes across different plant species. After applying our inclusion criteria and filters of data
161 quality, 14 independent studies were retained. These studies encompass eight different crop
162 species and included 882 individual plants belonging to maize (n = 129), wheat (n= 172), barley
163 (n= 55), sunflower (n= 127), tomato (n= 169), cotton (n= 31), bean (n= 39), and soybean (n= 160)
164 (Table S1). Except for bean, soybean, and sunflower, all crops were represented by more than
165 one accession of wild and domesticated plants (Table S1). With this dataset, we investigated two
166 primary hypotheses regarding the impact of plant domestication on root microbiomes: 1) the
167 reduction in microbial diversity resulting from the domestication process, and 2) the diminished
168 ability of host plants to control their microbiomes. This data did not experimentally assess plant
169 "control", hereafter we refer as "host influence" to the contribution of plants in shaping root
170 microbiome assemblies. Our analysis focused on the comparison between wild and domesticated
171 categories. To mitigate the potential bias introduced by merging amplicon sequence data from
172 disparate studies, our comparative analyses were restricted to within-study wild and domesticated
173 pairs (W-D pairs). We defined W-D pairs as plants cultivated concurrently and inoculated with the
174 same soil. Typically, each paper contributed a single W-D pair to our analysis. However, in
175 instances where a study investigated more than one soil type, the number of W-D pairs was
176 equivalent to the number of different soil treatments applied (we analyzed a total of 22 different
177 W-D pairs).

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183 ***Domesticated plants show increased microbiome diversity***

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185 Contrary to the common expectation of reduced diversity in the microbiomes as a result of
186 domestication, on average, domesticated plants had higher alpha diversity according to the
187 Shannon diversity index (Fig. 1 - Shannon index, $p < 0.001$, Fig. S1 - Chao1 index, $p = 0.134$).
188 Most pairwise comparisons (pairs of wild and domesticated plants from the same study, species,
189 and treatment) showed differences in alpha diversity, but the effects were highly variable across
190 species. Among the 22 Wild-Domesticated (W-D) pairs evaluated, only four (18%) exhibited no
191 statistical difference. Of the 18 (81%) pairs that showed statistically significant differences, 11
192 (61%) displayed increased diversity in domesticated plants, whereas seven (38.8%) had higher
193 diversity in their wild counterparts. Notably, the effect of domestication appears to be species-
194 specific and related to functional groups. Crops like wheat, tomato, and cotton showed increased
195 diversity in their domesticated forms compared to their wild forms, whereas maize and legumes
196 (beans and soybeans) presented greater diversity in their wild forms. Despite these overall
197 patterns, we observed different outcomes, even within the same species. The only exception to
198 this inconsistency was observed in tomatoes, where all four comparisons consistently revealed
199 higher diversity in domesticated plants. The most variable crop species was soybean, where from
200 the six total W-D pairs analyzed, two resulted in higher diversity in domesticated versions, three
201 in higher diversity in wild plants, and one showed no statistically significant difference.

202

203 ***Host species effect is more important than domestication in shaping microbiome***
204 ***composition***

205

206 We then evaluated the effect of domestication on microbiome composition using PERMANOVA.
207 We found that the domestication category accounted for a relatively small fraction of the variation
208 across most of the crop species studied (Fig. 2 and Fig. S2), suggesting a potential species-

209 specific effect. For several species, including maize, wheat, barley, sunflower, tomato, and bean,
210 domestication explained approximately 10% of the observed variance in microbial community
211 composition. Notably, cotton and soybean (except for two pairs) stood out, with domestication
212 accounting for a larger portion of the variance, ranging from 12% to 25%. Additionally, we
213 leveraged experiments that used multiple soil types to inoculate plants, allowing us to test the
214 contribution of the inoculum to the final microbial community by separating wild and domesticated
215 plants independently (Table S3). Although only a small set of papers had different soil types, for
216 barley, different initial soils accounted for a minor portion of the total variation (9.8% for wild plants
217 and 10.9% for domesticated plants). In contrast, for soybeans, using soils from different origins
218 accounted for a significant percentage of the variation (~75% for wild and ~78% for domesticated
219 plants). Taken together, these results suggested that domestication could affect the degree of
220 host influence, but that there might be variation across species.
221
222 ***Wild relatives do not display systematic evidence of increased host influence of their***
223 ***microbiomes***
224
225 To evaluate the extent of host influence exerted by wild and domesticated plants over their
226 microbiomes, we leveraged beta diversity metrics, comparing the microbial communities
227 associated with plants to those from bulk soils, which served as a reference for the initial microbial
228 community (Fig. 3). We hypothesized that if plants have a relatively strong influence in the
229 assembly of their microbiomes, then we would expect to find: a) significant dissimilarity between
230 plant-associated microbiomes and bulk soil microbiomes (Fig. 3A), suggesting that plants are
231 selecting specific microbial assemblages distinct from the initial soil; b) low intra-group dispersion
232 (Fig. 3B), indicating that plant replicates of the same genotype consistently select for similar
233 microbial compositions; and c) less proportion of the final community explained by the initial bulk

234 soil (Fig. 3C), implying that the host plays a decisive role in shaping its microbiome, potentially
235 steering it away from the original soil community composition.
236
237 Overall, we did not identify a consistent pattern linking host influence to the category of
238 domestication. In general, we found greater dissimilarity to bulk in wild barley and cotton and in
239 domesticated wheat and soybean (Fig. 3A). Similarly, we found a diverse range of outcomes in
240 the intra-group dispersion analyses (Fig. 3B). Domesticated plants generally exhibited more intra-
241 group dispersion in barley and soybean, whereas we found the opposite effect (more intra-group
242 dispersion in wild varieties) in wheat, cotton, and bean. Maize, sunflower, and tomato showed no
243 differences. We didn't find a clear pattern in the contribution of bulk soil microbiomes to the final
244 plant-associated communities across different levels of domestication (Fig. 3C).
245
246 We explored if some other plant attribute could better account for our results. Grouping our results
247 by functional plant group rather than domestication status provided increased insight.
248 Grasses (maize, wheat, barley) consistently demonstrated a more distinct divergence from their
249 initial bulk soil microbiomes (Fig. 3A), with less than 50% of the final community composition
250 attributable to the bulk soil (Fig. 3C). Whereas the legume soybean presented final communities
251 closely aligned with the initial bulk soil, reflecting less than 50% differentiation.
252
253 ***Some microbial taxa differentially associate with plants across species according to the***
254 ***domestication status***
255
256 Finally, we investigated if, despite all the variation, we could identify microbial groups differentially
257 present in wild and domesticated crops (see methods; Fig. 4). We identified a few families
258 consistently enriched or depleted from domesticated crops (relative to their wild counterparts)
259 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. S3).

286 **DISCUSSION**

287

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

293

294 Contrary to the hypothesis that domestication generally reduces microbial diversity, we found a
295 small but significant reduction of microbiome diversity in the rhizosphere of wild relatives (Fig. 1,
296 Log Ratio of Means with Shannon index, $p < 0.001$). In fact, for the Shannon index, most of the
297 significant comparisons indicated that domesticated plants exhibited higher microbial diversity
298 than wild plants (Fig. 1). When using the Chao1 diversity index, we observed fewer differences
299 and mostly saw no change in diversity across comparisons. The hypothesis that domestication
300 leads to a reduction in microbiome diversity is largely based on the idea that domestication
301 reduces genetic diversity, potentially leading to the loss of alleles important for plant-microbe
302 interactions through selective bottlenecks (15) and from the hypothesis in domesticated plants, a
303 reduced investment in symbiosis, could be promoting the evolution of reduced symbiont
304 cooperation (16).

305

306 It is not entirely clear why the reduction of genetic diversity at the level of populations would lead
307 to reduced microbiome diversity at the level of individuals. Instead, we might expect a decrease
308 in microbiome diversity across hosts (less dispersion) in domesticated varieties compared to their
309 wild counterparts. However, our results highlight that this is only the case in some pairs –mostly
310 wheat, barley, and cotton. The opposite is true in some other pairs, including many of the soybean
311 comparisons (Fig. 3). One of the limitations in making clear conclusions about the impact of host

312 diversity on the microbiome is that host diversity is rarely controlled in these studies (many
313 domesticated accessions might be compared against a single population of wild relatives).
314
315 Even the loss of alleles important for plant-microbe interactions does not have to be associated
316 with decreased microbiome diversity. It has been proposed, for example, that a reduction in
317 genetic diversity might lead to less efficient microbiome filtering mechanisms, allowing
318 domesticated plants to be less selective and thus associate with a greater diversity of microbes
319 (15, 17, 18). This hypothesis has gained attention due to its potential implications for agriculture
320 and resilience to climate change. The variation in microbial communities explained by host
321 genotype represents the substrate upon which natural selection can act and, therefore, it is also
322 the variation that could be manipulated for agricultural improvement (19). Thus, a reduction in
323 host influence resulting from domestication could lead to decreased evolutionary potential within
324 microbial communities, potentially limiting the adaptive resources of plants in response to
325 environmental stresses (18). This hypothesis of less efficient filtering mechanisms in
326 domesticated crops is consistent with our alpha (within-plant) diversity results, where we observed
327 a small increase in microbiome diversity in domesticated varieties. But, contrary to this
328 expectation, we found little support for decreased host influence in domesticated plants. In most
329 pairs, the microbial community of the rhizosphere is equally distant to the bulk soil with soybean
330 being the only consistent example of increased distance to bulk soil in the domesticated varieties
331 (even if most are not significant) (Fig. 3).
332
333 Until recently (4), the existence of a domestication syndrome had promoted the idea that most
334 plant species that undergo domestication suffer the same phenotypic consequences. Likewise,
335 this could have also promoted the assumption that, for all crop species, domestication would
336 always negatively affect microbial diversity. In addition to finding, on average, increased microbial
337 diversity in domesticated plants, our analysis revealed significant variability inside species. For

338 many of the crops analyzed, there were instances where wild plants exhibited higher microbial
339 diversity, while in other cases, domesticated plants showed greater diversity. We found this
340 variability in different studies using the same plant accessions and even within the same study
341 when different initial soils were used, implying a significant role of genotype-by-environment (GxE)
342 interactions in shaping microbiome assembly.
343
344 It is known that microbial communities from different habitats (i.e., different initial soils) can exhibit
345 distinct responses to the same host genotype. It has been proposed that environmental
346 heterogeneity can decouple host genotypes from their microbiomes by altering genotype-microbe
347 relationships (20, 21), potentially promoting mixed results even when controlling for host
348 genotypes. Our results suggest that domestication's effects on microbial diversity are not
349 universally negative and may vary depending on the specific crop species and environmental
350 conditions involved. This observation is further supported when examining the impact of
351 domestication on microbial community composition. Although most comparisons showed
352 differences in beta diversity (Fig. S2), domestication status accounted for a relatively minor
353 fraction of the microbial variation across the analyzed species (Fig. 2). Instead, much of the
354 variation, at least in studies using more than one soil type, is explained by the soil origin (Table
355 S3). This finding aligns with the results of individual studies on rice (22), poplar (23), soybean
356 (24), and the legume *Medicago truncatula* (19), where soil origin is a stronger predictor of
357 community composition than host genotype. Some of these studies suggest that endophytes are
358 more sensitive to host genotype signals, while rhizosphere communities are more affected by the
359 environment (19). Future studies looking at the effect of domestication should aim to separate the
360 effects of domestication on different microbial functional groups.
361
362 Despite all the variation found between crops, we detected two members of the *Rhizobiaceae*
363 family consistently enriched in domesticated wheat, tomato, and soybean, and two members of

364 the *Burkholderiaceae* family enriched in wild wheat, tomato, bean, and soybean (Fig. 4). In some
365 crop species, domesticated plants exhibit increased photosynthetic capacity compared to wild
366 relatives, which is thought to support the development of larger grains or fruits (25, 26). This
367 greater carbon fixation could be increasing the root exudation in domesticated plants, potentially
368 attracting more rhizobia and other copiotrophs in the rhizosphere (27). Additionally, prolonged
369 nitrogen fertilization in agricultural systems is proposed to promote the evolution of less-
370 cooperative rhizobia in domesticated cultivars, potentially leading to higher root abundance of
371 *Rhizobiaceae* members, many of which may not contribute significantly to plant nutrition (24, 28).
372 Particularly, rhizobia with poor or null symbiotic effectiveness are more prevalent in agricultural
373 soils and are predominantly associated with cultivated crops (29). Thus, a high abundance of
374 those microbes does not necessarily indicate a higher nitrogen fixation in the rhizosphere of
375 domesticated crops.
376
377 On the other hand, the consistent enrichment of *Burkholderiaceae* family members in the wild
378 versions of multiple species aligns with previous research on common beans and rice (30, 31)).
379 Although the mechanisms behind wild plants harboring an increased abundance of those
380 particular microbes remain unknown, it has been shown that members of the *Burkholderiaceae*
381 family are known for their ability to promote plant growth and suppress soil-borne pathogens,
382 supporting plant health overall (32). One potential explanation is that compared to domesticated
383 plants, wild relatives produce a greater diversity of secondary metabolites, which may promote
384 the recruitment of these beneficial microbes (33). Nonetheless, for the case of the common bean,
385 positive associations with *Burkholderiaceae* family members were found only when using native
386 soils (e.g. versus agricultural soils) (30), suggesting potential local adaptation to microbes found
387 within the natural distribution of such species.
388
389

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 (34). The evolutionary consequences of this have been extensively studied from a genetic perspective (35), and as a source for key agronomic traits (36). Nonetheless, even though there is increasing evidence showing that hybridization impacts root microbiomes (37, 38), the consequences of introgression between wild and domesticated plants remain unclear. Recently, studies on rice (38) and maize (39) 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.

441 **MATERIALS AND METHODS**

442

443 *Data collection*

444

445 To investigate the impact of the domestication process on the diversity and composition of the
446 root microbiome across various crop species, we collected 16S rRNA sequence data from
447 common garden experiments reported in scientific publications. We conducted a comprehensive
448 search in the Scopus and Google Scholar databases using the Boolean search string: Wild [AND]
449 domesticated [AND] microbiome [OR] rhizosphere [AND] crop. Our search centered on
450 contemporary crops with well-identified crop wild relatives, including but not limited to maize,
451 potato, tomato, squash, bean, coffee, barley, soybean, cotton, and sunflower. Eligibility for
452 inclusion in our meta-analysis was based on the following selection criteria: 1) the experiments
453 must have been conducted under controlled environmental conditions typical of common garden
454 studies, 2) wild and domesticated variants of the same crop species must have been cultivated
455 concurrently, and inoculated with the same soil, and 3) sequencing must have been performed
456 using Illumina platforms. We excluded studies that assessed the impact of extreme environmental
457 stressors, such as herbivory, but we incorporated those that utilized different soil types (e.g.,
458 forest or agricultural soils) as inoculum.
459 Applying these criteria, we narrowed down the pool to 14 publications, representing eight crops:
460 barley, soybean, cotton, tomato, potato, sunflower, maize, and bean. A detailed overview of the
461 number of crop accessions, experimental replicates, and analyzed publications per crop is
462 available in Supplementary Table 1.

463

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465

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467 *Data processing*

468

469 16S rRNA raw sequences and metadata were downloaded from public repositories including
470 NCBI, MG-Rast, and Github, and were analyzed with the Quantitative Insights Into Microbial
471 Ecology (QIIME2) platform (40) version 2023.5. For each dataset corresponding to a specific
472 publication, paired-end sequences were analyzed independently. Sequence denoising and
473 quality filtering, including primers and adapters trimming, were performed with the DADA2 plug-
474 in (41). Given that all selected studies utilized primers targeting the V4 16S rRNA gene region
475 (e.g., V4, V3-V4, V4-V5), we standardized the analysis by extracting the V4 segment using the
476 515F–806R primer pairs (42). This approach minimized discrepancies arising from taxonomic
477 assignments across different regions of the 16S rRNA gene. The length of the V4 region
478 sequences was standardized to a range between 120 and 150 nucleotides, based on sequence
479 quality and primer positioning. This criterion was consistently applied across all datasets.
480 Taxonomic classification of the Amplicon Sequence Variants (ASVs) for each dataset was
481 conducted using the "sklearn-base" classifier within QIIME2, referencing the Greengenes 2
482 database (43) with a 99% sequence identity threshold. Mitochondrial, chloroplast, and ASVs that
483 were not assignable at the kingdom level were excluded from the analysis. Comprehensive details
484 on the total number of reads (before and after processing) and the count of ASVs for each study
485 have been compiled in Supplementary Table 1.

486

487 *Data Analysis*

488

489 Data analysis was performed on R software (v.4.3.0) using custom scripts and employing the
490 following packages: Phyloseq (v. 1.46.0) (44), ggplot2 (v. 3.5.1) (45), metafor (v. 4.2-0) (REF),
491 Vegan (v.2.6-4) (46), DESEq2 (v.1.40.2) (47), Source Tracker 2 (48). Prior to amplicon analysis,
492 each of the 14 datasets, corresponding to the individual studies included in our meta-analysis,

493 was rarified to a standardized depth of 15,000 reads per sample to ensure comparability across
494 datasets. Specifics on the number of samples and ASVs retained after rarification are available
495 in Supplementary Table 1. Our analysis focused on the comparison between wild and
496 domesticated categories. To mitigate the potential bias introduced by merging amplicon sequence
497 data from disparate studies, our comparative analyses were restricted to within-study wild and
498 domesticated pairs (W-D pairs). We defined W-D pairs as plants cultivated concurrently and
499 inoculated with the same soil. Typically, each paper contributed a single W-D pair to our analysis.
500 However, in instances where a study investigated more than one soil type, the number of W-D
501 pairs was equivalent to the number of different soil treatments applied.
502
503 **Alpha diversity analyses:** To assess the effect of plant domestication on the diversity of the
504 microbiome we calculated Chao1 and Shannon alpha diversity indexes using the *estimate*
505 *richness* function in the phyloseq package. To test for differences in these indexes we applied the
506 Log Ratio of Means (LogRM) analysis which compensates for the non-independence of the data
507 by calculating the effect size and variance for each study individually (49). We implemented this
508 test with the *metafor* package, using W-D pairs from all crops and the diversity of the wild plants
509 as a reference.
510
511 **Beta diversity analyses:** To explore potential differences in beta diversity between plant
512 categories and crops, we calculated the Bray-Curtis distance for every W-D pair in the dataset.
513 Dissimilarity matrices were analyzed with Permutational Multivariate Analysis of Variance
514 (PERMANOVA) using the *Adonis* function in the vegan package. From the PERMANOVA result,
515 we extracted the R^2 values to represent the proportion of variation (in terms of beta diversity)
516 explained by category for all W-D pairs and all crops. A dot plot was constructed with this
517 information using ggplot2.
518

Furthermore, from the dissimilarity matrices we calculated, for all crops, the mean dissimilarity to the bulk of both wild and domesticated plants, and the mean distance to centroid between groups using the *betadisper* function in the vegan package. We plotted both metrics for all W-D pairs in the different crops using ggplot2. Finally, a Bayesian microbial source tracker was performed with the SourceTracker2 to calculate the contribution of the bulk soil microbial community to the final root microbiome. All samples were rarefied to a depth of 15,000 reads per sample before the analysis.

Differentially enriched microbes between categories: We identified differentially enriched microbes between W-D pairs across all crops using the DESeq2 test, implemented in the DESeq2 package. This test was applied exclusively to W-D pairs (excluding bulk soil sequences), with a P-value cutoff of <0.01 and a False Discovery Rate (FDR) correction. Subsequently, we merged the results from all W-D comparisons with differentially abundant features at the family level and selected only features with a log2Fold change of ≤-5 or ≥5, or those present in two or more crops. The results were visualized by crop using forest plots constructed in ggplot2. Additionally, we plotted the individual abundances of microbial families enriched in the same category across multiple crop species (Supplementary Figure 3).

ACKNOWLEDGMENTS AND FUNDING SOURCES

We would like to thank Brandon Gaut, Eduardo Choreño-Parra, Santiago Rosas-Plaza, Celia Symons, and members of the COMMONS Lab for productive discussions about this work. AHT was supported by the Human Frontiers Science Program Long-Term Postdoctoral Fellowship (LT 0047/2023-L) and the University of California President's Postdoctoral Fellowship. AEH was supported by UNAM-PAPIIT: IN214124. MRG was supported by the School of Biological Sciences at the University of California, Irvine (UCI).

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

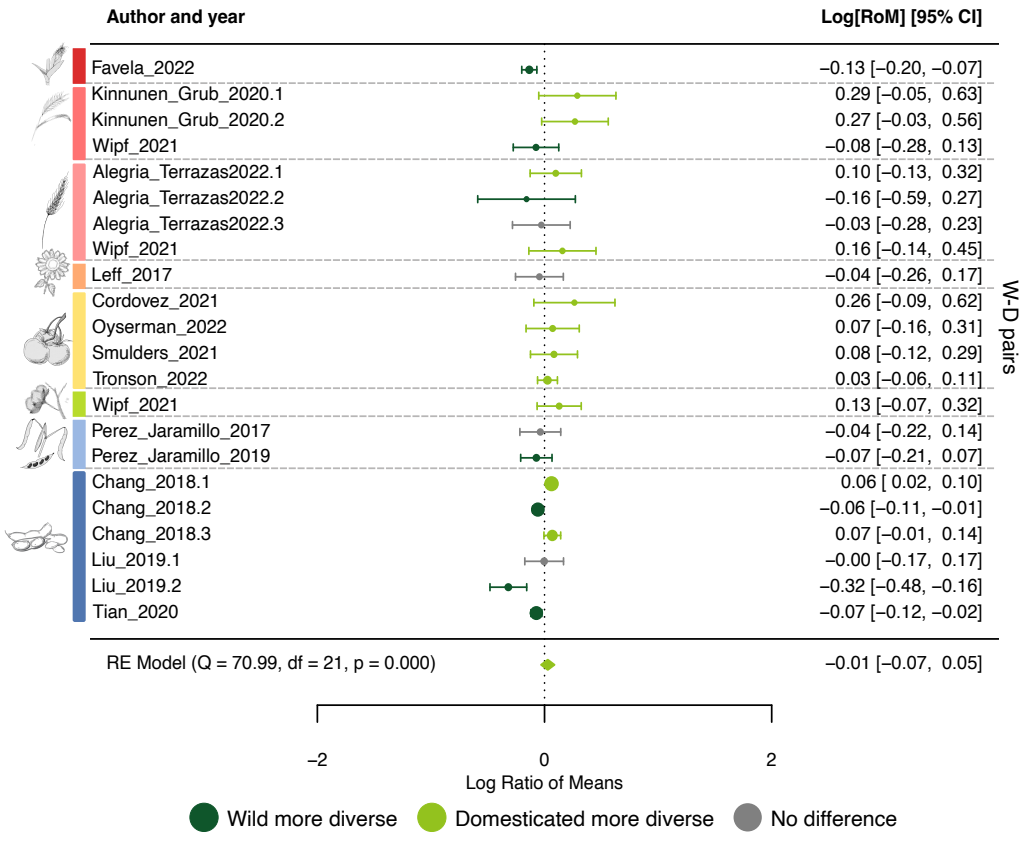

Figure 1. Effect of Domestication on Microbial Alpha Diversity Across Different Crop Species. Forest plot showing the log Ratio of Means (LROM) using Chao1 diversity index for all 22 Wild-Domesticated (W-D) pairs. The mean microbial diversity of wild plants was used as reference. Dashed lines denote the separation between different plant species. From top to down: Maize, Wheat, Barley, Sunflower, Tomato, Cotton, Bean, and Soybean. In the color code: red colors represent grasses and blue colors represent legumes. Bars represent the mean response ratios with 95% confidence intervals. Colors of the bars 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.

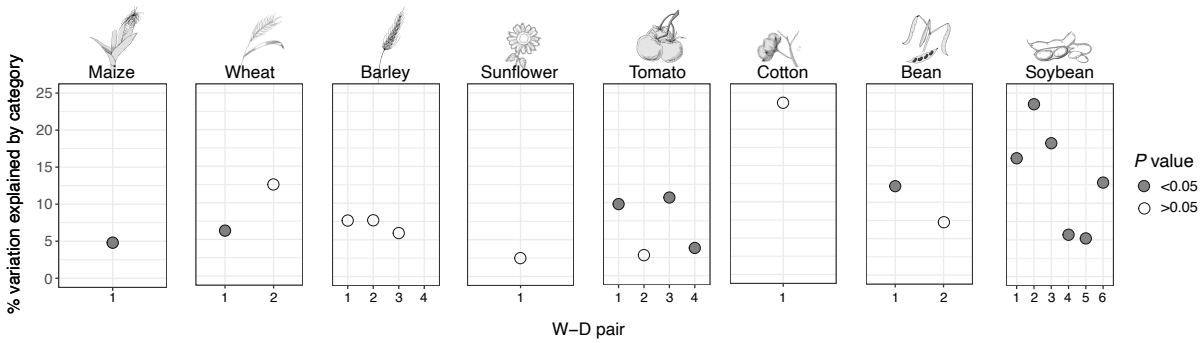

Figure 2. Effect of Domestication on Microbial Community Composition Across Different Crop Species. Dot plot showing the percentage of variation in microbial community composition explained by domestication category (wild vs. domesticated), according to the Bray-Curtis distance measure. Numbers in X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

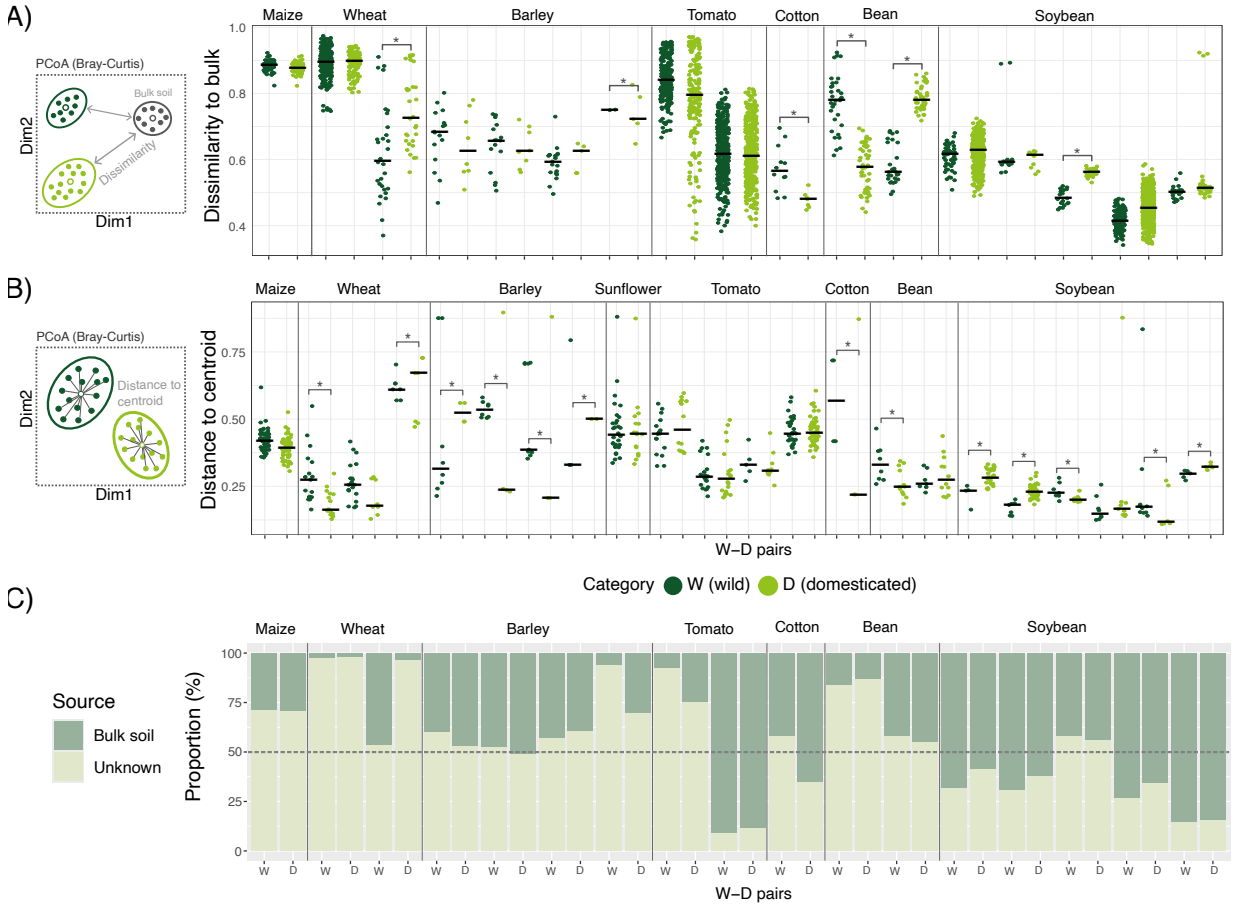

Figure 3. Impact of Domestication on Host Influence of the Microbiome

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.

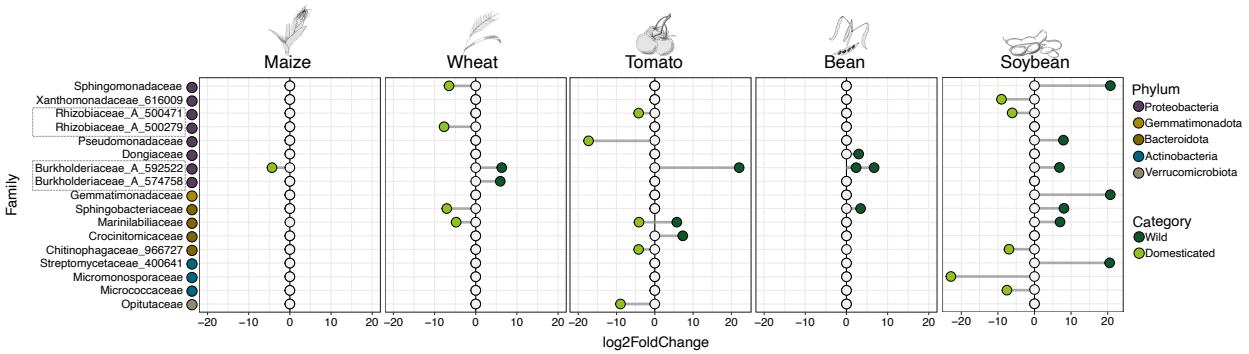

Figure 4. Differentially enriched microbial families between wild and domesticated plants across various crop species. DESeq analysis at the family level displaying microbes either enriched in two or more crop species or exhibiting a fold change greater than +5 or less than -5.

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