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Effects of Agricultural Management on Rhizosphere Microbial Structure and Function in Processing Tomato Plants

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ABSTRACT Agricultural management practices affect bulk soil microbial communities and the functions they carry out, but it remains unclear how these effects extend to the rhizosphere in different agroecosystem contexts. Given close linkages between rhizosphere processes and plant nutrition and productivity, understanding how management practices impact this critical zone is of great importance to optimize plant-soil interactions for agricultural sustainability. A comparison of six paired conventional-organic processing tomato farms was conducted to investigate relationships between management, soil physicochemical parameters, and rhizosphere microbial community composition and functions. Organically managed fields were higher in soil total N and NO_3-N, total and labile C, plant Ca, S, and Cu, and other essential nutrients, while soil pH was higher in conventionally managed fields. Differential abundance, indicator species, and random forest analyses of rhizosphere communities revealed compositional differences between organic and conventional systems and identified management-specific microbial taxa. Phylogeny-based trait prediction showed that these differences translated into more abundant pathogenesis-related gene functions in conventional systems. Structural equation modeling revealed a greater effect of soil biological communities than physicochemical parameters on plant outcomes. These results highlight the importance of rhizosphere-specific studies, as plant selection likely interacts with management in regulating microbial communities and functions that impact agricultural productivity.

IMPORTANCE Agriculture relies, in part, on close linkages between plants and the microorganisms that live in association with plant roots. These rhizosphere bacteria and fungi are distinct from microbial communities found in the rest of the soil and are even more important to plant nutrient uptake and health. Evidence from field studies shows that agricultural management practices such as fertilization and tillage shape microbial communities in bulk soil, but little is known about how these practices affect the rhizosphere. We investigated how agricultural management affects plant-soil-microbe interactions by comparing soil physical and chemical properties, plant nutrients, and rhizosphere microbial communities from paired fields under organic and conventional management. Our results show that human management effects extend even to microorganisms living in close association with plant roots and highlight the importance of these bacteria and fungi to crop nutrition and productivity.

KEYWORDS agricultural management, differential abundance, microbial communities, microbial ecology, rhizosphere-inhabiting microbes, structural equation modeling

Soil microbial communities mediate the provision of many ecosystem services by soils and are increasingly recognized as fundamental regulators of plant and environmental outcomes of agroecosystems. Agricultural practices such as nutrient

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inputs and tillage have been shown to shape bulk soil microbial communities and functions across spatial and temporal scales (1–4). Comparisons of bulk soil under different management strategies, i.e., organic (nutrients provided from sources other than synthetic inputs) versus conventional management, have revealed effects on soil properties that, in turn, drive variation in microbial communities at small and intermediate scales (5–8). Small-scale studies designed to minimize environmental heterogeneity, such as long-term experiments on a single site, show strong effects of management on soil physicochemical parameters (9, 10), microbial biomass (9), and habitat-specific bacterial and fungal taxa (11). At an intermediate spatial scale, such as paired fields within a region, contextual variables such as climate, soil type, and cropping system largely influence the soil physicochemical parameters and microbial processes that differ between conventional and organic fields. Organically managed processing tomato fields in California have higher levels of organic carbon, microbial abundance and diversity, and N mineralization potential than those of conventional fields, while soils under conventional management have higher inorganic N pools and salinity (7). However, these studies often have not extended to the rhizosphere, and the studies that have done so have not found universal predictors of rhizosphere community assembly across contexts and scales (4, 12–14).

While bulk soil communities affect recruitment and assembly of rhizosphere microbial communities (15), soil under the influence of plant roots represents a unique environment that must be studied separately (16). The rhizosphere is a hot spot of interactions where dynamic relationships between plant roots and soil microbial communities occur, allowing bacteria and fungi to break down and cycle organic matter and release nutrients (17), promote plant growth via direct and indirect mechanisms (18), and suppress pathogens (19). While linking agricultural management to large-scale outcomes such as nutrient fluxes or ecosystem services requires an analysis of bulk soil properties and processes, understanding the complex relationship between management practices and plant nutrition and productivity necessitates shifting the focus to the rhizosphere (20). Some evidence suggests that management can affect the ecosystem-level functions carried out by bulk soil microbial communities through impacts on microbial diversity (21), but the unique chemistry and microbial communities found in the rhizosphere (22) are more closely linked to plant outcomes of agricultural importance (23). Because rhizosphere soil is shaped by complex interactions between plant and bulk soil processes, the effects of agricultural management on rhizosphere communities and the functional implications are not always easy to predict.

The few studies that have addressed this question have concluded that differences in bulk soil microbial and protist communities do carry over to some extent to rhizosphere communities (22, 24). However, such studies have frequently been conducted on long-term research stations (22, 24), leaving open the questions of scale and context. Do management effects on rhizosphere microbial communities extend to an intermediate scale, such as paired fields within a region? If so, what soil properties are most closely linked to microbial variation, and how do differences in rhizosphere microbial communities influence plant health and productivity?

A regional-scale study of paired organic and conventional processing tomato fields in northern California was conducted to (i) characterize impacts of agricultural management on rhizosphere microbial community composition in California processing tomato agroecosystems at an intermediate spatial scale, (ii) identify how taxonomic shifts affected predicted metabolic and ecological functions carried out by these communities, and (iii) explore the effects of management-induced microbial variation on crop nutrition and productivity. To address the first objective of identifying variation in rhizosphere microbial communities, we employed three complementary approaches, namely, differential abundance, indicator species, and random forest analyses. Differential abundance analysis of microbial communities adapts transcriptome sequencing (RNA-seq) methodology used for gene expression to identify taxa whose abundance varies significantly among groups of samples (25). Indicator species analysis, an alter-

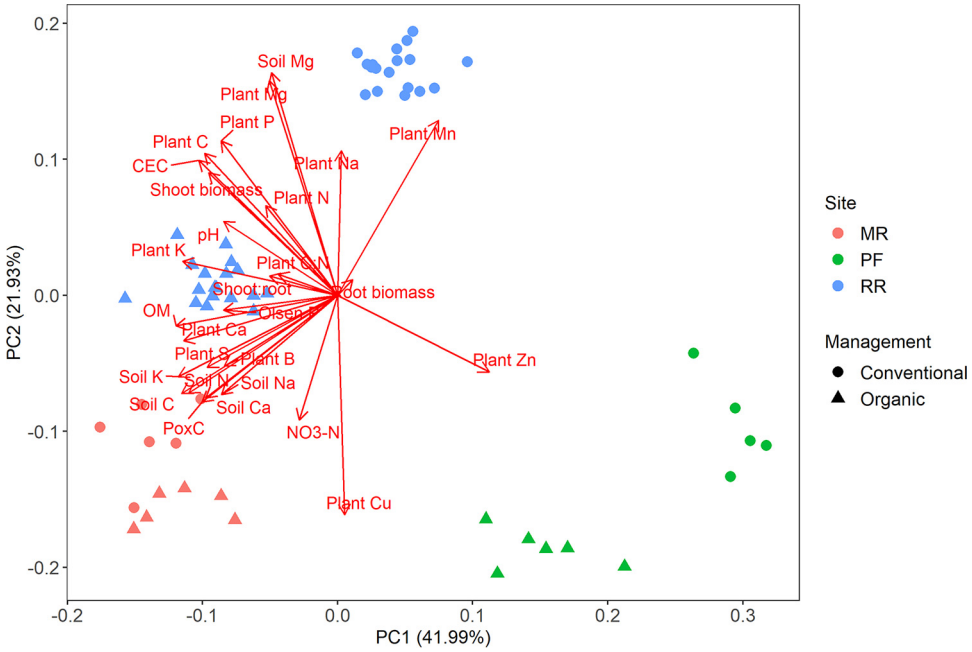

FIG 1 PCA of soil and plant variables measured in six processing tomato fields. Soil physicochemical parameters and plant variables separated primarily by site along PC1, which explained 42% of variation. Samples separated secondarily by management within site, and a significant site × management interaction was observed.

native approach, detects taxa preferentially associated with a given habitat or sample group based on a combination of specificity and fidelity rather than relative abundance alone (26). Random forest analysis (27), a machine learning method, approaches the microbe-sample group linkage from the opposite direction than the differential abundance and indicator species approaches, identifying key taxa whose abundance can be used to assign samples to the appropriate group.

The second objective, determining whether agricultural management induces shifts in rhizosphere microbial functions, was addressed using phylogeny-based trait prediction. This method predicts metagenomic data, such as genes involved in key agroecological functions, from 16S amplicon sequencing data (28). Structural equation modeling (SEM), a statistical technique to test hypothesized relationships among variables (29), was used to address our final objective of exploring linkages between soil properties, microbial communities, and plant nutrition and productivity. We hypothesized that rhizosphere community structure and function would differ between conventional and organic systems and that divergent microbial communities would relate to the variation in plant traits within and between fields.

RESULTS

Site and management drive variation in soil and plant variables. Site had a stronger influence on bulk soil and plant variables than management category (organic versus conventional) (site $R^2 = 0.54$, $P = 0.001$; management $R^2 = 0.17$, $P = 0.001$) and the site × management interaction was significant ($R^2 = 0.09$, $P = 0.001$). Two principal components (PCs) explained 41.99% (PC1) and 21.93% (PC2) of variation among samples, respectively (Fig. 1). Samples tended to cluster primarily by site along PC1, which was affected by numerous plant and soil nutrients, and secondarily by management within each site. PC2 was primarily influenced by plant Cu, Mg, and Mn as well as soil Mg and NO_3-N (Fig. 1; Table 1).

Management system significantly affected soil physicochemical variables ($P < 0.001$). Soil parameters that were higher in organically managed fields included total N ($P < 0.001$), C ($P < 0.001$), NO_3-N ($P = 0.008$), Olsen-extractable P ($P = 0.008$), K ($P = 0.0087$), Na ($P < 0.001$), organic matter (OM) ($P = 0.0022$), and permanganate-

TABLE 1 Factor loadings of scaled soil and plant variables contributing to PC1 and PC2

Variable	PC1	PC2
Soil N (total)	−0.243	−0.159
Soil C (%)	−0.254	−0.159
Soil NO_3-N (ppm)	−0.063	−0.202
Soil P (Olsen) (ppm)	−0.186	−0.024
Soil K (ppm)	−0.260	−0.132
Soil Na (ppm)	−0.190	−0.161
Soil Ca (ppm)	−0.215	−0.166
Soil Mg (ppm)	−0.108	0.361
CEC (meq/100 g)	−0.227	0.219
SOM[a]	−0.264	−0.050
pH	−0.186	0.120
PoxC (mg C/kg soil)	−0.221	−0.173
Shoot mass (g)	−0.211	0.199
Root mass (g)	0.025	0.025
Shoot:root ratio	−0.111	0.031
Plant C (%)	−0.217	0.230
Plant N (%)	−0.118	0.145
Plant C:N	−0.097	0.034
Plant P (%)	−0.190	0.250
Plant K (%)	−0.253	0.055
Plant Ca (%)	−0.251	−0.074
Plant Mg (%)	−0.112	0.347
Plant S (%)	−0.213	−0.117
Plant Mn (mg/kg)	0.165	0.284
Plant Cu (mg/kg)	0.012	−0.356
Plant B (mg/kg)	−0.185	−0.114
Plant Zn (mg/kg)	0.248	−0.124
Plant Na (mg/kg)	0.006	0.234

[a]Soil organic matter.

oxidizable carbon (PoxC) ($P < 0.001$), while pH was higher in conventionally managed fields ($P = 0.044$). Soil physicochemical properties were highly correlated with one another (Table 2). Magnesium was correlated with only NO_3-N, cation exchange capacity (CEC), and pH, but other macronutrients and key soil properties tended to vary together. Management also affected plant nutrients ($P < 0.001$), many of which were correlated with one another (Table 3). Concentrations of Ca ($P = 0.0004$), S ($P < 0.001$), and Cu ($P < 0.001$) were all higher in plants from organically managed fields.

Rhizosphere microbial community composition responds to management practices. The species composition of both bacterial and fungal rhizosphere communities varied according to site and management (Fig. 2), and these effects were also observed when phylogenetic relatedness of bacterial communities was considered (see Fig. S1 in the supplemental material). Tests of multivariate homogeneity of group dispersions (betadisper function of the vegan package) showed that dispersions did not

TABLE 2 Correlations among soil physicochemical properties based on the Pearson correlation coefficient[a]

	Correlation with:											
Variable	N	C	NO_3-N	P	K	Na	Ca	Mg	CEC	OM	pH	PoxC
N	1.00	0.96***	0.46***	0.66***	0.84***	0.79***	0.64***	−0.04	0.37**	0.82***	0.25	0.84***
C		1.00	0.26	0.50***	0.90***	0.71***	0.76***	−0.02	0.45***	0.90***	0.41**	0.89***
NO_3-N			1.00	0.62***	0.32*	0.56***	0.22	−0.44***	−0.24	0.06	−0.29*	0.21
P				1.00	0.57***	0.55***	0.36**	0.13	0.35**	0.43**	0.12	0.35**
K					1.00	0.63***	0.88***	0.02	0.55***	0.85***	0.61***	0.79***
Na						1.00	0.53***	−0.13	0.23	0.55***	0.22	0.59***
Ca							1.00	−0.11	0.48***	0.76***	0.60***	0.67***
Mg								1.00	0.81***	0.25	0.51***	−0.06
CEC									1.00	0.68***	0.79***	0.36**
OM										1.00	0.55***	0.76***
pH											1.00	0.34**
PoxC												1.00

[a]*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 3 Correlations among plant variables based on the Pearson correlation coefficient[a]

	Correlation with:															
Variable	Shoot biomass	C	N	P	K	Ca	Mg	S	Mn	Cu	B	Zn	Na	C:N	Root biomass	Shoot:root
Shoot biomass	1.00	0.75***	0.39**	0.86***	0.69***	0.55***	0.77***	0.48***	−0.03	−0.38**	0.27*	−0.74***	0.29*	0.28*	−0.01	0.33*
C		1.00	0.58***	0.80***	0.73***	0.54***	0.71***	0.32*	−0.04	−0.57***	0.35**	−0.87***	0.20	0.31*	−0.21	0.36**
N			1.00	0.53***	0.52***	0.33*	0.40**	0.06	0.03	−0.44***	0.13	−0.36**	0.40**	−0.59***	−0.17	0.30*
P				1.00	0.72***	0.53***	0.84***	0.42**	0.10	−0.47***	0.17	−0.69***	0.39**	0.17	−0.05	0.33*
K					1.00	0.78***	0.42**	0.55***	−0.43**	−0.13	0.60***	−0.74***	0.03	0.17	−0.09	0.38**
Ca						1.00	0.20	0.81***	−0.57***	0.27*	0.59***	−0.64***	−0.14	0.20	−0.21	0.42**
Mg							1.00	0.14	0.42**	−0.69***	−0.14	−0.52***	0.60***	0.19	0.00	0.24
S								1.00	−0.59***	0.45***	0.25	−0.47***	−0.08	0.31*	−0.19	0.26
Mn									1.00	−0.58***	−0.53***	0.20	0.37**	−0.16	0.07	−0.16
Cu										1.00	0.15	0.35**	−0.49***	0.00*	−0.10	0.02
B											1.00	−0.57***	−0.48***	0.26*	−0.01	0.20
Zn												1.00	0.08	−0.46***	0.18	−0.37**
Na													1.00	−0.31*	0.11	−0.02
C:N														1.00	0.00	−0.01
Root biomass															1.00	−0.62***
Shoot:root																1.00

[a]*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

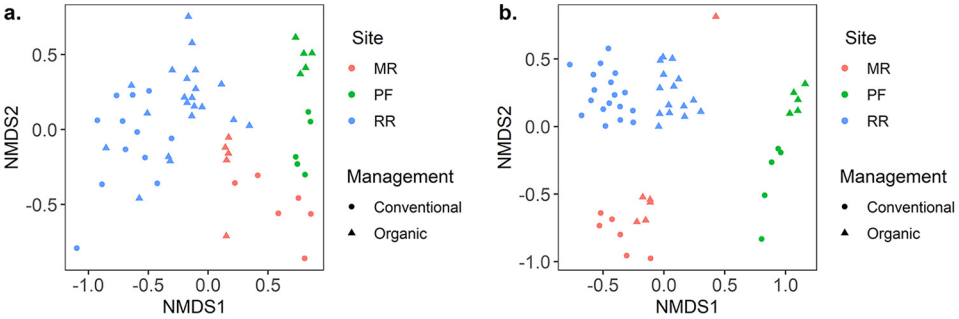

FIG 2 NMDS ordination of microbial communities sampled from the rhizosphere of processing tomatoes. Non-metric multidimensional scaling based on Bray-Curtis dissimilarity matrices revealed bacterial (a) and fungal (b) communities separated primarily by site and secondarily by management.

differ among sites or management types (both $P > 0.05$). Management influenced rhizosphere microbial communities but to different extents depending on the site identity (site codes MR, PF, and RR are used to indicate three distinct sites) (Fig. 2, site × management interaction bacteria $R^2 = 0.12$, $P < 0.01$; fungi $R^2 = 0.10$, $P < 0.01$). Management accounted for the greatest proportion of variation (53%) in bacterial communities at the MR site ($R^2 = 0.53$, $P = 0.02$), slightly more than at the PF site ($R^2 = 0.43$, $P = 0.02$) and nearly three times as much as at the RR site ($R^2 = 0.19$, $P = 0.01$). Fungal communities were also affected by management, which accounted for 22% of variation at the MR site ($R^2 = 0.22$, $P = 0.02$), 38% at the RR site ($R^2 = 0.38$, $P = 0.01$), and 43% at the PF site ($R^2 = 0.43$, $P = 0.02$).

Bacterial diversity was affected by the site × management interaction ($P < 0.001$). The Shannon index was higher in organically managed fields than conventionally managed fields at all sites except the MR site (Table 4). Fungal diversity was affected by site ($P < 0.001$) and management ($P < 0.001$) but not the interaction. Fungal diversity was higher in organically managed fields at all sites and higher at the PF site than RR or MR (Table 4).

Forty-eight bacterial amplicon sequence variants (ASVs) differed in abundance between the rhizospheres of conventionally and organically managed plants at the $\alpha = 0.01$ level (Fig. 3a). ASVs more abundant in organically managed rhizospheres included two members of the genus *Pseudomonas*, while ASVs more abundant in conventionally managed rhizospheres included six members of the genus *Flavobacterium* and three members of the genera *Devosia* and *Lysobacter*. An ASV belonging to the genus *Pseudomonas* had the highest relative abundance in organically managed fields, and an ASV belonging to the genus *Chryseobacterium* had the highest relative abundance in conventionally managed fields.

Nineteen fungal ASVs differed in abundance between management systems at the $P = 0.01$ level, only one of which was more abundant in conventionally managed fields (Fig. 3b). ASVs more abundant in organically managed plant rhizospheres included three members of the genus *Holtermanniella*, three members of the genus *Mucor*, and two members of the genus *Pyrenochaetopsis*. The ASV that was more abundant in conventionally managed rhizospheres was identified as *Plectosphaerella cucumerina*. *Mucor hiemalis* was most abundant in organic systems relative to conventional.

TABLE 4 Alpha diversity of bacterial and fungal communities by site and management[a]

	Value by site					
	MR		RR		PF	
Community	Conventional	Organic	Conventional	Organic	Conventional	Organic
16S	3.50 ± 0.14	2.90 ± 0.09	2.72 ± 0.05	2.99 ± 0.08	3.28 ± 0.08	3.58 ± 0.07
ITS	2.60 ± 0.08	2.62 ± 0.18	2.68 ± 0.08	3.12 ± 0.06	2.89 ± 0.16	3.32 ± 0.07

[a]Values reported are Shannon index ± standard error.

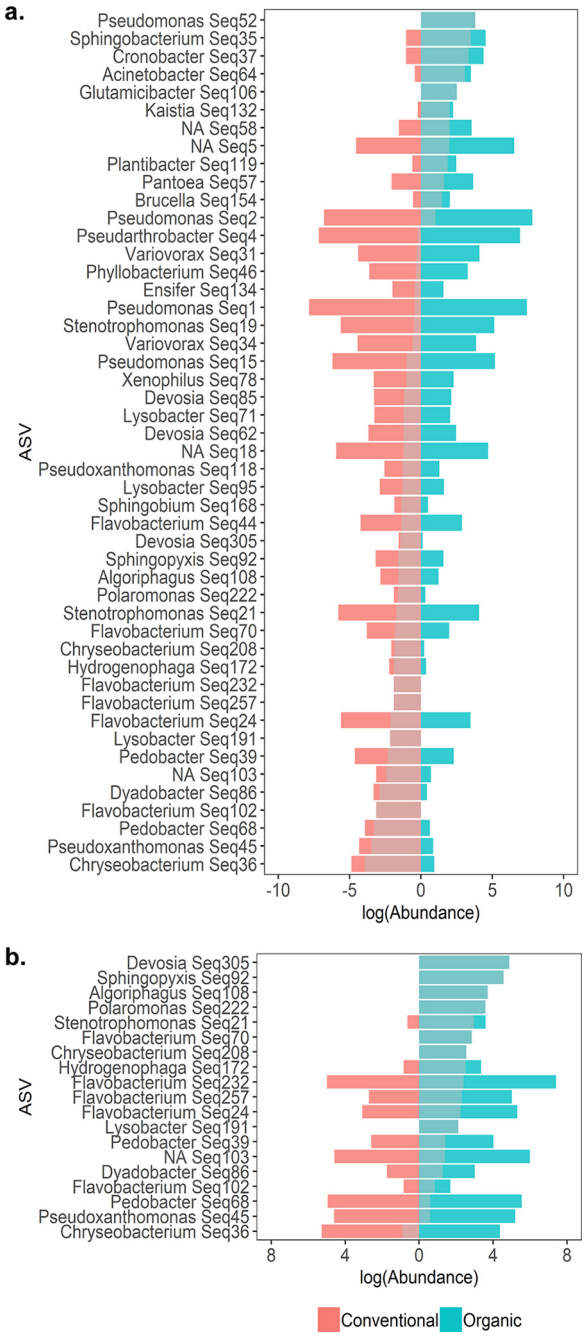

FIG 3 Differentially abundant microbial taxa. (a) A total of 48 bacterial and (b) 19 fungal taxa differed in abundance between conventional and organic management systems at the $\alpha = 0.01$ level. Colored bars represent the natural logarithm of abundance of each taxon, and gray bars represent the ratio of abundance in the organic system to abundance in the conventional system. Multiple strains or species within genus are shown. NA indicates that sequences could not be identified at the genus level.

Since system management has a strong impact on multiple soil properties, we conducted redundancy analysis (RDA) with forward selection to identify which soil physicochemical properties have the greatest influence on rhizosphere bacterial and fungal community composition. After site and management, Ca was the most significant driver of both bacterial and fungal community composition. Bacterial community composition also responded to Mg levels, while fungi were significantly influenced by Na and K.

Indicator species of rhizosphere communities differ between systems. Indicator species analysis showed 57 system-specific bacterial ASVs, namely, 35 with the conventional system and 22 with the organic system (see Table S3 in the supplemental material). Members of the genera *Flavobacterium* (8), *Pedobacter* (4), *Lysobacter* (3), and *Pseudomonas* (3) had the greatest number of sequences in the conventional system and *Pseudomonas* (4) in the organic system. Fewer fungal indicator taxa were discovered, with only 4 fungal ASVs associated with the conventional system but 17 with the organic system. The four ASVs associated with the conventional system came from different genera, while *Holtermanniella* (6) and *Mucor* (4) were the most represented indicator genera in the organic system. Fifteen of the 78 taxa identified by indicator species analysis were also differentially abundant. Because the indicator value (IndVal) index represents the probability of finding a given species in the environment of interest, taxa with a high relative abundance in the environment will generally score high on the fidelity component of the IndVal index. This was the case for *Flavobacterium* spp. in the conventional system and *Pseudomonas*, *Holtermanniella*, and *Mucor* spp. in the organic system.

Random forest (RF) analysis was used to identify ASVs that could be used to discriminate between management systems. ASVs belonging to the genera *Lysobacter* and *Gibellulopsis* had the greatest impact on the mean decrease in accuracy and mean decrease in Gini coefficient of the random forest model (see Fig. S2 in the supplemental material). Substantial overlap was observed between the results of RF analysis and differential abundance analysis. Eleven of the 20 most significant ASVs from the RF analysis had also been identified through differential abundance analysis, although ASVs, such as *Gibellulopsis* spp., that had a significant impact on the RF model only slightly differed in abundance between systems (Fig. 3).

Management induces changes in predicted rhizosphere bacterial functions. Of the total number of genes predicted, 4.8% (169) differed in abundance between the rhizosphere of organic and conventional plants. Of those genes, 79 were more abundant in the organic system and 90 were enriched in the conventional system. Functions corresponding to cellular processes including quorum sensing, biofilm formation, and chemotaxis showed the greatest difference between systems, with only two peroxisome functions upregulated in the organic system and 31 upregulated in the conventional system (Fig. 4). Genes with the highest relative abundance in the organic system were distributed across a variety of functions, including ABC transporters (12), two-component systems (8), biosynthesis of siderophores (5), starch and sucrose metabolism (5), and type I polyketide structures (5). A component of the *trcR*/*trcS* two-component regulatory system, *trcR* (K07672), was upregulated by the greatest ratio in organic systems. Genes with greater relative abundance in the conventional system tended to be associated with biosynthesis of amino acids (19), two-component systems (18), quorum sensing (10), ABC transporters (9), and biofilm formation (9) (Fig. 4).

Structural equation modeling identifies key linkages among plant, soil, and microbial variables. Hypothetical links between bulk soil physicochemical parameters, plant nutrition, rhizosphere microbial communities, and plant biomass were tested using structural equation modeling (SEM) across management systems (Fig. 5a). Bacterial and fungal communities were represented by two vectors each (PC1B, PC2B, PC1F, and PC2F) that were derived from principal-component analysis shown in Fig. 2. Plant biomass was most strongly positively correlated with plant P, which, in turn, was most strongly correlated with fungi from the PC2F vector (Fig. 5b). The taxa that contributed most to PC2F were *Vishniacozyma victoriae* and an unidentified *Solicoccozyma* sp. Neither of these species were identified in the differential abundance analysis (Fig. 3b). Fungi from the PC1F vector had a slight positive influence on plant Na and included ASVs classified as *Alternaria* sp., *Cryptococcus aerius*, and *Plectosphaerella cucumerina*. PC1B, the first principal component of bacterial communities, was negatively correlated with shoot C:N ratio; the three ASVs with the greatest contribution to this component were a strain of *Pseudomonas* and two strains of *Stenotrophomonas*.

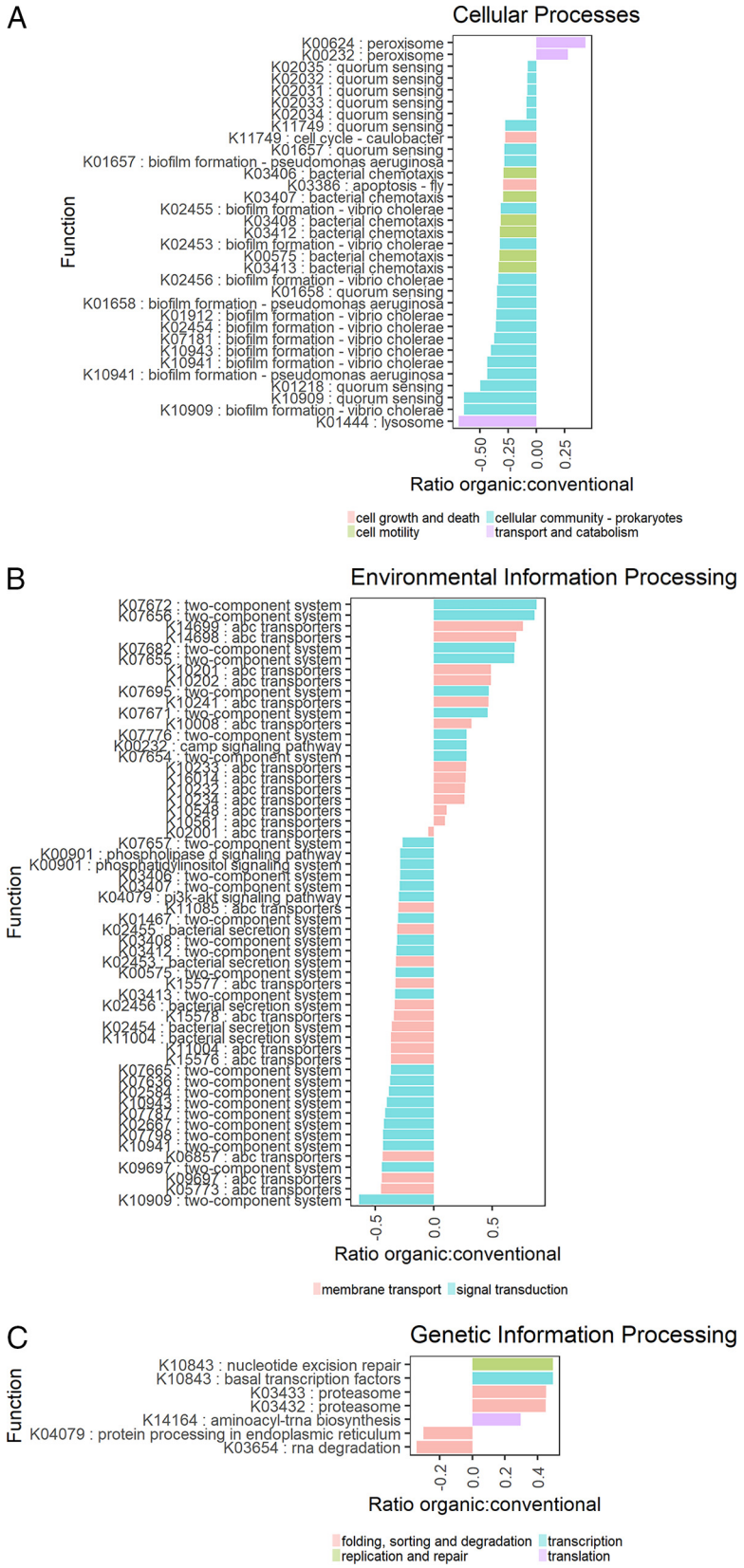

FIG 4 Differentially abundant functions. Phylogeny-based trait prediction revealed 169 functional genes that differed in abundance between the two systems at the $\alpha = 0.01$ level, of which 79 were more abundant in the organic system and 90 in the conventional system.

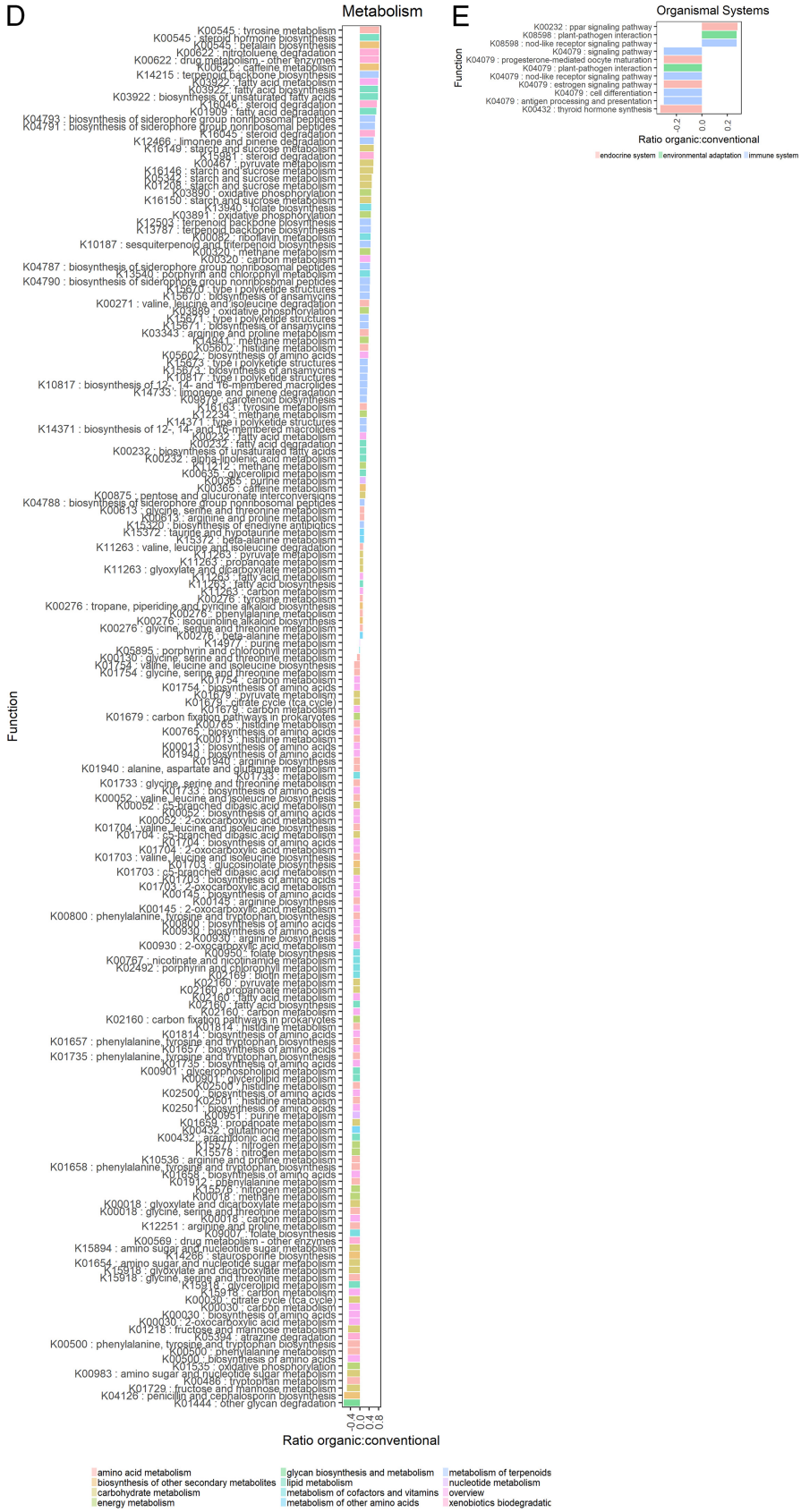

FIG 4 (Continued)

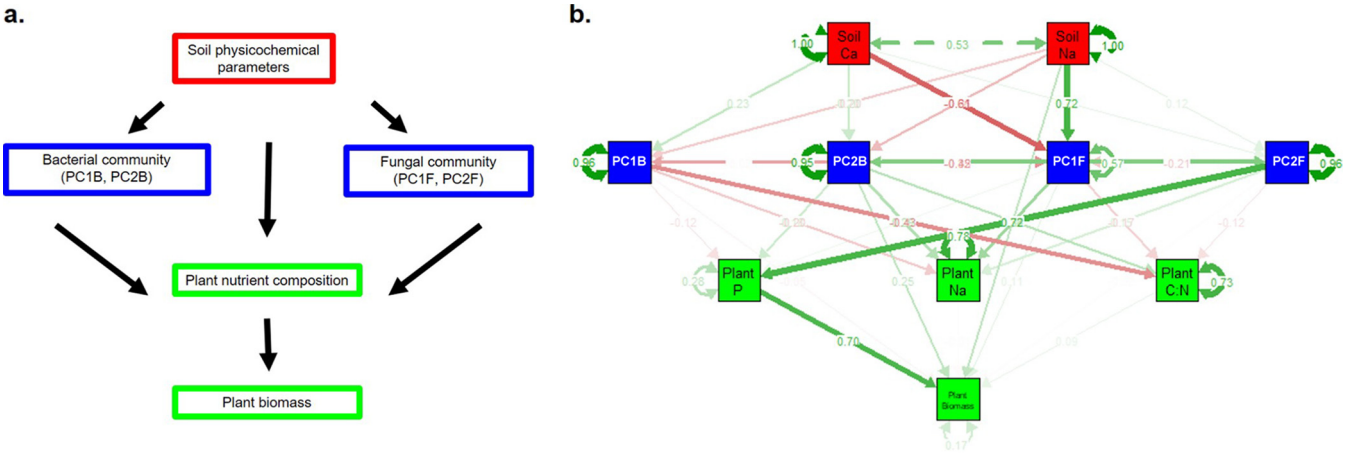

FIG 5 Structural equation model linking soil, plant, and microbial variables. (a) A hypothetical model linking soil, microbial, and plant parameters was tested using structural equation modeling. (b) The final SEM showed that microbial communities had a strong but indirect effect on plant biomass through a positive correlation between fungal community composition and plant P. Soil Ca and Na affected fungal communities more strongly than bacterial communities. Red represents soil variables, blue represents microbial variables (principal components 1 and 2 extracted from PCA of bacterial and fungal communities, respectively), and green represents plant variables. Dashed lines represent fixed parameters.

The second principal component of bacterial communities (PC2B) was slightly positively correlated with plant biomass, P, Na, and C:N ratio; four of the five ASVs with the greatest contribution to this component were classified as *Pseudomonas* sp.

The final SEM had a χ^2 test statistic of 1.907 with 3 degrees of freedom, giving a χ^2/v ratio of 0.64, root mean square error of approximation (RMSEA) of 0.000 (90% confidence interval $0.000 \leq x \leq 0.195$), comparative fit index (CFI) of 1.000, Tucker Lewis index (TLI) of 1.062, and standardized root mean square residual (SRMR) of 0.016. A low χ^2/v ratio indicates a good model, although this test statistic does not perform well with small sample sizes (30). The CFI and TLI model indices perform well with small sample sizes and are above the acceptable threshold (0.95 for a good model [31]). An SRMR less than 0.08 generally indicates that a model fits the data well (32).

DISCUSSION

Our objectives were to explore how management practices implemented in organic and conventional tomato production systems shape rhizosphere microbial composition, infer how taxonomic shifts affected microbe-mediated functions, and identify linkages between management-induced shifts in soil physicochemical parameters, rhizosphere microbial communities, and plant nutrition and productivity. In support of our hypotheses, we identified specific taxa that differed in abundance between management systems and predicted the functional implications of those shifts in community composition (Fig. 3, 4, and S2). Some differentially abundant taxa were confirmed as indicator species that could be used to distinguish communities between management systems. More importantly, phylogeny-based trait prediction showed that management-induced differences in rhizosphere bacterial community composition translated into agriculturally relevant outcomes, particularly with regard to plant nutrition and pathogen-related functions such as quorum sensing and biofilm formation (33, 34) (Fig. 4 and 5). Although our techniques could not examine the contribution of fungi to predicted function, it is likely that observed compositional shifts in fungal communities increase divergence in functional outcomes between systems.

Bacterial diversity was higher in the rhizospheres of organically managed plants at all sites except MR, and fungal diversity was higher in the organic system across sites, consistent with other studies finding increased microbial diversity under organic management (2, 7, 35–37). Numerous bacterial ASVs belonging to the genus *Pseudomonas*, which contains members known to possess plant-growth-promoting properties (18, 19), had a higher relative abundance in organic systems (Fig. 3a). Sixteen of

the 17 differentially abundant fungal ASVs were found at higher abundance in the rhizosphere of plants growing in the organic system; these included numerous members of the genera *Holtermanniella* and *Mucor* (Fig. 3b). *Holtermanniella* is a small, cold-tolerant genus of potentially parasitic fungi (38) that includes species able to metabolize diverse carbon compounds and generate unique fatty acid profiles (39). *Mucor* is a genus of starch-decomposing fungi (40) that are capable of metabolizing a wide range of complex carbohydrates (41). Although a long-term comparison of conventional and organic management found no difference in the relative abundance of *Mucor* sp. in bulk soils (42), potential shifts in the rhizosphere have not been shown. In addition, predicted potential community functions also differed between soils under different management systems. Although our approach relies on predicted potential (DNA-based) functions rather than genomic or transcriptomic information from the strains found at these sites, Tax4Fun performs well in comparison with shotgun metagenomic data from soils (28), suggesting that broad patterns may be informative. Bacterial community shifts in the rhizospheres of organically managed plants were associated with a higher abundance of predicted genes involved in starch and sucrose metabolism and biosynthesis of siderophores, which can increase the availability of micronutrients such as iron (Fig. 4). Other enzymes with high relative abundance in the organic system catalyze reactions involved in the metabolism of tyrosine, carotenoids, and other complex organic compounds (Fig. 4).

Rhizosphere diversity was generally lower under conventional management, and community composition and functions were notably different. ASVs belonging to the genera *Flavobacterium*, *Devosia*, and *Lysobacterium* had higher relative abundances in the conventional system. The *Flavobacterium* genus has been found elsewhere to increase in abundance in response to 6 years of intensive organic vegetable production (43), suggesting that individual species within the genus may respond differently to conventional and organic management. Members of *Lysobacterium* have been shown to degrade complex aromatic compounds (44). *Plectosphaerella cucumerina*, a known pathogen that causes rots on a variety of horticultural species (45), was the only fungal ASV found to be more abundant in the conventional system. Perhaps due to the greater abundance of this pathogen, functions upregulated in the conventional system included genes related to quorum sensing and biofilm formation (Fig. 4).

Management practices and sites had a strong influence on soil chemical properties, which, in turn, affected bacterial and fungal community composition. Forward selection revealed that the two kingdoms responded to different sets of soil physicochemical parameters, namely, bacterial community composition was affected by Ca and Mg, while fungal community composition was affected by Ca, Na, and K. These predictors are notably different from variables commonly accepted as important for microbial community composition, such as organic matter (46, 47), pH (48, 49), and N. The failure of organic matter and N to predict microbial community structure is surprising at first glance, given that scarce C and N availability can limit rates of microbial growth and functions such as mineralization and that the abundance of N-cycling microbial taxa often varies with C and inorganic N species. However, this result is consistent with multiple studies showing no effect of N on microbial community composition (50–52). Agricultural management might outweigh the effects of variation in these parameters since Ca and Mg were not affected by management. It may also be that low variation in organic matter, pH, and soil N within the context of this study reduced the ability of these parameters to explain variation in community composition (see Table S2 in the supplemental material).

Soil Ca and Na have similarly appeared elsewhere as significant predictors of microbial community composition. In another comparison of management systems, soil Ca was higher in soils receiving organic amendments than in soils receiving synthetic amendments and was among the parameters correlated with microbial community composition (53). Ca was also a primary driver of microbial community composition in a multiyear study of a soil amended with composted tannery sludge (54). Salinity frequently drives variation in microbial community composition, especially

in irrigated systems, although most commonly when a stronger salinity gradient is present due to environmental filtering based on salinity tolerance (55–57).

SEM tied together this observed variation in microbial community composition with soil and plant variables and tested a hypothetical model linking plant and soil biological and physicochemical parameters with plant biomass (Fig. 5). Management was not retained in the final model, suggesting that management effects were indirect and captured by other included variables at these sites. Other studies have similarly found that soil type and physicochemical parameters affect microbial community composition and catabolic functions more than long-term agricultural management practices (58). Within this study, it appears that rhizosphere microbial communities were more closely linked to differences in bulk soil properties created by management systems than to the management practices themselves.

SEM revealed a greater relative influence of rhizosphere biological communities than bulk soil physicochemical characteristics on plant nutrient content and biomass (Fig. 5). A strong indirect linkage was observed between microbial communities and plant biomass: fungal community composition was strongly positively correlated with plant P, which, in turn, strongly correlated with shoot biomass (Fig. 5). The link between plant P and fungal communities is particularly striking given the absence of sequences belonging to the phylum Glomeromycota, which contains mycorrhizal fungi (data not shown). The lack of mycorrhizal sequences may be partly explained by the choice of amplicon or primer bias (59). Since the length of the amplified region differs for mycorrhizae compared with the more abundant Ascomycota and Basidiomycota (60), it is unlikely that mycorrhizae were truly absent from all samples. Nonetheless, even nonmycorrhizal fungi can improve plant P status through solubilization, mineralization, and direct transfer of phosphate (61). Members of genera such as *Aspergillus* and *Penicillium* release organic acids that can solubilize phosphate, potentially rendering it available for direct uptake by plants or mycorrhizae (62).

PC2B was slightly positively correlated with plant Na, and PC1B was negatively correlated with C:N ratio. The correlation between bacterial community composition and plant Na could be the direct effect of microbial interference in plant metabolism, or changes in soil parameters could foster unique microbial communities and also increase plant Na. While limitations of the measured data do not allow us to distinguish between these explanations in this context, microbial influence on plant Na has been reported elsewhere; certain bacterial strains are capable of plant tissue-specific regulation of sodium transporters that increases salt tolerance in *Arabidopsis* spp. (63), while other bacterial strains reduce salt accumulation in salinity-stressed plants (64). A negative correlation with C:N ratio indicates that the bacterial populations improved plant N content, a result that could be due to increased N availability via N fixation or mineralization of organic matter.

This study identified rhizosphere microbial taxa and functions affected by agricultural management and illuminated unexpected linkages between soil, microbes, and crop nutrition and productivity, but compelling questions remain. Organic certification encompasses a diverse set of management practices, and variation in cover crop species, green manure inputs, or crop rotation complexity and duration likely leads to diverse effects on soil microbes. To translate the broad, extensive conventional-organic literature into tangible recommendations, future studies should focus on causal relationships between specific inputs or techniques and key soil physicochemical parameters. This could be achieved in part by employing SEM with a much larger data set (a sample size of at least 200 [65] and data satisfying the requirement of multivariate normality [66]) to allow the incorporation of additional variables (e.g., crop genotype, N fertility source and rate, and tillage) and improve the predictive power of the model. Such analysis would add nuance to the results of this study and enable the development of management systems that foster agricultural productivity by maximizing beneficial plant-soil-microbe interactions in the rhizosphere.

Our results add an additional layer of complexity to previous investigations of the effects of agricultural management on microbial communities. Others have noted the

importance of scale in determining how soil properties relate to microbial community composition or function, as geographic scale alters the relative importance of factors such as environmental heterogeneity and distance that influence microbial distribution (67, 68). We emphasize the importance of integrating plants and rhizosphere processes into these discussions of microbial biogeography, particularly at intermediate scales, as plants exert strong influence on rhizosphere communities and may modulate management effects on rhizosphere communities. Management of plant-microbe-soil interactions in the rhizosphere is a critical step toward building more resource-efficient and resilient agricultural systems, and our study indicates that soil management has strong and consistent effects on landscape-level variation in the rhizosphere composition and predicted function.

MATERIALS AND METHODS

Sample collection. Samples were collected from 6 paired fields under conventional and organic management on Yolo silt loam during the 2017 growing season (details of sites and management practices can be found in Table S1 in the supplemental material). Plant and soil samples were collected ~6 weeks after transplanting on the same date at paired fields. Samples were taken from six locations per field (two on the exterior margins of the field and four internal). At each location, two entire plants were excavated and shoot and root samples were separated by clipping at the base of the shoot. A bulk soil sample was collected from the upper 10 cm of soil immediately adjacent to each plant. Roots were separated from bulk soil, stored in paper bags, and transported to the lab on ice. Twelve root fragments from each plot (6 from each individual plant) were pooled and rhizosphere soil was collected using a shaking wash in an 0.9% NaCl/0.01% Tween 80 (vol/vol) solution followed by centrifugation. Because this volume of soil was insufficient for full textural and nutrient analysis, we assumed that rhizosphere soil characteristics such as texture and organic matter would be similar to the parameters measured for the corresponding bulk soil. Shovels and other sampling implements were cleaned thoroughly between samples. The remaining roots and shoots were dried at 60°C and weighed.

Plant and soil analysis. Dried bulk soil samples and aboveground dried biomass were homogenized and analyzed for total nitrogen (N) and carbon (C) via combustion analysis (69). Soil nitrate was measured using a flow injection analyzer (70), soil extractable phosphorus (P) was determined according to Olsen and Sommers (71), and other soil nutrients were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (72). Soil organic matter content was determined via the loss-on-ignition method (73). Soil pH was measured on a saturated paste extract. Bulk soil properties can be found in Table S2 in the supplemental material.

Dried aboveground biomass was ground thoroughly to pass a 2-mm sieve. Plant leaf samples were analyzed for N, P, K, Ca, Mg, Mn, Fe, Cu, B, and Zn at the Agricultural Analytical Services Lab of Pennsylvania State University. Total N was analyzed via combustion (74), and concentrations of the remaining elements were determined via hot block acid digestion (75).

Microbial community analysis. DNA was extracted from rhizosphere samples using the MoBio PowerSoil kit (Qiagen). At least 5 ng of DNA from each sample was sent for library preparation and sequencing using a MiSeq instrument at Dalhousie IMR facility. The V4-V5 region of the 16S rRNA region was sequenced to characterize bacterial communities and the internal transcribed spacer (ITS) region of the rRNA gene was sequenced to characterize fungal communities (76, 77). Negative controls were also extracted and submitted, but no reads were recovered. All statistical analyses were carried out using R software (78). Reads were error-corrected and assembled into amplicon sequence variants (ASVs) using DADA2 v.1.8 (79), with taxonomy assigned using SILVA v.128 for bacteria (80) and UNITE database (2017 release) for fungi (81). Taxa without a taxonomic assignment or assigned to Archaea, mitochondria, or chloroplasts were removed from this data set. Those not assigned to the kingdom Fungi were removed from the fungal data set. Sequence abundance was rarefied to 15,310 sequences per sample for bacteria and 13,000 per sample for fungi, and all samples approached saturation.

Nonmetric multidimensional scaling (NMDS) was used to ordinate samples in two-dimensional space (ordinate function of the phyloseq package using method = "NMDS"). Two outliers were removed from this and subsequent analyses in order to minimize the stress function. A second NMDS ordination was performed based on weighted UniFrac distances (distance function of phyloseq package with "wunifrac" command) to determine whether the phylogenetic distance among samples was affected by site and management. Shannon diversity was calculated for each sample using the estimate_richness function (measures = "Shannon") of the phyloseq package.

Differential abundance of microbial taxa. Differential abundance of bacterial and fungal taxa in the rhizosphere of plants grown in organically and conventionally managed systems was carried out using the DESeq2 package (25). Although applying this analysis to compositional data sets obtained from sequencing microbial communities has been critiqued (82), the method has been shown to be effective when library sizes are similar across groups and sample size is small (<50 samples per group) (83), as was the case here. Sequences occurring in fewer than three samples were filtered out prior to the analysis to avoid bias due to rare taxa (filter_taxa function of phyloseq package). Dispersions were fit to the mean intensity using a gamma-family generalized linear model (GLM) by setting the parameter fitType = "parametric," and significance was assessed using the Wald test with a significance threshold of $\alpha = 0.01$.

Indicator species analysis. Indicator species analysis was conducted to identify specific rhizosphere microbial taxa that were associated with the conventional or organic system using the indicspecies package (84). Briefly, the indicator value (IndVal) index was calculated for each ASV-system combination as the product of specificity and fidelity indices (84). The highest IndVal index for each ASV was tested for significance with 999 permutations [multipatt function of indicspecies package using duleg = TRUE and control = how(nperm = 999)]. A Bonferroni correction was used to control the family-wise error rate at $\alpha = 0.01$.

Random forest analysis. We complemented the indicator species analysis with a random forest approach, which identifies bacterial and fungal ASVs that could be used to classify samples by management system through a machine learning algorithm. Random forest analysis was conducted using the randomForest package (27). The data set was split into subsets for training (70% of observations) and validation (30% of observations). Model parameters were adjusted to minimize the error rate, but the default parameters for ntree (ntree = 500) and mtry (mtry = $\sqrt{p}$, with p representing the number of model parameters) resulted in the lowest error rate (6.52%). The classification accuracy was calculated to be 95%, indicating high prediction accuracy. ASVs with the greatest contribution to the classification algorithm were identified according to the highest scores for mean decrease in accuracy or mean decrease in the Gini coefficient (importance function of randomForest package).

Phylogeny-based functional trait prediction. We determined potential shifts in rhizosphere microbial functions with management and soil properties using functional trait prediction of 16S communities with the themetagenomics package (85). Briefly, this package implements Tax4Fun (28) to predict functions from the KEGG Orthology database that are associated with provided abundance tables, sample metadata, and phylogenetic information. Phylogeny is assigned according to the SILVA rRNA database project (80). To identify functions that differed in abundance between systems, predicted functions were subjected to differential abundance analysis using the DESeq2 package. Parameters were identical to those described previously and the significance threshold was set at $\alpha = 0.01$.

Principal-component analysis of plant and soil variables. Principal-component analysis (PCA) was used to reduce the dimensions of the multivariate data set containing scaled soil and plant variables, visualize samples in two-dimensional space, and calculate factor loadings (prcomp function of stats package). Outliers for individual soil and plant variables were identified with Grubb's test (grubbs.test function of outliers package) and removed from the data set prior to PCA. The multivariate homogeneity of group dispersions (betadisper function of vegan package) was tested to determine whether variances differed among sampling sites. The effect of management on soil and plant variables was tested with multivariate analysis of variance (MANOVA) using the manova function of the stats package (78).

Permutational multivariate analysis of variance. Permutational multivariate analysis of variance was used to test the effect of the interaction between site and management on microbial community composition (adonis function of vegan package), separately for bacteria and fungi. If the interaction was significant, the magnitude of the management effect was then tested within each site. If the interaction was not significant, permutational multivariate analysis of variance (PERMANOVA) was used to test the relative magnitude of site and management effects. Redundancy analysis (RDA) was conducted to identify soil physicochemical properties with the greatest influence on rhizosphere microbial community composition. Parameters that significantly explained variation in bacterial or fungal community composition were identified using forward selection (ordistep function of vegan package).

Structural equation modeling. SEM was used to test a hypothetical model linking soil, plant, and microbial variables that affect shoot biomass (Fig. 5a). Parameters included in the model were chosen using forward selection of a linear model with shoot biomass as the response variable and all other soil, microbial, and plant parameters as independent variables (step function of stats package) (78). The model was established using the sem function of the lavaan package (86) and visualized with the semPlot package (87). The model was then refined by sequentially removing variables with poor explanatory power ($R^2 < 0.50$). Management (organic versus conventional) was originally included as a variable but was ultimately removed because management significantly and consistently decreased the fit statistics for the model, perhaps because the variables retained in the model were good indicators of management differences.

Although the variables identified by forward selection (soil Na, soil Ca, plant P, plant C:N, and plant Na) were not consistent with a hypothesis of multivariate normality, the sample size was too small to permit the exclusion of outliers. The first two principal components of microbial species composition, which accounted for 31% and 15% of bacterial variation (PC1B and PC2B, respectively) and 26% and 21% of fungal variation (PC1F and PC2F, respectively), were used to represent microbial communities in the model (Fig. 5). The maximum likelihood (ML) method was used to estimate model fit test statistics. The goodness of fit of the model was tested using standard model fit indices, namely, the ratio of the chi-square statistic to degrees of freedom (χ^2/v), root mean square error of approximation (RMSEA), comparative fit index (CFI), Tucker-Lewis index (TLI), and standardized root mean square residual (SRMR) (88).

Data availability. Sequencing data are available in the NCBI SRA data repository under the project accession number PRJNA539989.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01064-19.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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