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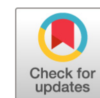

Agricultural Soil Management Practices Differentially Shape the Bacterial and Fungal Microbiomes of *Sorghum bicolor*

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ABSTRACT Soils play important roles in biological productivity. While past work suggests that microbes affect soil health and respond to agricultural practices, it is not well known how soil management shapes crop host microbiomes. To elucidate the impact of management on microbial composition and function in the sorghum microbiome, we performed 16S rRNA gene and ITS2 amplicon sequencing and metatranscriptomics on soil and root samples collected from a site in California's San Joaquin Valley that is under long-term cultivation with (i) standard (ST) or no tilling (NT) and (ii) cover cropping (CC) or leaving the field fallow (NO). Our results revealed that microbial diversity, composition, and function change across tillage and cover type, with a heightened response in fungal versus bacterial communities. Surprisingly, ST harbored greater microbial alpha diversity than NT, indicating that tillage may open niche spaces for broad colonization. Across management regimes, we observed class-level taxonomic level shifts. In addition, we found significant functional restructuring across treatments, including enrichment for microbial lipid and carbohydrate transport and metabolism and cell motility with NT. Differences in carbon cycling were also observed, with increased prevalence of glycosyltransferase and glycoside hydrolase carbohydrate active enzyme families with CC. Lastly, treatment significantly influenced arbuscular mycorrhizal fungi, which had the greatest prevalence and activity under ST, suggesting that soil practices mediate known beneficial plant-microbe relationships. Collectively, our results demonstrate how agronomic practices impact critical interactions within the plant microbiome and inform future efforts to configure trait-associated microbiomes in crops.

IMPORTANCE While numerous studies show that farming practices can influence the soil microbiome, there are often conflicting results on how microbial diversity and activity respond to treatment. In addition, very little has been published on how the corresponding crop plant microbiome is impacted. With bacteria and fungi known to critically affect soil health and plant growth, we concurrently compared how the practices of no and standard tillage, in combination with either cover cropping or fallow fields, shape soil, and plant-associated microbiomes between the two classifications. In determining not only the response to treatment in microbial diversity and composition, but for activity as well, we demonstrate here the significance of agronomic practice in modulating plant-microbe interactions, as well as encourage future work on the mechanisms involved in community assemblages supporting similar crop outcomes.

KEYWORDS conservation agriculture, reduced disturbance, cover crops, microbiome, sorghum, amplicon sequencing, metatranscriptomics

The soil underfoot is intimately tied to the wealth and wellbeing of our nations, where numerous, vital ecosystem services are provided by the approximate one fourth of the world's biodiversity that is hosted by soil (1). Soil health is defined as "the

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capacity of soil to function as a living system" (2) and is characterized by the ability to sustain biological productivity, promote plant and animal health, and preserve air and water quality (3). In particular, healthy soils can improve crop yields by promoting nutrient cycling, water retention, pest and disease control, and the storing, filtering, and transformation of a wide array of compounds (4). Despite this key involvement in the degree and maintenance of agricultural productivity, soil health is on the decline and about one third of land globally is impacted by soil degradation (5).

Agronomic soil management practices are a critical factor in determining short- and long-term soil health (6). Tillage is one long-standing method that has been conventionally used to control weeds and loosen soil in preparation for planting. With intensive, mechanical agitation of soil to depths up to 45 cm, tillage typically leaves less than 15% of the previous year's crop residue on the soil surface (7). While standard tillage (ST) can more evenly distribute organic matter and nitrogen, remove unwanted plants and potential pathogens, and aerate the soil, it can also lead to soil compaction below the depth of tillage, erosion, an increased need for fertilizer application, decreased carbon sequestration, and increased rates of soil moisture loss (8–11). One alternative is reduced or no tillage (NT), which leaves 15 to 30% (reduced tillage) or more (no tillage) of the previous crop residue on the soil surface. This method can abate runoff and erosion by facilitating water and fertilizer infiltration, improve carbon sequestration, decrease soil temperature fluctuations, and requires fewer fuel and labor inputs than ST (7, 12, 13). In addition, NT has been correlated with increased nutrient levels—including soil total nitrogen, phosphorus, and exchangeable potassium and calcium, as well as reduced exchangeable magnesium, cation exchange capacity, and zinc—compared to ST (14–16). The potential disadvantages of NT, however, can include increased herbicide dependence for weed control and soil health benefits that manifest over multiple growing seasons (7). An additional practice that can benefit soil health and is often used in conjunction with both ST and NT is cover cropping (CC), where one or more crops are grown in off-season months as an alternative to letting fields lie fallow (NO). CC can reduce soil erosion, hinder weeds, and enrich soils with nitrates and organic material, as well as augment biological diversity, increase crop yields, improve water availability, and propagate arbuscular mycorrhizal fungi (AMF) (17–23). Moreover, management practices employed in combination can have synergistic impacts on increasing crop yield over time (24–26).

One research direction that may yield important insights into the mechanisms that support soil functioning and improve crop performance is investigating the impact of agricultural practices on the microbiomes of plant hosts and the surrounding soil. It is well known that plant root-associated microbiomes can alter plant fitness and that plant root microbiomes are largely derived from the surrounding soil microbiome (27–30). What the combined effects of these soil practices are on agroecosystem-associated microbial communities has been less studied. Past work has shown that NT and CC can broadly increase soil microbial diversity and abundance, as well as reduce the amounts of fungal pathogens, compared to the conventional practices of ST and NO (31–37). In comparison to soils, it is even less clear what impact tillage and CC have on plant microbiome diversity, composition, and function. In the last few years, it has been demonstrated that wheat rhizosphere bacterial communities are indeed influenced by tillage (38). There is also some evidence suggesting that AMF are thought to be negatively impacted by intense tillage and monocultures, with higher spore density and active hyphal length reported in reduced tillage systems (39–41), and Rosner et al. observed that reduced tillage with CC may increase AMF root colonization in some plant hosts (*Helianthus annuus* L.), but not others (*Triticum aestivum* L.) (42).

Furthermore, relatively few studies have compared the responses between bacterial and fungal communities across agricultural soil management regimes, despite evidence of important functional complementation and antagonistic interactions existing between classifications (43). Coinoculations of multiple bacteria and fungi have been shown to improve plant growth, survival, and productivity versus single inoculations

(44, 45), and changes in community balances can be linked to disease in associated hosts (44, 46). In addition, tillage has been shown to decrease the soil biomass of fungi while that of bacteria increased (47), suggesting that bacteria and fungi may respond differently to soil management. Indeed, when comparing various tillage intensities in combination with conventional and organic management systems, soil bacterial communities were largely structured by tillage, while fungi were primarily impacted by system type (48). While one study also found that soil bacterial community richness and composition were more impacted by tillage than fungi (49), another suggested that bacteria may be more impacted by practices other than tillage, such as crop rotation (50). Lastly, within wheat roots, fungi may be more impacted by tillage than bacterial communities (48). Collectively, this work demonstrates that agricultural practices can significantly impact agriculture-associated microbiomes and elicit different responses in the bacterial and fungal fractions of these communities. However, more research is needed to understand how combinatorial agricultural soil management practices impact the assembly and activity of crop microbiomes, as well as their relation to plant fitness.

To address these knowledge gaps, we utilized a field site in California's San Joaquin Valley. It has been managed for close to two decades with a combination of (i) NT or ST practices and (ii) CC or NO to test how these management practices impact the composition and function of sorghum root microbial communities. Using 16S rRNA and ITS2 amplicon sequencing, in addition to metatranscriptomics, we test four specific hypotheses regarding the impact of these practices on the crop microbiome. (i) Fungal communities are influenced by management regime to a greater extent than bacteria, due to the vulnerability of hyphal networks to damage from tillage. (ii) NT differentially impacts composition and function in microbial communities, compared to ST, and will reflect greater niche differentiation due to reduced disturbance. (iii) Greater amounts of carbon cycling activity occur with ST than NT, as past crop residue is shredded and buried for rapid degradation with ST management. (iv) CC promotes increased microbial diversity and enriches for AMF, over NO, due to increased amounts of resources regularly provided by the plants grown in the off season.

RESULTS

Minimal variation in measured plant and soil chemical characteristics across soil management types. To address our hypotheses on how soil management practices impact crop plant-microbe interactions, we surveyed the bacterial and fungal communities of soil and the rhizospheres and roots of *Sorghum bicolor* grown at the West Side Agricultural Research Station in Five Points, California, where plots are under long-term management with NT or ST and CC or NO (see Fig. S1 in the supplemental material). To allow for comparisons between the effects of agronomic practice on soil and plant-associated microbiomes, as well as investigate developmental and temporal variability, samples were collected from three replicate plots before and after flowering in the summer of 2016 and before flowering in 2017 (see Fig. S1). At the time of each sampling, plants from which roots and rhizospheres were harvested were phenotyped (see Fig. S2). We observed that management practices did impact the above-ground plant phenotype (see Fig. S2), where variation in fresh shoot biomass was significantly explained by tillage and cover type ($P < 0.05$) for before flowering time points (see Table S1). However, both treatment types were statistically nonsignificant for plant height and yield variation (see Table S1).

To characterize soil environmental factors that could contribute to describing our microbial community results, soils sampled in 2016 were chemically profiled (see Table S2 and Fig. S3). We found that the majority of parameters measured varied slightly across treatment, including percent organic matter, which averages ranged between 2.46% (STNO) and 2.90% (STCC) (see Fig. S3). We further observed that variation in soils was only statistically significant for aluminum concentration (tillage type: $F = 10.67$, $P = 0.031$; cover type: $F = 10.67$, $P = 0.031$) and calcium base saturation (tillage type: $F = 22.857$, $P = 0.009$). However, these levels of aluminum (8.33 to 10.00 ppm) are not

expected to impact root and shoot growth in soil pH levels above 5 (51). In addition, past work demonstrates that this range in calcium base saturation (77.67 to 82.00%) does not differentially impact plant growth (52). Taken together, these results suggest that plant growth—in particular, shoot biomass accumulation—is impacted by soil management to a slight degree, and soil physicochemistry varies minimally across treatments in this field site.

Microbial community diversity and composition are significantly altered by management practice, with greater impacts for fungi. To survey the impacts of tillage and cover type on plant-associated bacterial and fungal microbiomes, community composition was investigated for each sample type (soil, rhizosphere, and root) using Illumina MiSeq sequencing of the V3-V4 region of the 16S rRNA gene and 5.85Fun-ITS4Fun region of the internal transcribed spacer 2 (ITS2) gene. We predicted that less mechanical soil disturbance would promote niche differentiation over time, and this would be reflected with higher diversity in samples collected from NT managed fields. Surprisingly, our results indicated that Shannon's diversity is greater in fields managed by ST, versus NT ($P < 0.001$; means, bacteria: 5.61 versus 5.48 and fungi: 2.61 versus 2.26) for soil (both classifications; see Fig. S4a and Table S3a) and rhizosphere (fungi only) (Fig. 1a and Table 1) samples in time point 1. We also observed that tillage type structured the alpha diversity of fungal communities to a greater extent than bacteria ($F = 31$, $P < 0.001$; $F = 13$, $P < 0.001$, respectively), while the factor of cover type was significant for bacteria only ($F = 6$, $P = 0.02$) across all time points (see Table S4a). As predicted, bacterial and fungal Shannon's diversity was generally higher on average with CC for rhizosphere and root-associated communities as well (Fig. 1a and d; see also Fig. S5a and c). Interestingly, in addition to time point, we found that tillage type was highly statistically significant in structuring Shannon's diversity in soils (bacteria: $F = 13$, $P < 0.001$; fungi: $F = 54$, $P < 0.001$) and, to a lesser extent, in rhizospheres (bacteria: $F = 3$, $P = 0.07$, fungi: $F = 53$, $P < 0.001$).Within roots, tillage type was statistically significant only at the after flowering time point (TP2) for fungi ($F = 12$, $P = 0.003$), while bacterial root communities are significantly structured by cover type only ($F = 4$, $P = 0.04$). Taken together, these results demonstrate that tillage and cover type influence the alpha diversity of sorghum-associated microbiomes, with increased Shannon's diversity under ST and CC, and suggest that tillage type shapes fungal soil communities in particular to a much greater degree than bacteria.

Tillage and cover type are expected to influence a number of soil characteristics that can impact microbial fitness (53), including soil pore size, nutrient bioavailability, and moisture. We therefore hypothesized that treatment would significantly structure the beta diversity of hosted microbial communities. In addition, we predicted that tillage type would impact fungi to a greater extent than bacteria, due, in part, to their formation of extensive hyphae networks. To visualize and quantify the differences between microbial communities (beta diversity), we used unconstrained principal coordinate analysis, canonical analysis of principal coordinates (CAPs), and permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis dissimilarity distances. The beta diversity was significantly explained by tillage and cover type ($P < 0.001$) (see Table S4b), and greater variation was attributed to tillage type for fungal communities, versus bacterial communities, for all compartments (soil, rhizosphere, and roots) in preflowering 2016 samples (TP1) (Fig. 1b and e and Table 1; see also Fig. S4b and e and Table S3b). A pattern of greater variation due to tillage type within fungal communities versus bacteria was again observed in postflowering (TP2) rhizospheres and soils and preflowering roots the following year (TP3) (see Fig. S5b and d and S6b and e). In addition, management treatment appears to have had a greater impact during initial stages of rhizosphere colonization, since the variation in beta diversity attributed to tillage and cover type is higher for samples collected at the preflowering time points (Fig. 1b; see also Fig. S4d) compared to the postflowering samples (see Fig. S4b). These analyses further indicate that (i) bacterial and fungal communities are distinctly shaped by soil management type, with greater shifts across tillage type predominantly occurring in fungi, and that (ii) plants likely buffer

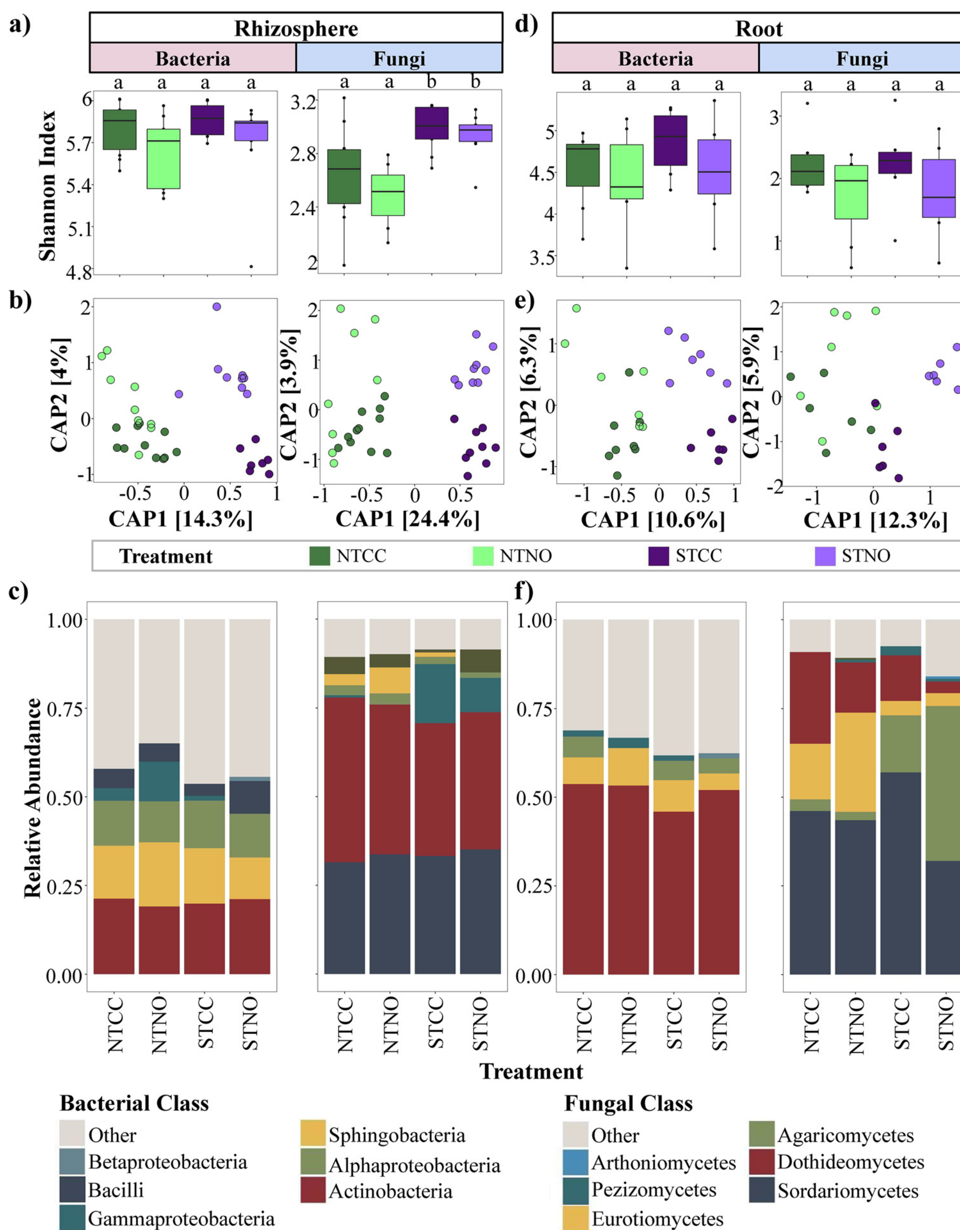

FIG 1 Rhizosphere and root microbial communities are differentially impacted by soil management practice. Amplicon data from time point 1 for bacterial and fungal communities in the rhizosphere (a to c) and root (d to f) are shown. (a and d) Boxplots of Shannon diversity for bacteria and fungi with letters representing statistical significance (analysis of variance, $P < 0.05$) between treatments within each sample type. (b and e) Constrained ordinations for canonical analysis of principal coordinates tillage (*x* axis, CAP1) and cover type (*y* axis, CAP2) with Bray-Curtis dissimilarity for bacteria and fungi. (c and f) Stacked bar plots of the six most abundant classes for bacteria and fungi. Treatments no tillage with cover cropping (NTCC), no tillage with leaving the field fallow during the off-season months (NTNO), standard tillage with cover cropping (STCC), and standard tillage with leaving the field fallow (STNO) are represented with dark green, light green, dark purple, and light purple, respectively, in panels a, b, d, and e.

community shifts, with the strongest response to soil management type found in soils and then the rhizosphere and roots.

We further analyzed community composition to determine whether certain taxonomic lineages demonstrate a preference for any of the four management practices. In addition to detectable shifts in class-level relative abundances for both bacteria and fungi between soil management types within each sample type (soil, rhizosphere, and

TABLE 1 Tillage and cover type significantly structure alpha and beta diversity for a number of compartments (rhizosphere and root)[a]

		16S							ITS2						
Sample type	Factor	Df	Sum Sq	Mean Sq	F	R^2	P	S	Df	Sum Sq	Mean Sq	F	R^2	P	S
Shannon index															
Rhizosphere	Tillage_Type	1	0.011	0.011	0.205		0.655		1	1.348	1.348	20.490		0.000	***
	Cover_Type	1	0.219	0.218	4.092		0.054	•	1	0.135	0.135	2.056		0.162	
	Block	2	0.227	0.114	2.126		0.141		1	0.036	0.036	0.539		0.469	
	Tillage_Type:Cover_Type	1	0.006	0.006	0.110		0.743		1	0.024	0.024	0.364		0.551	
	Tillage_Type:Block	2	0.023	0.011	0.212		0.810		1	0.025	0.025	0.375		0.545	
	Cover_Type:Block	2	0.311	0.155	2.910		0.074	•	1	0.002	0.002	0.031		0.862	
	Tillage_Type:Cover_Type:Block	2	0.011	0.006	0.106		0.900		1	0.059	0.059	0.903		0.350	
	Residuals	24	1.281	0.053					29	1.908	0.066				
Root	Tillage_Type	1	0.273	0.273	1.486		0.241		1	0.005	0.005	0.011		0.919	
	Cover_Type	1	0.371	0.371	2.015		0.175		1	1.502	1.502	2.999		0.101	
	Block	2	1.883	0.941	5.120		0.019	*	1	0.009	0.009	0.018		0.894	
	Tillage_Type:Cover_Type	1	0.013	0.013	0.068		0.797		1	0.007	0.007	0.013		0.910	
	Tillage_Type:Block	2	0.594	0.297	1.616		0.230		1	0.015	0.015	0.031		0.863	
	Cover_Type:Block	2	0.456	0.228	1.241		0.315		1	0.257	0.257	0.513		0.484	
	Tillage_Type:Cover_Type:Block	2	0.662	0.331	1.801		0.197		1	1.275	1.275	2.546		0.129	
	Residuals	16	2.942	0.184					17	8.513	0.501				
Bray-Curtis															
Rhizosphere	Tillage_Type	1	0.511	0.511	6.672	0.138	0.001	***	1	1.265	1.265	12.663	0.245	0.001	***
	Cover_Type	1	0.162	0.162	2.120	0.044	0.003	**	1	0.216	0.216	2.160	0.042	0.036	*
	Block	2	0.307	0.153	2.002	0.083	0.001	***	1	0.127	0.127	1.274	0.025	0.226	
	Tillage_Type:Cover_Type	1	0.171	0.171	2.233	0.046	0.002	**	1	0.186	0.186	1.862	0.036	0.056	•
	Tillage_Type:Block	2	0.208	0.104	1.356	0.056	0.084	•	1	0.096	0.096	0.962	0.019	0.431	
	Cover_Type:Block	2	0.270	0.135	1.763	0.073	0.003	**	1	0.208	0.208	2.079	0.040	0.041	*
	Tillage_Type:Cover_Type: Block	2	0.230	0.115	1.498	0.062	0.037	*	1	0.172	0.172	1.725	0.033	0.096	•
	Residuals	24	1.839	0.077		0.497			29	2.896	0.100		0.561		
	Total	35	3.697			1.000			36	5.166			1.000		
Root	Tillage_Type	1	0.311	0.311	4.086	0.104	0.001	***	1	0.968	0.968	3.488	0.116	0.001	***
	Cover_Type	1	0.193	0.193	2.538	0.065	0.005	**	1	0.575	0.575	2.071	0.069	0.006	**
	Block	2	0.507	0.254	3.334	0.170	0.001	***	1	0.389	0.389	1.403	0.046	0.126	
	Tillage_Type:Cover_Type	1	0.133	0.133	1.747	0.045	0.048	*	1	0.423	0.422	1.523	0.050	0.094	•
	Tillage_Type:Block	2	0.168	0.084	1.106	0.057	0.290		1	0.343	0.343	1.237	0.041	0.214	
	Cover_Type:Block	2	0.275	0.137	1.807	0.092	0.013	*	1	0.379	0.379	1.366	0.045	0.120	
	Tillage_Type:Cover_Type: Block	2	0.173	0.087	1.138	0.058	0.269		1	0.581	0.581	2.095	0.069	0.007	**
	Residuals	16	1.217	0.076		0.409			17	4.717	0.277		0.563		
	Total	27	2.976			1.000			24	8.375			1.000		

[a]PERMANOVA results conducted for Shannon index (top part of table) and Bray-Curtis dissimilarity (bottom part of table) with bacterial (16S) and fungal (ITS2) amplicon sequencing data for time point 1. Abbreviations: Df, degrees of freedom in the factor; Sum Sq, sum of squares due to the factor; Mean Sq, mean sum of squares due to the factor; F, F-statistic value; *P*, *P* value; S, significance (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; •, $P < 0.10$).

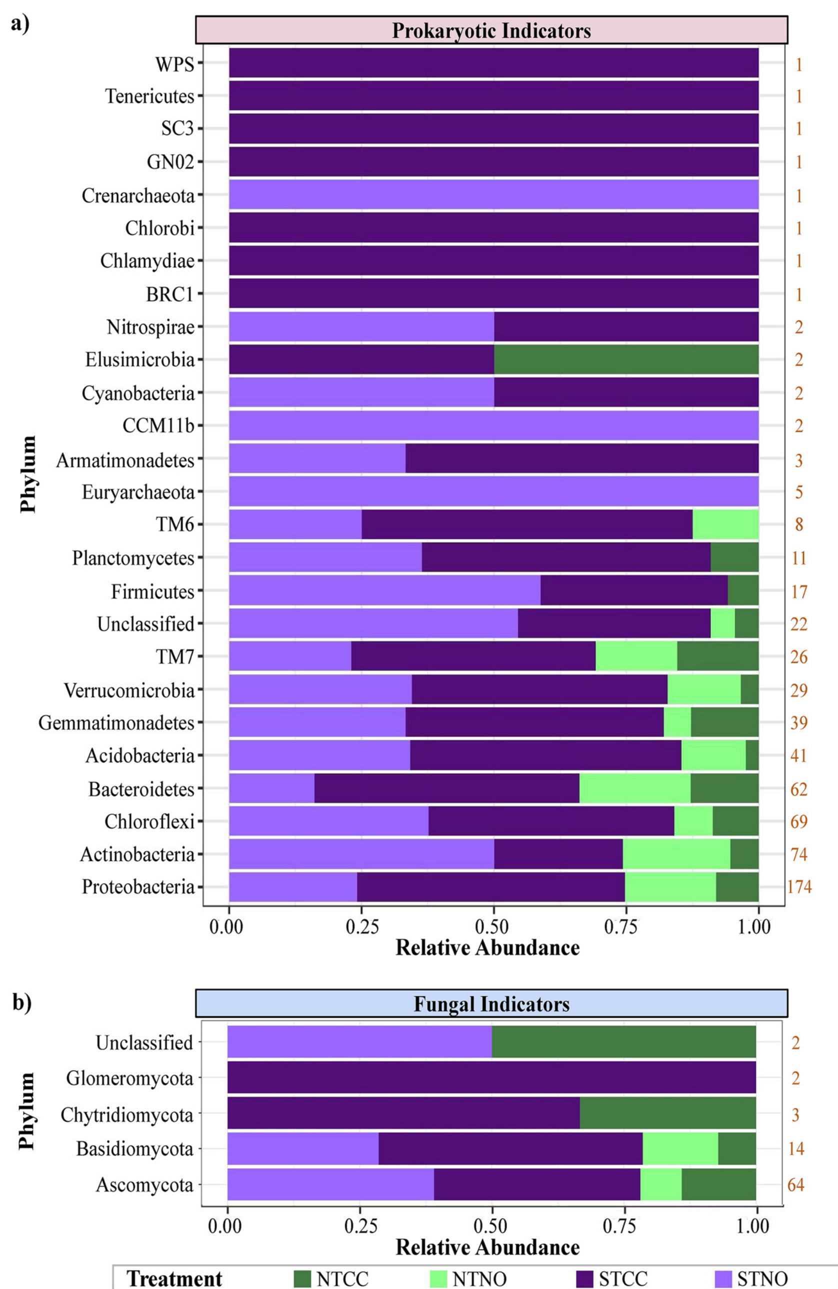

FIG 2 Soil management indicators partially cluster by phylogeny and correspond most to standard tillage treatments. (a and b) Relative abundance plots of prokaryotic (a) and fungal (b) OTUs that are indicator species ($P \leq 0.001$) for treatment type of no tillage with cover cropping (NTCC, dark green), no tillage with leaving the field fallow during the off-season months (NTNO, light green), standard tillage with cover cropping (STCC, dark purple), or standard tillage with leaving the field fallow (STNO, light purple). The total number of indicators is indicated to the right of the plots in orange.

roots) (Fig. 1c and f; see also Fig. S6 and S7), we found that the relative abundance of fungi shifted to a greater extent than bacteria across soil management type in preflowering 2016 roots in particular (Fig. 1f). Observable shifts across treatment included a greater overall prevalence of *Bacilli* with NO (Fig. 1c; see also Fig. S4c and 6c) and *Gammaproteobacteria* with NT (Fig. 1c and f; see also Fig. S6f and S7a and b), in addition to a greater relative abundance of *Agaricomycetes* with ST in preflowering root fungal communities (Fig. 1f; see also Fig. S7b). In addition, *Eurotiomycetes* and *Dothideomycetes*,

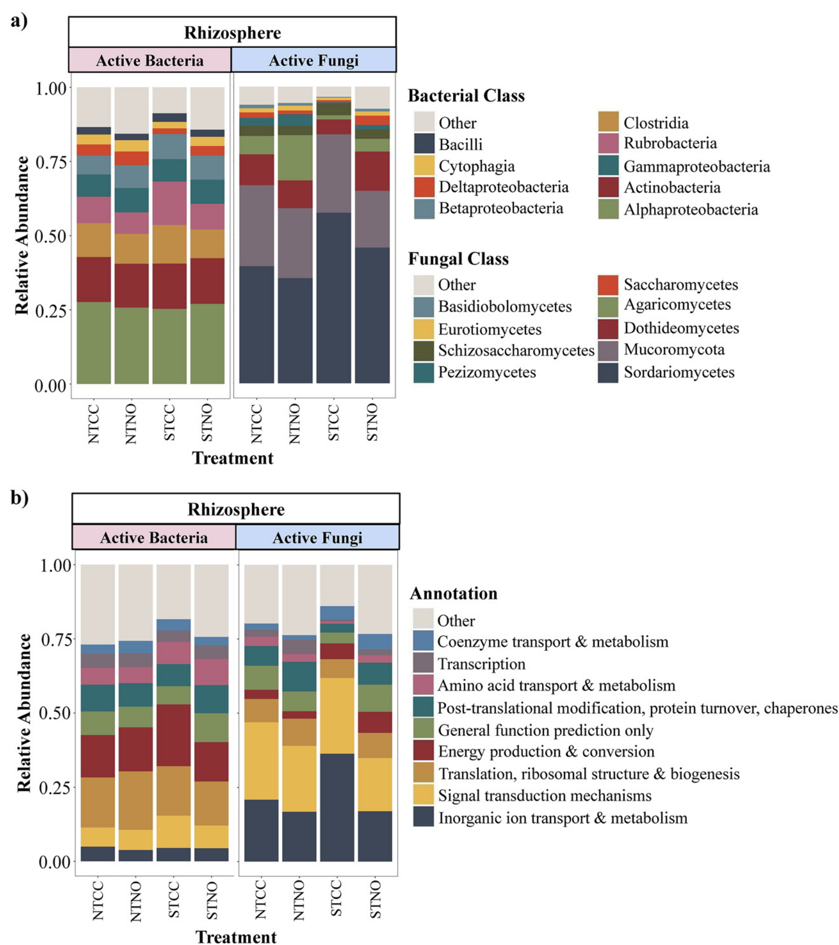

FIG 3 Soil and rhizosphere microbial activity varies with soil management practice. (a and b) Stacked bar plots of the relative abundances of the top nine most active bacterial and fungal classes (a), calculated as the sum of all transcripts corresponding to each class, and functions (b), as annotated and grouped by COG (Clusters of Orthologous Groups of proteins) category, in sorghum rhizosphere samples collected during time point 3 meta-transcriptome sampling. Treatment corresponds to no tillage with cover cropping (NTCC), no tillage with leaving the field fallow during the off-season months (NTNO), standard tillage with cover cropping (STCC), and standard tillage with leaving the field fallow (STNO).

when present, were generally at higher relative abundances in samples from NT plots (Fig. 1c and f; see also Fig. S4, S6c, and S7), with the exception of time point 3 soils. To further explore taxonomic patterns, we performed indicator species analyses ($P \leq 0.001$) across all sample types to determine whether certain operational taxonomic units (OTUs) correlated with a specific tillage (NT and ST) and cover (CC and NO) type (see Fig. S8 to S10), as well as treatment (NTCC, NTNO, STCC, and STNO) (Fig. 2; see also Fig. S9 and S10). For both microbial classifications, we observed that a greater number of indicators are present in ST over NT (bacteria: 539 versus 350; fungi: 50 versus 36) and CC over NO (bacteria: 77 versus 34; fungi: 21 versus 7) (see Fig. S8). Interestingly, the vast majority of bacterial phyla consisted of indicators largely specific to STCC and STNO treatment, including *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* (Fig. 2a). For fungi, the majority of indicator OTUs were in the phylum Ascomycota (Fig. 2b), and treatment indicators appeared to phylogenetically cluster (see Fig. S10). Taken together, these results indicate that (i) management impacts plant-associated bacterial and fungal community composition on a class level, with greater community shifts attributed to changes in tillage versus cover type, (ii) ST may support greater diversification than NT, and (iii) fungal communities demonstrate an overall heightened sensitivity to soil management practice than bacteria.

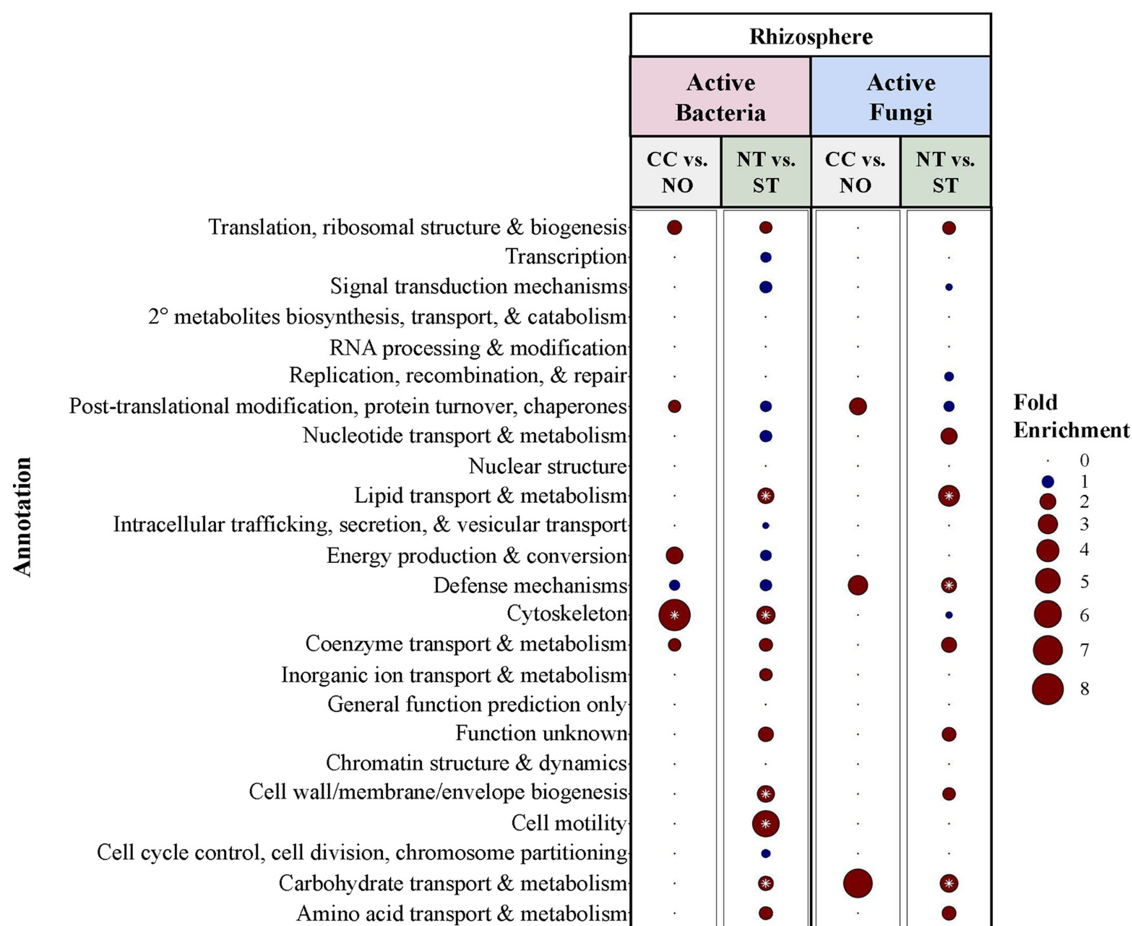

FIG 4 Soil management type enriches for numerous functions in rhizosphere bacterial and fungal communities. COG category enrichment analysis of differentially expressed bacterial and fungal genes was individually performed for rhizosphere samples to determine what genes are enriched with cover cropping in comparison to when the field is left fallow (CC versus NO) and with no tillage compared to standard tillage (NT versus ST). The size of the circle represents the degree of fold enrichment, which is calculated as the percentage of total differentially expressed genes (DEGs) that have a significant ($P \leq 0.01$) positive $\log_2$ fold change in a given COG category out of all DEGs, divided by the percentage of total genes in that COG category. The color of the circle represents whether the fold enrichment is ≤1 (blue) or >1 (red), and a white asterisk within the circle represents a statistically significant ($P \leq 0.05$) fold enrichment, as determined by a hypergeometric test.

Microbial activity varies across soil practices, with the greatest changes observed between tillage types and within fungal communities. Changes in community composition determined by amplicon sequencing may, or may not, reflect changes in the active fraction of the microbiome. In order to evaluate treatment effects on the portion of the microbial community that is active, we sequenced and annotated via Joint Genome Institute's Integrated Microbial Genomes & Microbiomes Expert Review (IMG/MER) system the metatranscriptomes of soil and rhizosphere communities at time point 3, corresponding to a vegetative stage of rapid growth in sorghum development. Since NT retains past crop residues and ST is a major soil disturbance, we hypothesized that management practice would correspond to distinct functional changes between samples. To first broadly survey impacts on the active microbial community, we investigated bacterial and fungal beta diversity, as determined by Bray-Curtis dissimilarity (see Fig. S11). We found that tillage type was a significant factor for both classifications within soil and rhizosphere ($P \leq 0.045$) (see Table S5), where it contributed to 29.1% (bacteria) and 36.3% (fungi) of beta diversity variation in soils and 15% (bacteria) and 19.8% (fungi) in rhizospheres (see Fig. S11). Cover type was borderline significant in the soil only (bacteria: P value = 0.061; fungi: P = 0.072) (see Table S5), correlating to 13.4% (bacteria) and 12.7% (fungi) of variation in beta diversity (see Fig.

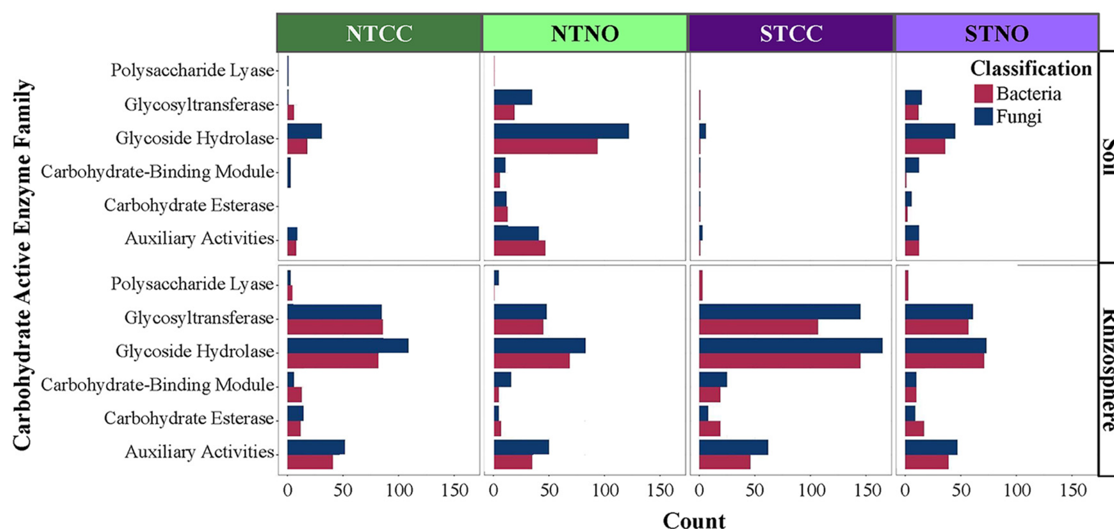

FIG 5 Soil management regimes differentially alter carbon cycling dynamics. Bar plots depict the number of transcripts for carbohydrate active enzyme (CAZy) genes attributed to bacteria (pink) and fungi (blue) in the soil and rhizosphere across treatments, in the following family classifications: polysaccharide lyases, glycosyltransferases, glycoside hydrolases, carbohydrate-binding modules, carbohydrate esterases, and auxiliary activities. Treatments include the following: no tillage with cover cropping (NTCC, dark green), no tillage with leaving the field fallow during the off-season months (NTNO, light green), standard tillage with cover cropping (STCC, dark purple), or standard tillage with leaving the field fallow (STNO, light purple).

S11). These analyses indicate that treatment affects active soil and rhizosphere communities and tillage type affects diversity to a greater extent in fungal communities than for bacteria, as was observed in our amplicon sequencing results.

Within both soil and rhizospheres, we observed treatment responses in the class-level relative abundances of active microbes (Fig. 3a; see also Fig. S12a), as well as in relative functional activity, as described using the Clusters of Orthologous Groups of proteins (COG) database (54) (Fig. 3b; see also Fig. S12b). Bacterial community activity is largely attributed to the classes *Alphaproteobacteria* and *Actinobacteria* (Fig. 3a; see also Fig. S12a), and we detected several classes in the rhizosphere that vary across treatment, including STCC hosting the highest relative levels of rubrobacterial and clostridial activity (Fig. 3a). Similar to our amplicon data results, we also found evidence of a greater response to treatment in active fungal classes, compared to bacterial classes, in both sample types (Fig. 3a; see also Fig. S12a). In particular, NT hosted greater relative activity by classes *Agaricomycetes*, *Pezizomycetes*, and *Basidiobolomycetes* than ST (Fig. 3a; see also Fig. S12a). While bacterial activity on the level of COG category varied somewhat across treatments, we observed that ST fungal communities harbored relatively greater levels of energy production and conversion and coenzyme transport and metabolism, and NT activity profiles were characterized by relatively higher transcription, amino acid transport and metabolism, and posttranslational modification, protein turnover, and chaperone activity (Fig. 3b; see also Fig. S12b). CC also correlated with relatively higher levels of fungal inorganic ion transport and metabolism in the rhizosphere (Fig. 3b). Unsurprisingly, the greatest shifts in activity were between sample types, with the following COG categories more represented in the rhizosphere: energy production and conversion (for bacteria), translation, ribosomal structure, and biogenesis (bacteria), inorganic ion transport and metabolism (fungi), and signal transduction mechanisms (both) (Fig. 3b; see also Fig. S12b). These analyses reveal that soil management not only has distinct impacts on what taxonomic groups are active in the soil and rhizosphere, but also affects the relative levels of activity occurring in bacterial and fungal communities; furthermore, metatranscriptome analyses demonstrate heightened responses to treatment within the fungal microbiome, compared to bacteria.

To further characterize treatment impacts on microbial communities and determine

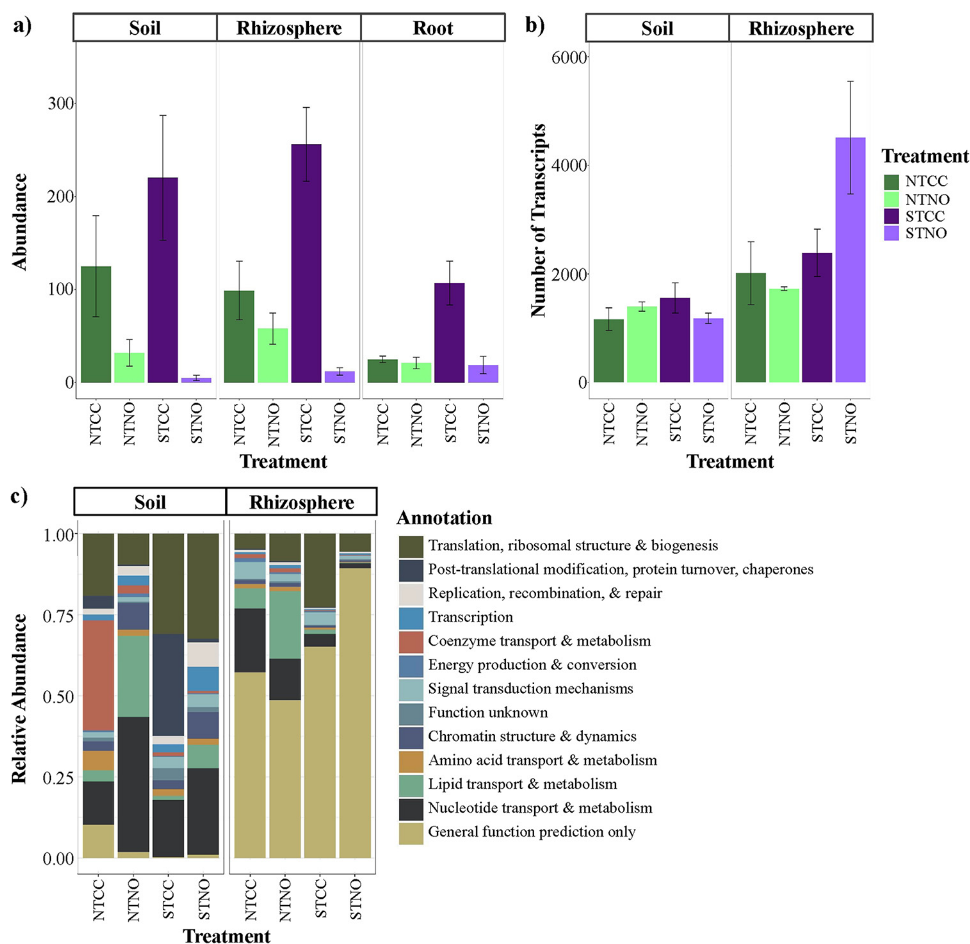

FIG 6 Abundances and activity of agriculturally important fungal symbionts vary across soil management regimes. (a) Bar plot showing total counts of arbuscular mycorrhizal fungi (phylum Glomeromycota) OTUs from amplicon data across all time points. (b) Bar plot showing the total number of transcripts corresponding to arbuscular mycorrhizal fungi from metatranscriptomic data collected in time point 3. (c) Stacked bar plot of the relative abundances of all COG categories attributed to metatranscriptomic data from Glomeromycota. Treatments included the following: no tillage with cover cropping (NTCC, dark green), no tillage with leaving the field fallow during the off-season months (NTNO, light green), standard tillage with cover cropping (STCC, dark purple), and standard tillage with leaving the field fallow (STNO, light purple).

whether treatment significantly selects for specific functions, we analyzed the fold enrichment (Fig. 4; see also Fig. S13) and depletion (see Fig. S14) of genes from NT versus ST, as well as CC versus NO, in soil and rhizosphere samples. As anticipated, tillage type impacted a broader range of functions than cover type; however, the functions that are impacted by cover type are generally enriched and depleted to a higher fold degree than those for tillage type (Fig. 4; see also Fig. S13 and S14). In addition, bacterial and fungal communities responded to treatment with significant fold changes in largely the same COG categories; for instance, both classifications are enriched under NT for energy production and conversion in soils (see Fig. S13) and carbohydrate and lipid transport and metabolism in rhizospheres (Fig. 4). In addition, within several COG categories a set of genes was significantly enriched, while a different set was significantly depleted (data not shown), including (i) cell motility, (ii) cell wall, cell membrane, and cell envelope biogenesis, and (iii) translation, ribosomal structure and biogenesis. These findings indicate that NT and—to a lesser degree—CC significantly impact a range of functions in bacterial and fungal communities. Interestingly, gene activity may also fluctuate considerably more for bacteria than fungi, where a greater number of statistically significant fold changes occur under NT and CC management types;

however, this may be due to how a greater number of sample reads originate from bacteria versus fungi.

Furthermore, we performed an indicator species analysis ($P \leq 0.05$) on metatranscriptomes to determine whether particular microbial functions uniquely characterize the soil and rhizosphere from plots managed by NT, ST, CC, and/or NO (see Fig. S15 to S18). Similar to our amplicon data analyses, we observed more indicators for ST (including STCC and STNO) versus NT (including NTCC and NTNO), as well as for NO over CC, treatments (see Fig. S15 to S18). Interestingly, the majority of indicators did not have a COG annotation, and we found no specific treatment-based patterns in function categories (see Fig. S16 and S18). When we explored which COG-annotated indicators arise in cover type, however, NO indicators were present to a greater degree than those for CC and comprise a range of activity, including genes in the COG categories: carbohydrate and amino acid transport and metabolism (see Fig. S16c and S18c). Together, these analyses further demonstrate that soil management uniquely affects microbial gene activity, as well as suggest that ST and NO practices correlate to a greater extent to distinct gene activity than NT and CC, where a larger number of indicator genes in a greater number of COG categories correlate with ST and NO. In addition, our findings suggest that a broader range of unique activities may be affected by soil treatment in bacterial versus fungal communities, although this may be explained by how a greater number of sample reads originate from bacteria than fungi.

Patterns of microbially driven carbon cycling vary across soil management types. In order to test our hypothesis that ST practices of shredding crop residue to incorporate into soils would significantly alter microbial community dynamics in relation to carbon cycling, we performed an analysis of transcripts corresponding to carbohydrate active enzyme (CAZy) genes (Fig. 5). While it was unsurprising to find that rhizospheres correlated with a relatively greater level of transcripts for CAZy genes than soils, we curiously discovered that NO systems corresponded to more CAZy gene transcriptional activity than CC in soils and less in rhizospheres (Fig. 5). Interestingly, we also detected relatively similar amounts of CAZy gene transcriptional activity for bacteria and fungi within treatments and sample types (Fig. 5), though more sample reads were of bacterial origin than fungal (see Table S6). The largest CAZy transcript-based responses to treatment were found in two families, glycosyltransferases and glycoside hydrolases (Fig. 5), which are involved in the biosynthesis of sugar polymers (disaccharides, oligosaccharides, and polysaccharides) and the hydrolysis of glycosidic bonds in polysaccharides (e.g., cellulose, hemicellulose, pectin, and chitin), respectively (55). Based on past work (56), we also analyzed the putative substrates for the CAZy genes detected on a transcript level (see Fig. S19) and found similar patterns across treatments and sample types. Furthermore, compared to other soils, NTNO soils appeared particularly active in degrading a wider variety of carbon sources, including xylan, oligosaccharides, glycogen, lignin, and cellulose (see Fig. S19). These analyses suggest that microbes in fields managed with CC are primed for plant-based carbon usage in the rhizosphere, whereas NO supports microbial communities that utilize a greater diversity of carbon substrates in soils, which may have implications for soil carbon sequestration.

Collectively, these results demonstrate that agricultural practices of NT, ST, CC, and NO impact bacterial and fungal community activity, and these shifts are demonstrated not only in the soil communities, but in sorghum rhizospheres as well. Our data demonstrate shifts in taxonomic groups and a suite of COG categories and transcripts of CAZy genes across soil management types. Furthermore, tillage type significantly impacts a greater number of functions than cover type, and bacterial and fungal communities have slightly different enriched and depleted COG categories with NT, versus ST, as well as with CC versus NO. Lastly, transcript levels of microbial CAZy genes vary across soil management treatment, with opposite patterns occurring in rhizospheres versus soils for overall activity amounts associated with tillage and cover type, which may be indicative of further differences in microbial community specialization to the varying soil environment.

Arbuscular mycorrhizal fungi differentially respond to soil management type. Some soil practices can prime soils for plant-microbe mutualisms (57). In order to determine how soil management regime may impact plant-microbe interactions known to promote plant health, we compared the amount and activity of AMF across management treatments from amplicon and metatranscriptomic data (Fig. 6). We observed a greater abundance of AMF with CC, and we also surprisingly found that AMF were most prevalent in samples collected from plots managed with STCC (Fig. 6a). Furthermore, while AMF were least prevalent in STNO samples (Fig. 6a), STNO corresponded with the most overall AMF activity (Fig. 6b). Functional profiles also varied widely across treatments and sample types, where AMF activity was characterized by the greatest relative levels of (i) coenzyme transport and metabolism in both soils and rhizospheres managed with NT, (ii) lipid and nucleotide transport and metabolism in NTNO soils and rhizospheres, and (iii) translation, ribosomal structure, and biogenesis in ST soils and STCC rhizospheres (Fig. 6c). Taken together, these results show evidence that soil management practices greatly influence the abundance and activity of one of the most well-known fungal symbionts of agricultural importance (58).

DISCUSSION

Our study provides an initial look at how the agricultural soil management practices of standard and no tillage, in combination with cover cropping and leaving fields fallow, shape the assembly and activity of crop-associated bacterial and fungal communities during sorghum development. Past work suggests that microbial communities are influenced by soil management, where microbial diversity can increase with reduced tillage (59–61) and CC (61, 62), and CC can promote microbial abundance (59, 63), but combined practice impacts on the microbiome are not well characterized and understood. We hypothesized that we would find reduced fungal and bacteria diversity with ST, reasoning that NT could allow for the construction of distinct niches for colonization by distinct groups of microorganisms over time. Surprisingly, we found that ST was associated with greater diversity for the majority of sample types and time points, as well as larger numbers of indicator OTUs. To our knowledge, only one other study has reported that intensive tillage is associated with increased bacterial and fungal diversity compared to reduced- and no-tillage regimes (48). A possible explanation for this increased diversity with ST may involve the impacts of disturbance, where tillage may increase nutrient availability and open niches for colonization that may otherwise have been inaccessible due to competitive exclusion (64, 65). Furthermore, disturbance has been shown to be an important driver in endophyte community variation (66) and to influence microbial diversity (67). As such, ST here may present an intermediate level of disturbance that may support a greater variety of specialist and generalist microbes (64, 68). Past studies do suggest that disturbance can lead to fungal or bacterial community shifts in host niches, where antibiotic use has been associated with overgrowth of the fungus *Candida albicans* in animal guts and root-associated fungi were detrimental to plant hosts in the absence of commensal bacterial (reviewed by Getzke et al. [43]). Similar results have been observed in other systems as well: one study found increased microbial diversity with disturbance in marine sediments (69), and combinations of temperature and physical disturbance have been correlated with increased coral microbiome diversity (70). Others have observed that the adaptive diversification of microbial communities was significantly greater in the absence of an established community, which NT systems may engender (71). We recommend that future studies sample a range of time points post-tillage to help further our understanding of how ST systems impact diversity and what factors microbial diversification is dependent on. In addition, as increased diversity has been shown to promote community recovery after environmental disturbance (72), we recommend more studies to test whether this increased diversity in managed soil is beneficial to plant yield and growth under various climate and nutrient conditions, as well as explore what costs to host fitness may

occur with increased diversity, in order to better understand how resident diversity may inhibit or promote resilience in the face of changing environmental conditions.

Several other factors may explain, in part, this response in microbial diversity. One factor is sampling depth. Recent work on the soil microbiome shows diversity increases under NT with increasing soil depth (59, 73, 74), and soil management types can favor distinct microbial life strategies (59). For example, distinct bacterial communities and differing dominant taxa have been found in no-tillage systems across depths up to 100 cm from the soil surface (74). Soil fungal communities in no-tillage systems have also been shown to be highly stratified across depths of 0 to 100 cm, with lower diversity and a greater proportion of pathotrophic or symbiotrophic taxa, in addition to saprotrophic, at greater depths (73). For these reasons, future efforts in this area should consider, including depth as an additional experimental parameter. Another factor that may influence patterns drawn from community characterization is the taxonomic level at which analyses are performed. One study that explicitly explored this found increased microbial diversity in no-tillage systems only when looking beyond the phylum level (34), and future studies may consider a finer scale and a wider range of analyses (75), including indicator species and/or functional guild analyses. Lastly, one additional factor that may potentially contribute to reduced microbial diversity in NT systems may relate to how soils managed by NT are often wetter than those managed by ST (76); wetness can reduce the number of microaggregates and therefore microhabitats that are conducive to the establishment and maintenance of microbial diversity (77).

There is evidence that reduced and no tillage can improve carbon sequestration and reduce atmospheric carbon release compared to ST (78–81). We thus hypothesized that samples from plots under NT management would support a distinct functional profile that would include smaller amounts of carbon cycling than with ST, due, in part, to greater amounts of more intact past crop residue in NT plots that, consequently, may require a longer time frame to break down (82). In addition, we sampled to depths of 15 cm, and while some redistribution of nutrients does occur with earthworm populations (83, 84), a significant amount of crop residue in NT plots is generally localized to the soil surface (85). Consequently, microbial activity was likely stratified and the overall signal from any one layer reduced with our sampling method. We further hypothesized that CC would also correspond to a functional profile that differs from NO due to an enrichment of plant-associated microbes and evidence that CC favors moderately fast-growing microbes with a relatively high metabolic range (59). Cover crops have been found to increase plant and microbial biomass and alter soil microbiomes (86–90), due, in part, to increased inputs of organic carbon from exudates and litter, as well as influences on soil aggregation and moisture (91, 92). Indeed, our results demonstrate that each treatment type corresponds to a distinct functional profile, which corroborates prior work (62), in which agricultural soil management practices shifted fungal functional composition, with no tillage corresponding to increased relative proportions of symbiotrophs, whereas saprotrophs decreased. However, another study found similar microbial activity in no-tillage and conventionally tilled soils (93). We recommend that additional studies in different fields and across time points also consider collecting metatranscriptomic analysis to better understand how microbial activity is influenced by soil management. Furthermore, we found that treatment correlates with much greater changes in function than composition, highlighting both the importance of metatranscriptomic analysis to better characterize microbiome responses to the environment and that amplicon data alone is not sufficient in determining whether, and to what degree, a microbiome is changing in response to a disturbance.

With NT often employed with the aims of improving soil health, we further investigated how soil management impacted carbon cycling dynamics using the CAZy database. Carbohydrate active enzyme classes glycosyltransferase and glycoside hydrolase (involved with the making and breaking of glycosidic bonds, respectively) were present

at high abundances for all treatments and are important for energy mobilization, defense, signaling, symbiosis, and secondary plant metabolism (94). While minimal activity was detected in soils with CC, we surprisingly found high CAZy gene levels characterize NO treatments, with the highest levels from NTNO samples. This may be due to selective pressures in NTNO soils that favor the prolonged breakdown of complex carbohydrates in soil residue as a primary energy source, and we suggest more studies to determine the implications on overall carbon sequestration ability. In rhizospheres, CC corresponded to higher levels of carbohydrate-related activity than NO, with the highest in STCC, suggesting that CC selects for microbes that readily utilize plant-based photosynthates and STCC systems may be sourced with more available carbon from both the living plant and the shredded past crop residue. Future work may include metabolomics and radiolabeled substrate to determine how carbon break down and usage may differ across management treatments.

Evidence from recent studies suggests that bacterial and fungal communities differ in sensitivity to environmental parameters (48, 95, 96). Given that (i) tillage is a major physical disturbance, (ii) many soil fungi grow in extended hyphal networks across considerable distances (97), and (iii) fungi are considered dominant decomposers of crop residue (98), which is present to a greater extent in NT and CC systems, we hypothesized that fungal communities would be altered to a greater extent than bacteria by implemented soil management practices. As predicted, we found that fungal communities did shift across soil management to a greater extent than bacteria. Specifically, tillage type (NT and ST) was more highly attributed to impacting beta diversity in fungal communities than bacteria. In addition, management type (NTCC, NTNO, STCC, and STNO) corresponded to greater shifts in relative abundances for fungal taxonomic and functional categories than bacteria. This included a shift of at least 20% between management types for all six of the top six most abundant fungal classes (versus the five for the top bacterial classes) and STCC being represented by twofold more relative amounts of inorganic ion transport and metabolism activity than any other management type in fungal communities, a magnitude of change not observed in bacterial communities for any category. Furthermore, many fungal community members at high taxonomic levels responded similarly to tillage type, suggesting that tilling structures communities by acting on traits broadly shared within the classification, while bacteria are less sensitive, more resilient, and/or inhabit more diverse niche spaces than fungal communities. Our findings contradict other studies that compare bacterial and fungal responses to agricultural soil management using amplicon sequencing, where it was observed that the bacterial communities of soil and wheat roots are more sensitive than fungi to tillage type (48, 49, 59). Many more studies have explored soil management effects on soil bacteria and fungi with regard to biomass, but reported conflicting results; some observed increased ratios of fungal to bacterial biomass in no-tillage systems (99–101), while others found either decreased ratios or no changes at all in fungal/bacterial biomass ratios (33, 102–104). In addition, other studies have found an increase or no change in fungal (105, 106) and bacterial (87) diversity with no tillage. These differences could partly be explained by a range in sampling locations and times throughout the year, which can correspond to various climatic factors, soil moisture levels, and chemical and physical soil characteristics—all of which may influence associated community dynamics (102, 107). There is also evidence that length of time from when the practice of no tillage is first adopted, to date of sampling, can also influence results, where it can take several years to see a stabilization in soil parameters and improvement in plant yield (12). When analyzing the active community, we similarly discovered that the activity and composition of fungal communities shifts to a greater extent than bacterial communities across treatments. Fungi have been shown to have higher carbon/nitrogen ratios (108), slower biomass turnover (109), and broader enzymatic capabilities at large (110) than bacteria, and there is some evidence showing that differences between bacterial and fungal physiology may result in large scale impacts on carbon cycling (111–114). Despite broad-level differences between

bacterial and fungal activity across treatment, we detected a similar number of CAZy transcripts for fungi and bacteria, suggesting that both classifications may have a similar level of carbon usage in soils and rhizospheres at the sampled time point. This joins other evidence of some functional redundancy across microbial classifications regarding the breakdown of organic matter (115). We recommend that future studies take into account multikingdom responses to agricultural soil management practices and consider sampling at multiple time points spanning multiple years. Inclusion of such design elements would help advance our understandings of how microbial dynamics vary over time with these management practices and in accordance with important stages of plant development, as well as how crop-associated microbial communities can be modulated by soil management for climate-smart agriculture (116) and promote plant growth and yield.

We predicted an enrichment for AMF with CC, compared to fallow treatments, due to CC increasing selection pressure for plant-associated microbes and providing continuous photosynthate energy resources in the off season, where cover crops have been shown to have species-specific effects on microbial communities (89) and can promote AMF associations (40, 117–120). CC appeared to make the most difference in overall AMF abundance when fields were also managed with ST, suggesting that STCC management may be more conducive to AMF associations. This may be due to less competition with other mutualists, where AMF species have been shown to compete for root colonization, with interspecies impacts on plant growth (121–123). Interestingly, relative activity of AMF varied significantly across treatments in both the soil and rhizosphere, suggesting different roles are being played for the host, depending on proximity (from soil to rhizosphere). Future work exploring how soil treatment impacts the performance and proliferation of other plant-growth-promoting microbes, including those introduced by a microbial biostimulant, is suggested, in order to determine whether certain soil practices and communities can promote mutualisms.

Lastly, all four soil management treatments supported surprisingly similar soil chemistry profiles and plant growth and yield phenotypes, although their associated microbial communities significantly varied. We found that microbial activity varied across treatment as well, and the increased relative abundances of various COG categories, including inorganic ion and coenzyme transport and metabolism, with tillage may relate to a greater prevalence of macro- and micronutrients. Although the slight variation in soil chemistry that we detected across treatments unlikely contributes to major shifts in microbial diversity (124, 125), nutrient additions (in particular, nitrogen and phosphorus) are known to significantly impact bacterial and fungal community composition in a variety of soils (124–126). Shifts in microbial life history strategies (copiotroph/oligotroph ratios; competitors, stress tolerators, ruderals) have also been reported in investigations of NT and ST soils (16, 73, 74, 127), as well as with specific nutrient additions to soil (124). There is evidence of certain soil parameters increasing under NT, compared to tillage, including percent organic matter, soil pH, exchangeable calcium, and available phosphorus (12, 128–130). The extent of these shifts can vary considerably across studies, however, and discrepancies likely correspond to differences in sampling and quantification methodologies, as well as soil characteristics like mineralogy (12, 131). Variability may also relate specifically to soil microbial metabolic activity, where higher levels of various enzymes, including phosphatase, have been found in no tillage systems (132). Conditions such as residue quantity and quality, as well as the environment, have been shown to influence the functional diversity of soil microbial communities (133). Furthermore, certain soil factors regulate microbial community activity to a greater degree than others, where, for instance, aluminum and pH levels considerably shape microbial carbon usage (134). In order to better understand how microbial composition and metabolic processes shape the biogeochemistry of agroecosystems, we suggest that soil chemistry is monitored long term and is related to ongoing microbial activity at several depths to the surface. In particular, past studies using the same field site has shown redistribution of nutrients to the soil surface layer

(0 to 5 cm) with no tillage, and at greater depths, significant differences between no tillage and standard tillage are not seen (129, 130). In addition, we recommend that future work investigates the mechanisms at play in providing plant benefits in lower diversity systems, compared to higher diversity systems, as could be achieved with transcriptomics in plants and metabolomics. Lastly, determining the rate of host colonization in different management regimes, by utilizing a series of time points earlier in and across plant development, would add to our understanding of microbial agroecology and how farming practices influence plant-microbe interactions. This study would also help determine whether host colonization is slower in NT systems, particularly with CC, with the potentially increased presence of more competitors in the microbial community.

Conclusion. Utilizing the ability of microbial assemblages to promote plant growth is one promising means of improving crop performance and has attracted interest in both academia and industry. Despite the importance of the plant microbiome in promoting plant yield and numerous studies demonstrating that the plant microbiome is influenced by the local soil community, not much is known how current agricultural soil management practices influence the assembly and activity of plant-associated microbial communities. Here, we employed 16S rRNA gene and ITS2 amplicon sequencing and metatranscriptomics to characterize bacterial and fungal communities in sorghum roots and rhizospheres and soils managed by standard and no tilling, in conjunction with cover cropping and leaving a field fallow during the off-season months. We observed that standard tilling and cover cropping correlated with increased microbial diversity, fungi were more sensitive than bacteria to tillage type, as evidenced by shifts in composition and activity, and the activity and association of arbuscular mycorrhizal fungi varied with treatment type. This study furthers our understanding of how microbial communities respond to soil management practices and provides direction for how we might better optimize soil environments for beneficial plant-microbe interactions.

MATERIALS AND METHODS

Field experimental design. This study was conducted at a 3.6 ha field site maintained by the University of California West Side Research and Extension Center in Five Points, California (36°20′29″N, 120°7′14″W), in which plots have been managed by standard (ST) and no-tilling (NT) practices from 1999 to the present day. Prior to the start of tilling treatments, a barley (*Hordeum vulgare* L.) crop was grown and removed in 1998 to reduce any potential differences in soil fertility and moisture from previous land usage. A yearly tomato-cotton rotation was then planted across four replicate blocks. In 2014, this tomato-cotton rotation was switched for a yearly rotation of sorghum (*Sorghum bicolor*) and garbanzo beans (*Cicer arietinum*).

The site consists of 32 plots that are each 9.1 m wide by 30.5 m long, with either a 9.1-m buffer or a border plot between treatment plots (see Fig. S1 in the supplemental material). Each treatment plot consists of six planting beds. Plots are divided into four soil management treatments. ST treatment has been described in detail previously (85), and it entails past crop residue shredding, multiple soil diskings to mix residues to a depth of 20 cm (cm), use of a subsoil shank at about 30.5 to 45.7 cm, additional disking to 20 cm to soil clods, and pulverization of the surface 20 cm of soil. In addition, planting beds were broken down and remade following each harvest. During the first 8 years, the NT treatment limited soil disturbances to shallow weed removal and tractor traffic was restricted to certain furrows. In 2012, NT fields became true no-tillage systems with the only soil disturbance occurring at the time of seeding or transplanting. During the entire length of this study, the location of NT planting beds was preserved, and past crop biomass was left on the field. An adapted overview of soil profiles when fields are managed by NT, versus ST, is provided in Fig. S20 (135).

Following each year's harvest, half the rows within the ST and NT treatments were either left fallow (NO) or planted with a cover crop mixture (CC) in October. This mixture was originally made up of Juan triticale (X *Triticosecale* Wittm.), Merced rye (*Secale cereale* L.), and common vetch (*Vicia sativa* L.); in 2010, pea (*Pisum sativum* L.), faba bean (*Vicia faba* l.), radish (*Raphanus sativus*), and Phacelia (*Phacelia tanacetifolia*) were added to the mix. Cover crops were cut in mid-March of the following year using a Buffalo Rolling Stalk Chopper (Buffalo Equipment, Columbus, NE). In the STCC system, the chopped cover crop was disked into the soil to a depth of about 20 cm. The cut cover crop in the NTCC system was sprayed with a 2% solution of glyphosate [*N*-(phosphonomethyl)glycine] and left on the surface as a mulch. Dry fertilizer (11-52-0 N-P-K) was applied in equal amounts to each plot preplanting of sorghum at 89.2 kg ha^{-1} (9.8 kg ha^{-1} N and 46.4 kg ha^{-1} P) using a standard straight fertilizer shank at depths of 15 cm. Additional N (urea) was side dress applied at 111.5 kg ha^{-1} for a total of 51.3 kg N ha^{-1} in two lines about 18 cm from the transplants and about 15-cm deep about 4 weeks after transplanting.

The average surface residue that typically remains following the combination of these four treatment types follows a decreasing gradient, with over 90% for NTCC, between 40 and 70% for NTNO, between 10 and 20% for STCC, and below 5% for STNO (85).

Sample collection and processing. In the summer of 2016, we sampled at two time points corresponding to before and after flowering, i.e., 40 and 80 days after planting, respectively, for sorghum (see Fig. S1). Specifically, for each treatment plot (NTCC, NTNO, STCC, and STNO), rhizosphere and roots were collected from three plants positioned 20.4 m apart in the same row and three beds into each plot. Soil was collected 20 cm away from harvested plants, in furrows, using a soil auger to sample 0 to 15 cm from the soil surface. We collected three rhizosphere, root, and soil samples each per treatment per three replicate blocks for a total of 108 samples: 3 samples $\times$ 3 sample types $\times$ 4 treatment types $\times$ 3 blocks. In addition, at each position we collected topsoil for each treatment per three replicate blocks (12 samples in all) to total approximately 500 g, in order to determine soil organic matter, pH, cation exchange capacity, and macro- and micronutrient levels, as analyzed by the Soil and Plant Nutrient Testing Laboratory at the University of Massachusetts, Amherst. Plant height and fresh shoot weight were measured for each plant harvested.

The following summer, we similarly sampled soils and sorghum roots and rhizospheres at a before flowering time point, i.e., 38 days after planting. We collected from two plants that were located 20.4 m into the row that was, as was done the year prior, three beds into each plot, sampling per treatment per three replicate blocks (see Fig. S1). One plant was sampled for amplicon sequencing, while the other for metatranscriptomics, in order to better correlate community composition with activity, as well as help validate year one results. Plant height and fresh shoot weight were again measured for each plant harvested. Plant yield was estimated when sorghum was harvested in October of those years (12 October 2016 and 16 October 2017) by harvesting 15-m lengths of one row in each plot with a 46-cm bundle plot thresher (Kincaid Equipment Manufacturing, Haven, KS).

DNA extraction and library preparation. Samples (108 per time points 1 and 2, 36 for time point 3 [252 total]) were transported from the field to the laboratory on dry ice and DNA extracted using a Power Soil DNA isolation kit (catalog no. 12888-100; MoBio, Carlsbad, CA). Concentrations were measured with a Qubit 3 fluorometer, and dilutions were then made to 5 ng/μl. To construct 16S rRNA gene and internal transcribed spacer 2 (ITS2) amplicon libraries, DNA from each sample was amplified using a dual-indexed 16S rRNA and ITS2 Illumina iTags primer set specifically to the V3-V4 16S rRNA (136) and 5.85Fun-ITS4Fun ITS2 region (137), respectively, using 5-Prime Hot Master Mix (catalog no. 2200410), according to protocols and conditions detailed previously (138, 139). Replicates were pooled, and the DNA concentration for each sample was then quantified using a Qubit 3 fluorometer. Using 100 ng of each PCR product, the amplicons were next pooled. Before submitting for sequencing, pooled samples were cleaned with 1.0$\times$ volume Agencourt AMPureXP (Beckman-Coulter, West Sacramento, CA) beads, according to the manufacturer's directions, except for the modifications of using 1.0$\times$ rather than 1.6$\times$ volume beads per sample, dispensing 1,500 μl of 70% ethanol rather than 200 μl, and eluting in 100 μl of DNase-free H_2O rather than 40 μl. An aliquot of the pooled amplicons was diluted to 10 nM in a 30-μl total volume and then submitted to the QB3 Vincent J. Coates Genomics Sequencing Laboratory for sequencing, using the Illumina MiSeq platform's 300-bp paired-end reads with v3 chemistry.

Amplicon sequence data processing. The resulting 16S amplicon libraries produced on average approximately 38,135, 44,906, and 37,852 reads per sample for soils, rhizospheres, and roots, respectively. The resulting ITS2 amplicon libraries produced on average approximately 44,848, 26,598, and 23,158 reads per sample for soils, rhizospheres, and roots, respectively. The sequencing depth was moderate across most samples, where read depth ranged between 4,826 and 146,220 and between 2,006 and 117,597 for the 16S and ITS2 amplicon libraries, respectively. The resulting read data were processed with the custom pipeline iTagger from the Joint Genome Institute in Qiime2, as detailed by Deng et al. (140). To remove low-abundance OTUs that are, in many cases, artifacts generated through the sequencing process, we removed OTUs without at least four reads in at least three samples. We also removed samples that had fewer than 10,000 bacterial and fungal reads, which yielded 3,155 bacterial and 454 fungal high-abundance OTUs for downstream analyses. To account for differences in sequencing read depth across samples, all samples were rarefied to 10,000 reads per sample for specific analyses.

RNA extraction and library preparation. For the summer 2017 field season, we collected soil and rhizosphere samples from one plant per treatment per block (three plants per treatment, for 12 rhizosphere and 12 soil samples in all) for metatranscriptomic analysis. Samples were harvested just before flowering (the second growth stage with panicle formation, 38 days after transplanting) to determine what microbial processes are ongoing close to a critical period for grain production (60). Soil and rhizosphere samples were flash frozen with liquid nitrogen and stored on dry ice.

RNA from samples was extracted and cDNA synthesized using the PowerMax soil DNA isolation kit for RNA extraction, as the MoBio PowerMax (catalog no. 12988-10) and PowerSoil kit use the same silica membrane, with a modified protocol provided by MoBio, as detailed previously (141). The resultant RNA was washed with 70% ethanol and resuspended in 100 μl of RNase-free H_2O, and the remaining DNA digested using a DNase Max kit (Qiagen, catalog no. 15200-50), according to the manufacturer's protocol. RNA was purified using an RNeasy PowerClean Pro Cleanup kit (MoBio), and rRNA from bacteria was removed with a Ribo-Zero rRNA removal kit (Bacteria, Illumina, catalog no. MRZB12424) according to the manufacturers' instructions. The concentration and quality were then assessed using a Qubit 3 fluorometer (Invitrogen, Carlsbad, CA) and Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA), respectively. Subsequently, a TruSeq Stranded Total RNA Library Prep Plant SetA kit (Illumina, catalog no. 20020610) was used, according to the manufacturer's instructions, to make 300- to 500-bp fragment libraries for sequencing on the Illumina HiSeq4000 platform with 150-bp paired end reads at the QB3 Vincent J.

Coates Genomics Sequencing Laboratory. Returned reads were assembled with Trinity and then submitted to Integrated Microbial Genomes & Microbiomes at the Joint Genome Institute for annotation and taxonomic assignment (pipeline, version 4.16.5 [137]).

Metatranscriptome sequence data processing. The metatranscriptome data analysis pipeline is detailed in reference 141 and read statistics, sequencing depth, rRNA and tRNA contamination levels from archaea, bacteria, and eukaryotes are reported in Table S6 in the supplemental material. In brief, we performed a quality control of raw fastq data with the software FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and then cut sequencing adaptors and removed short reads that were less than 35 bp, as well as reads with more than three Ns (flags: -q 15,15 -m 35 –max-n 3), with CUTADAPT, version 1.9 (142). The remaining reads were compared to the Silva and Rfam database for identification of rRNA (18S, 28S, 16S, 23S, 5.8S, 12S, and 5S) and tRNA, which were removed with BBmap (v35.34; sourceforge.net/projects/bbmap/). All remaining clean mRNA reads from all of our data sets were combined with MEGAHIT (version 1.1.1) with odd numbered k-mers from length 21 to 99 (Li et al., 2015). Contig coverage was determined by mapping the initial unassembled reads to the combined assembled contigs using BBMap, where the pileup.sh script (BBMap) was used to calculate contig-wise average coverage. The assembled contigs with lengths of ≥200 bp and a covered percentage of ≥50% were submitted to JGI IMG (https://img.jgi.doe.gov) for gene calling, functional annotation, and taxonomy assignment, as described previously (143). In brief, the IMG pipeline performs an additional quality control and protein-coding genes are identified using a consensus of four different *ab initio* gene prediction tools: prokaryotic GeneMark.hmm 265 (v2.8) (144), MetaGeneAnnotator (v.Aug 2008) (145), Prodigal (v.2.6.2) (146), and FragGeneScan (v.1.16) (147). The predictions from all tools are combined, and protein-coding genes with translations shorter than 32 amino acids are deleted. Functional annotation of associated protein-coding genes is performed with COGs, Pfams, KO terms, EC numbers, and phylogenetic lineage for contigs (148). For phylogenetic lineage analysis, the best hit for genes with >30% identity to a defined taxonomic lineage was picked for further analysis (this threshold permitted classification of ~63.7% of all genes). Genes classified as belonging to either bacteria or fungi were picked manually and used for relative abundance calculations in R. Of the assembled reads assigned taxonomy (51%) in our data set, 48.47% are from bacteria, 48.33% from eukaryota (25% from Fungi alone), 2.30% from archaea, and 0.89% from viruses. The relative gene expression levels were determined by counting the number of reads that were assigned to a particular protein-encoding gene. The relative gene expression was then quantified for each taxon. Normalization was obtained by dividing each gene count by the total mRNA read count of each data set. For taxonomy assignment based on the core gene set, we calculated relative abundances based only on the abundances of nine core genes (*gyrA*, *recA*, *rpoB*, *rpoA*, *gyrB*, *gap*, *rho*, *ftsZ*, and *secA*) commonly represented across a large percentage of bacterial lineages from the IMG Bacterial Database. Since MEGAHIT does not report the specific reads incorporated into the assembled contigs, we used BBMap to map the mRNA reads back to contigs to calculate the percentage of reads assembled per sample (bbwrap.sh with flags: kfilter = 22 subfilter = 15 maxindel = 80). Kallisto (https://pachterlab.github.io/kallisto/) was used to map the high-quality mRNA reads per sample to these IMG-derived gene sequences to provide a per-gene coverage estimate with "sequence-based bias correction" and "strand specific reads, first read reverse" flags. The raw gene counts table was passed to edgeR (149) to perform the normalization by calcNormFactors function with the TMM method (150) and to calculate which genes were differentially expressed with quasi-likelihood F tests ($P \leq 0.05$). Hypergeometric tests were used to calculate the enrichment of functional categories and subcategories ($P \leq 0.05$).

Data availability. All raw sequences are deposited in Sequence Read Archive with the accession codes PRJNA683037 and PRJNA682819, and all scripts used for the statistical analysis of data in the manuscript are included as part of a public repository on GitHub (https://github.com/colemanderr-lab/Wipf-2020/tree/main/Agricultural-Management-Practices). In addition, an assembly of the metatranscriptome can be accessed and downloaded via IMG/MER (https://img.jgi.doe.gov/cgi-bin/m/main.cgi) using the IMG ID 3300029287.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 5.1 MB.

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REFERENCES

1. Schloss PD, Handelsman J. 2006. Toward a census of bacteria in soil. PLoS Comput Biol 2:e92. https://doi.org/10.1371/journal.pcbi.0020092.
2. FAO. 2011. Save and grow: a policymaker's guide to the sustainable intensification of smallholder crop production. Food and Agriculture

Organization of the United Nations, Rome, Italy. http://www.fao.org/3/a-i2215e.pdf.
3. Koch A, McBratney A, Adams M, Field D, Hill R, Crawford J, Minasny B, Lal R, Abbott L, O'Donnell A, Angers D, Baldock J, Barbier E, Binkley D, Parton W, Wall DH, Bird M, Bouma J, Chenu C, Flora CB, Goulding K, Grunwald S, Hempel J, Jastrow J, Lehmann J, Lorenz K, Morgan CL, Rice CW, Whitehead D, Young I, Zimmermann M. 2013. Soil security: solving the global soil crisis. Glob Policy 4:434–441. https://doi.org/10.1111/1758-5899.12096.
4. Stavi I, Bel G, Zaady E. 2016. Soil functions and ecosystem services in conventional, conservation, and integrated agricultural systems: a review. Agron Sustain Dev 36. https://doi.org/10.1007/s13593-016-0368-8.
5. Panel ME. 2014. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services: Ecosystem Services. IPBES, Bonn, Germany.
6. Turmel M-S, Speratti A, Baudron F, Verhulst N, Govaerts B. 2015. Crop residue management and soil health: a systems analysis. Agric Syst 134:6–16. https://doi.org/10.1016/j.agsy.2014.05.009.
7. Derpsch R. 2003. Conservation tillage, no-tillage and related technologies, p 181–190. *In* García-Torres L, Benites J, Martínez-Vilela A, Holgado-Cabrera A (ed), Conservation agriculture: environment, farmers experiences, innovations, socio-economy, policy. Springer, Dordrecht, Netherlands.
8. Mann L, Tolbert V, Cushman J. 2002. Potential environmental effects of corn (*Zea mays* L.) stover removal with emphasis on soil organic matter and erosion. Agric Ecosyst Environ 89:149–166. https://doi.org/10.1016/S0167-8809(01)00166-9.
9. Wilhelm WW, Johnson JMF, Hatfield JL, Voorhees WB, Linden DR. 2004. Crop and soil productivity response to corn residue removal. Agron J 96:1–17. https://doi.org/10.2134/agronj2004.0001.
10. Moebius-Clune BN, van Es HM, Idowu OJ, Schindelbeck RR, Moebius-Clune DJ, Wolfe DW, Abawi GS, Thies JE, Gugino BK, Lucey R. 2008. Long-term effects of harvesting maize stover and tillage on soil quality. Soil Sci Soc Am J 72:960–969. https://doi.org/10.2136/sssaj2007.0248.
11. Shah AN, Tanveer M, Shahzad B, Yang G, Fahad S, Ali S, Bukhari MA, Tung SA, Hafeez A, Souliyanonh B. 2017. Soil compaction effects on soil health and crop productivity: an overview. Environ Sci Pollut Res Int 24:10056–10067. https://doi.org/10.1007/s11356-017-8421-y.
12. Busari MA, Kukal SS, Kaur A, Bhatt R, Dulazi AA. 2015. Conservation tillage impacts on soil, crop and the environment. Int Soil Water Conserv Res 3:119–129. https://doi.org/10.1016/j.iswcr.2015.05.002.
13. Hobbs PR, Sayre K, Gupta R. 2008. The role of conservation agriculture in sustainable agriculture. Philos Trans R Soc Lond B Biol Sci 363:543–555. https://doi.org/10.1098/rstb.2007.2169.
14. Thomas GA, Dalal RC, Standley J. 2007. No-till effects on organic matter, pH, cation exchange capacity and nutrient distribution in a Luvisol in the semi-arid subtropics. Soil Tillage Res 94:295–304. https://doi.org/10.1016/j.still.2006.08.005.
15. Smith CR, Blair PL, Boyd C, Cody B, Hazel A, Hedrick A, Kathuria H, Khurana P, Kramer B, Muterspaw K, Peck C, Sells E, Skinner J, Tegeler C, Wolfe Z. 2016. Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. Ecol Evol 6:8075–8084. https://doi.org/10.1002/ece3.2553.
16. Rahman M, Okubo A, Sugiyama S, Mayland H. 2008. Physical, chemical and microbiological properties of an Andisol as related to land use and tillage practice. Soil Tillage Res 101:10–19. https://doi.org/10.1016/j.still.2008.05.006.
17. Lal R, Regnier E, Eckert DJ, Edwards WM, Hammond R. 1991. Expectations of cover crops for sustainable agriculture, p 1–11. *In* Hargrove WL (ed), Cover crops for clean water. Soil and Water Conservation Society, Ankeny, IA.
18. Shirley C, Bowman G, Cramer C. 1998. Managing cover crops profitably. Sustainable Agriculture Publications, Burlington, VT.
19. Strock JS, Porter PM, Russelle MP. 2004. Cover cropping to reduce nitrate loss through subsurface drainage in the northern US Corn Belt. J Environ Qual 33:1010–1016. https://doi.org/10.2134/jeq2004.1010.
20. Campbell CA, McConkey BG, Zentner RP, Selles F, Curtin D. 1996. Tillage and crop rotation effects on soil organic C and N in a coarse-textured Typic Haploboroll in southwestern Saskatchewan. Soil Tillage Res 37:3–14. https://doi.org/10.1016/0167-1987(95)01002-5.
21. Wortman SE, Francis CA, Lindquist JL. 2012. Cover crop mixtures for the western corn belt: opportunities for increased productivity and stability. Agron J 104:699–705. https://doi.org/10.2134/agronj2011.0422.
22. Dodd JC, Jeffries P. 1986. Early development of vesicular-arbuscular mycorrhizas in autumn-sown cereals. Soil Biol Biochem 18:149–154. https://doi.org/10.1016/0038-0717(86)90019-2.
23. Kabir Z, Koide RT. 2002. Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of sweet corn in Pennsylvania, USA. Plant Soil 238:205–215. https://doi.org/10.1023/A:1014408723664.
24. Calegari A, Hargrove WL, Rheinheimer DDS, Ralisch R, Tessier D, de Tourdonnet S, de Fatima Guimarães M. 2008. Impact of long-term no-tillage and cropping system management on soil organic carbon in an Oxisol: a model for sustainability. Agron J 100:1013–1019. https://doi.org/10.2134/agronj2007.0121.
25. Chávez-Romero Y, Navarro-Noya YE, Reynoso-Martínez SC, Sarria-Guzmán Y, Govaerts B, Verhulst N, Dendooven L, Luna-Guido M. 2016. 16S metagenomics reveals changes in the soil bacterial community driven by soil organic C, N-fertilizer and tillage-crop residue management. Soil Tillage Res 159:1–8. https://doi.org/10.1016/j.still.2016.01.007.
26. Miguez FE, Bollero GA. 2005. Review of corn yield response under winter cover cropping systems using meta-analytic methods. Crop Sci 45:2318–2329. https://doi.org/10.2135/cropsci2005.0014.
27. Lareen A, Burton F, Schäfer P. 2016. Plant root-microbe communication in shaping root microbiomes. Plant Mol Biol 90:575–587. https://doi.org/10.1007/s11103-015-0417-8.
28. Bergelson J, Mittelstrass J, Horton MW. 2019. Characterizing both bacteria and fungi improves understanding of the *Arabidopsis* root microbiome. Sci Rep 9:24. https://doi.org/10.1038/s41598-018-37208-z.
29. Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. 2012. Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499. https://doi.org/10.1007/s00374-012-0691-4.
30. Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC, Berendsen RL, Bakker PAHM, Feussner I, Pieterse CMJ. 2018. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. Proc Natl Acad Sci U S A 115:E5213–E5222. https://doi.org/10.1073/pnas.1722335115.
31. Dumontet S, Mazzatura A, Casucci C, Perucci P. 2001. Effectiveness of microbial indexes in discriminating interactive effects of tillage and crop rotations in a Vertic Ustorthens. Biol Fertil Soils 34:411–416. https://doi.org/10.1007/s00374-001-0424-6.
32. Peters RD, Sturz AV, Carter MR, Sanderson JB. 2003. Developing disease-suppressive soils through crop rotation and tillage management practices. Soil Tillage Res 72:181–192. https://doi.org/10.1016/S0167-1987(03)00087-4.
33. Helgason BL, Walley FL, Germida JJ. 2009. Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. Soil Sci Soc Am J 73:120–127. https://doi.org/10.2136/sssaj2007.0392.
34. Dorr de Quadros P, Zhalnina K, Davis-Richardson A, Fagen JR, Drew J, Bayer C, Camargo FAO, Triplett EW. 2012. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. Diversity 4:375–395. https://doi.org/10.3390/d4040375.
35. Souza RC, Cantão ME, Vasconcelos ATR, Nogueira MA, Hungria M. 2013. Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. Appl Soil Ecol 72:49–61. https://doi.org/10.1016/j.apsoil.2013.05.021.
36. Murugan R, Koch H-J, Joergensen RG. 2014. Long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths. Biol Fertil Soils 50:487–498. https://doi.org/10.1007/s00374-013-0871-x.
37. Sharma-Poudyal D, Schlatter D, Yin C, Hulbert S, Paulitz T. 2017. Long-term no-till: a major driver of fungal communities in dryland wheat cropping systems. PLoS One 12:e0184611. https://doi.org/10.1371/journal.pone.0184611.
38. Yin C, Mueth N, Hulbert S, Schlatter D, Paulitz TC, Schroeder K, Prescott A, Dhingra A. 2017. Bacterial communities on wheat grown under long-term conventional tillage and no-till in the Pacific Northwest of the United States. Phytobiomes J 1:83–90. https://doi.org/10.1094/PBIOMES-09-16-0008-R.
39. Gosling P, Hodge A, Goodlass G, Bending GD. 2006. Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35. https://doi.org/10.1016/j.agee.2005.09.009.
40. Kabir Z, O'Halloran IP, Fyles JW, Hamel C. 1997. Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization. Plant Soil 192:285–293. https://doi.org/10.1023/A:1004205828485.
41. Borie F, Rubio R, Rouanet JL, Morales A, Borie G, Rojas C. 2006. Effects of tillage systems on soil characteristics, glomalin and mycorrhizal propagules in a Chilean Ultisol. Soil Tillage Res 88:253–261. https://doi.org/10.1016/j.still.2005.06.004.

42. Rosner K, Bodner G, Hage-Ahmed K, Steinkellner S. 2018. Long-term soil tillage and cover cropping affected arbuscular mycorrhizal fungi, nutrient concentrations, and yield in sunflower. Agron J 110:2664–2672. https://doi.org/10.2134/agronj2018.03.0177.
43. Getzke F, Thiergart T, Hacquard S. 2019. Contribution of bacterial-fungal balance to plant and animal health. Curr Opin Microbiol 49:66–72. https://doi.org/10.1016/j.mib.2019.10.009.
44. Durán P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S. 2018. Microbial interkingdom interactions in roots promote *Arabidopsis* survival. Cell 175:973–983.e14. https://doi.org/10.1016/j.cell.2018.10.020.
45. van der Heijden MGA, Bruin SD, Luckerhoff L, van Logtestijn RSP, Schlaeppi K. 2016. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition, and seedling recruitment. ISME J 10:389–399. https://doi.org/10.1038/ismej.2015.120.
46. Kim Y-G, Udayanga KGS, Totsuka N, Weinberg JB, Núñez G, Shibuya A. 2014. Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE2. Cell Host Microbe 15:95–102. https://doi.org/10.1016/j.chom.2013.12.010.
47. Cookson WR, Murphy DV, Roper MM. 2008. Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. Soil Biol Biochem 40:763–777. https://doi.org/10.1016/j.soilbio.2007.10.011.
48. Hartman K, van der Heijden MGA, Wittwer RA, Banerjee S, Walser J-C, Schlaeppi K. 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6:14. https://doi.org/10.1186/s40168-017-0389-9.
49. Anderson C, Beare M, Buckley HL, Lear G. 2017. Bacterial and fungal communities respond differently to varying tillage depth in agricultural soils. PeerJ 5:e3930. https://doi.org/10.7717/peerj.3930.
50. Acosta-Martínez V, Dowd SE, Bell CW, Lascano R, Booker JD, Zobeck TM, Upchurch DR. 2010. Microbial community composition as affected by dryland cropping systems and tillage in a semiarid sandy soil. Diversity 2:910–931. https://doi.org/10.3390/d2060910.
51. Rahman MA, Lee S-H, Ji HC, Kabir AH, Jones CS, Lee K-W. 2018. Importance of mineral nutrition for mitigating aluminum toxicity in plants on acidic soils: current status and opportunities. Int J Mol Sci 19:3073. https://doi.org/10.3390/ijms19103073.
52. Singh D, Jain P, Gupta A, Nema R. 2013. Soil diversity: a key for natural management of biological and chemical constitute to maintain soil health and fertility. Int J Biosci Biotechnol 5:41–50.
53. Hacquard S. 2016. Disentangling the factors shaping microbiota composition across the plant holobiont. New Phytol 209:454–457. https://doi.org/10.1111/nph.13760.
54. Tatusov RL, Galperin MY, Natale DA, Koonin EV. 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 28:33–36. https://doi.org/10.1093/nar/28.1.33.
55. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res 37:D233–D238. https://doi.org/10.1093/nar/gkn663.
56. Nuccio EE, Starr E, Karaoz U, Brodie EL, Zhou J, Tringe S, Malmstrom RR, Woyke T, Banfield JF, Firestone MK, Pett-Ridge J. 2020. Niche differentiation is spatially and temporally regulated in the rhizosphere. ISME J 14:999–1014. https://doi.org/10.1038/s41396-019-0582-x.
57. Hallama M, Pekrun C, Lambers H, Kandeler E. 2019. Hidden miners: the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. Plant Soil 434:7–45. https://doi.org/10.1007/s11104-018-3810-7.
58. Basu S, Rabara RC, Negi S. 2018. AMF: the future prospect for sustainable agriculture. Physiol Mol Plant Pathol 102:36–45. https://doi.org/10.1016/j.pmpp.2017.11.007.
59. Schmidt R, Gravuer K, Bossange AV, Mitchell J, Scow K. 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLoS One 13:e0192953. https://doi.org/10.1371/journal.pone.0192953.
60. Lupwayi NZ, Rice WA, Clayton GW. 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. Soil Biol Biochem 30:1733–1741. https://doi.org/10.1016/S0038-0717(98)00025-X.
61. Nivelle E, Verzeaux J, Habbib H, Kuzyakov Y, Decocq G, Roger D, Lacoux J, Duclercq J, Spicher F, Nava-Saucedo J-E, Catterou M, Dubois F, Tetu T. 2016. Functional response of soil microbial communities to tillage, cover crops, and nitrogen fertilization. Appl Soil Ecol 108:147–155. https://doi.org/10.1016/j.apsoil.2016.08.004.
62. Schmidt R, Mitchell J, Scow K. 2019. Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities. Soil Biol Biochem 129:99–109. https://doi.org/10.1016/j.soilbio.2018.11.010.
63. Chavarría DN, Verdenelli RA, Serri DL, Restovich SB, Andriulo AE, Meriles JM, Vargas-Gil S. 2016. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. Eur J Soil Biol 76:74–82. https://doi.org/10.1016/j.ejsobi.2016.07.002.
64. Cadotte MW. 2007. Competition-colonization trade-offs and disturbance effects at multiple scales. Ecology 88:823–829. https://doi.org/10.1890/06-1117.
65. Choi S, Song H, Tripathi BM, Kerfahi D, Kim H, Adams JM. 2017. Effect of experimental soil disturbance and recovery on structure and function of soil community: a metagenomic and metagenetic approach. Sci Rep 7:2260. https://doi.org/10.1038/s41598-017-02262-6.
66. Griffin EA, Harrison JG, Kembel SW, Carrell AA, Joseph Wright S, Carson WP. 2019. Plant host identity and soil macronutrients explain little variation in sapling endophyte community composition: is disturbance an alternative explanation? J Ecol 107:1876–1889. https://doi.org/10.1111/1365-2745.13145.
67. Christian N, Whitaker BK, Clay K. 2015. Microbiomes: unifying animal and plant systems through the lens of community ecology theory. Front Microbiol 6:869. https://doi.org/10.3389/fmicb.2015.00869.
68. Andrews JH, Harris RF. 1986. r- and K-selection and microbial ecology. Adv Microb Ecol 9:99–147. https://doi.org/10.1007/978-1-4757-0611-6_3.
69. Galand PE, Lucas S, Fagervold SK, Peru E, Pruski AM, Vétion G, Dupuy C, Guizien K. 2016. Disturbance increases microbial community diversity and production in marine sediments. Front Microbiol 7:1950. https://doi.org/10.3389/fmicb.2016.01950.
70. McDevitt-Irwin JM, Garren M, McMinds R, Thurber RV, Baum JK. 2019. Variable interaction outcomes of local disturbance and El Niño-induced heat stress on coral microbiome alpha and beta diversity. Coral Reefs 38:331–345. https://doi.org/10.1007/s00338-019-01779-8.
71. Gómez P, Buckling A. 2013. Real-time microbial adaptive diversification in soil. Ecol Lett 16:650–655. https://doi.org/10.1111/ele.12093.
72. Feng K, Zhang Z, Cai W, Liu W, Xu M, Yin H, Wang A, He Z, Deng Y. 2017. Biodiversity and species competition regulate the resilience of microbial biofilm community. Mol Ecol 26:6170–6182. https://doi.org/10.1111/mec.14356.
73. Schlatter DC, Kahl K, Carlson B, Huggins DR, Paulitz T. 2018. Fungal community composition and diversity vary with soil depth and landscape position in a no-till wheat-based cropping system. FEMS Microbiol Ecol 94. https://doi.org/10.1093/femsec/fiy098.
74. Schlatter DC, Kahl K, Carlson B, Huggins DR, Paulitz T. 2020. Soil acidification modifies soil depth-microbiome relationships in a no-till wheat cropping system. Soil Biol Biochem 149:107939. https://doi.org/10.1016/j.soilbio.2020.107939.
75. Wakita Y, Shimomura Y, Kitada Y, Yamamoto H, Ohashi Y, Matsumoto M. 2018. Taxonomic classification for microbiome analysis, which correlates well with the metabolite milieu of the gut. BMC Microbiol 18:188. https://doi.org/10.1186/s12866-018-1311-8.
76. Hill RL, Horton R, Cruse RM. 1985. Tillage effects on soil water retention and pore size distribution of two Mollisols. Soil Sci Soc Am J 49:1264–1270. https://doi.org/10.2136/sssaj1985.03615995004900050039x.
77. Bach EM, Williams RJ, Hargreaves SK, Yang F, Hofmockel KS. 2018. Greatest soil microbial diversity found in micro-habitats. Soil Biol Biochem 118:217–226. https://doi.org/10.1016/j.soilbio.2017.12.018.
78. de M Sá JC, Cerri CC, Dick WA, Lal R, Filho SPV, Piccolo MC, Feigl BE. 2001. Organic matter dynamics and carbon sequestration rates for a tillage chronosequence in a Brazilian Oxisol. Soil Sci Soc Am J 65:1486–1499. https://doi.org/10.2136/sssaj2001.6551486x.
79. McConkey B. 2003. Crop rotation and tillage impact on carbon sequestration in Canadian prairie soils. Soil Tillage Res 74:81–90. https://doi.org/10.1016/S0167-1987(03)00121-1.
80. Six J, Frey SD, Thiet RK, Batten KM. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70:555–569. https://doi.org/10.2136/sssaj2004.0347.
81. Sundermeier AP, Islam KR, Raut Y, Reeder RC, Dick WA. 2011. Continuous no-till impacts on soil biophysical carbon sequestration. Soil Sci Soc Am J 75:1779–1788. https://doi.org/10.2136/sssaj2010.0334.
82. Ambus P, Jensen ES. 1997. Nitrogen mineralization and denitrification as

influenced by crop residue particle size. Plant Soil 197:261–270. https://doi.org/10.1023/A:1004276631914.
83. Zachmann JE, Linden DR, Clapp CE. 1987. Macroporous infiltration and redistribution as affected by earthworms, tillage, and residue. Soil Sci Soc Am J 51:1580–1586. https://doi.org/10.2136/sssaj1987.03615995005100060032x.
84. Edwards WM, Norton LD, Redmond CE. 1988. Characterizing macropores that affect infiltration into nontilled soil. Soil Sci Soc Am J 52:483–487. https://doi.org/10.2136/sssaj1988.03615995005200020033x.
85. Mitchell JP, Shrestha A, Horwath WR, Southard RJ, Madden N, Veenstra J, Munk DS. 2015. Tillage and cover cropping affect crop yields and soil carbon in the San Joaquin Valley, California. Agron J 107:588–596. https://doi.org/10.2134/agronj14.0415.
86. De Graaff M-A, Classen AT, Castro HF, Schadt CW. 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytol 188:1055–1064. https://doi.org/10.1111/j.1469-8137.2010.03427.x.
87. Navarro-Noya YE, Gómez-Acata S, Montoya-Ciriaco N, Rojas-Valdez A, Suárez-Arriaga MC, Valenzuela-Encinas C, Jiménez-Bueno N, Verhulst N, Govaerts B, Dendooven L. 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol Biochem 65:86–95. https://doi.org/10.1016/j.soilbio.2013.05.009.
88. Ramirez-Villanueva DA, Bello-López JM, Navarro-Noya YE, Luna-Guido M, Verhulst N, Govaerts B, Dendooven L. 2015. Bacterial community structure in maize residue amended soil with contrasting management practices. Appl Soil Ecol 90:49–59. https://doi.org/10.1016/j.apsoil.2015.01.010.
89. Finney DM, Buyer JS, Kaye JP. 2017. Living cover crops have immediate impacts on soil microbial community structure and function. J Soil Water Conserv 72:361–373. https://doi.org/10.2489/jswc.72.4.361.
90. Drummelsmith J. 2020. The impact of cover crops and crop residue removal on soil microbial community abundance, diversity and soil health in a medium-term cover crop field trial in Southwest Ontario. MSc thesis. University of Guelph, Guelph, Ontario, Canada.
91. Bertin C, Yang X, Weston LA. 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83. https://doi.org/10.1023/A:1026290508166.
92. Vukicevich E, Lowery T, Bowen P, Úrbez-Torres JR, Hart M. 2016. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture: a review. Agron Sustain Dev 36:48. https://doi.org/10.1007/s13593-016-0385-7.
93. Carpenter-Boggs L, Stahl PD, Lindstrom MJ, Schumacher TE. 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. Soil Tillage Res 71:15–23. https://doi.org/10.1016/S0167-1987(02)00158-7.
94. Minic Z. 2008. Physiological roles of plant glycoside hydrolases. Planta 227:723–740. https://doi.org/10.1007/s00425-007-0668-y.
95. Likar M, Stres B, Rusjan D, Potisek M, Regvar M. 2017. Ecological and conventional viticulture gives rise to distinct fungal and bacterial microbial communities in vineyard soils. Appl Soil Ecol 113:86–95. https://doi.org/10.1016/j.apsoil.2017.02.007.
96. Peay KG, Kennedy PG, Talbot JM. 2016. Dimensions of biodiversity in the Earth mycobiome. Nat Rev Microbiol 14:434–447. https://doi.org/10.1038/nrmicro.2016.59.
97. Ritz K, Young IM. 2004. Interactions between soil structure and fungi. Mycologist 18:52–59. https://doi.org/10.1017/S0269915X04002010.
98. Neely CL, Beare MH, Hargrove WL, Coleman DC. 1991. Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. Soil Biol Biochem 23:947–954. https://doi.org/10.1016/0038-0717(91)90175-J.
99. Acosta-Martínez V, Burow G, Zobeck TM, Allen VG. 2010. Soil microbial communities and function in alternative systems to continuous cotton. Soil Sci Soc Am J 74:1181–1192. https://doi.org/10.2136/sssaj2008.0065.
100. Schutter M, Sandeno J, Dick R. 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. Biol Fertil Soils 34:397–410. https://doi.org/10.1007/s00374-001-0423-7.
101. Frey SD, Elliott ET, Paustian K. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biol Biochem 31:573–585. https://doi.org/10.1016/S0038-0717(98)00161-8.
102. Feng Y, Motta AC, Reeves DW, Burmester CH, van Santen E, Osborne JA. 2003. Soil microbial communities under conventional-till and no-till continuous cotton systems. Soil Biol Biochem 35:1693–1703. https://doi.org/10.1016/j.soilbio.2003.08.016.
103. Mathew RP, Feng Y, Githinji L, Ankumah R, Balkcom KS. 2012. Impact of no-tillage and conventional tillage systems on soil microbial communities. Appl Environ Soil Sci 2012:1–10. https://doi.org/10.1155/2012/548620.
104. Mbuthia LW, Acosta-Martínez V, DeBruyn J, Schaeffer S, Tyler D, Odoi E, Mpheshea M, Walker F, Eash N. 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: implications for soil quality. Soil Biol Biochem 89:24–34. https://doi.org/10.1016/j.soilbio.2015.06.016.
105. Wang Y, Xu J, Shen J, Luo Y, Scheu S, Ke X. 2010. Tillage, residue burning and crop rotation alter soil fungal community and water-stable aggregation in arable fields. Soil Tillage Res 107:71–79. https://doi.org/10.1016/j.still.2010.02.008.
106. Dong W, Liu E, Yan C, Zhang H, Zhang Y. 2017. Changes in the composition and diversity of topsoil bacterial, archaeal and fungal communities after 22 years conventional and no-tillage managements in Northern China. Arch Agron Soil Sci 63:1369–1381. https://doi.org/10.1080/03650340.2017.1281392.
107. Fierer N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15:579–590. https://doi.org/10.1038/nrmicro.2017.87.
108. Wallenstein MD, McNulty S, Fernandez IJ, Boggs J, Schlesinger WH. 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. For Ecol Manage 222:459–468. https://doi.org/10.1016/j.foreco.2005.11.002.
109. Rousk J, Bååth E. 2011. Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiol Ecol 78:17–30. https://doi.org/10.1111/j.1574-6941.2011.01106.x.
110. Boer W. d, de Boer W, Folman LB, Summerbell RC, Boddy L. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29:795–811. https://doi.org/10.1016/j.femsre.2004.11.005.
111. Waring BG, Averill C, Hawkes CV. 2013. Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. Ecol Lett 16:887–894. https://doi.org/10.1111/ele.12125.
112. Whitaker J, Ostle N, Nottingham AT, Ccahuana A, Salinas N, Bardgett RD, Meir P, McNamara NP, Austin A. 2014. Microbial community composition explains soil respiration responses to changing carbon inputs along an Andes-to-Amazon elevation gradient. J Ecol 102:1058–1071. https://doi.org/10.1111/1365-2745.12247.
113. Sun R, Li W, Dong W, Tian Y, Hu C, Liu B. 2018. Tillage changes vertical distribution of soil bacterial and fungal communities. Front Microbiol 9:699. https://doi.org/10.3389/fmicb.2018.00699.
114. Treseder KK, Lennon JT. 2015. Fungal traits that drive ecosystem dynamics on land. Microbiol Mol Biol Rev 79:243–262. https://doi.org/10.1128/MMBR.00001-15.
115. Banerjee S, Kirkby CA, Schmutter D, Bissett A, Kirkegaard JA, Richardson AE. 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biol Biochem 97:188–198. https://doi.org/10.1016/j.soilbio.2016.03.017.
116. Cordovil C. d S, Bittman S, Brito LM, Goss MJ, Hunt D, Serra J, Gourley C, Aarons S, Skiba U, Amon B, Vale MJ, Cruz S, Reis R, Dalgaard T, Hutchings N. 2020. Climate-resilient and smart agricultural management tools to cope with climate change-induced soil quality decline, p 613–662. *In* Prasad MNV, Pietrzykowski M (ed), Climate change and soil interactions. Elsevier, Amsterdam, Netherlands.
117. Hontoria C, García-González I, Quemada M, Roldán A, Alguacil MM. 2019. The cover crop determines the AMF community composition in soil and in roots of maize after a ten-year continuous crop rotation. Sci Total Environ 660:913–922. https://doi.org/10.1016/j.scitotenv.2019.01.095.
118. Kabir Z, O'Halloran IP, Hamel C. 1997. Overwinter survival of arbuscular mycorrhizal hyphae is favored by attachment to roots but diminished by disturbance. Mycorrhiza 7:197–200. https://doi.org/10.1007/s005720050181.
119. Kabir Z, Rhamoun M, Lazicki P, Horwath W. 2008. Cover crops and conservation tillage increase mycorrhizal colonization of corn and tomato roots. SAFS Newl 9:1. http://safs.ucdavis.edu/newsletter/v09n1/page3.htm.
120. Neelam AA, Gaur A, Bhalla E, Gupta SR. 2010. Soil aggregate carbon and

diversity of mycorrhiza as affected by tillage practices in a rice-wheat cropping system in northern India. Int J Ecol Environ Sci 36:233–243.
121. Thonar C, Frossard E, Smilauer P, Jansa J. 2014. Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. Mol Ecol 23:733–746. https://doi.org/10.1111/mec.12625.
122. Knegt B, Jansa J, Franken O, Engelmoer DJP, Werner GDA, Bücking H, Toby Kiers E. 2016. Host plant quality mediates competition between arbuscular mycorrhizal fungi. Fungal Ecol 20:233–240. https://doi.org/10.1016/j.funeco.2014.09.011.
123. Engelmoer DJP, Behm JE, Toby Kiers ET. 2014. Intense competition between arbuscular mycorrhizal mutualists in an *in vitro* root microbiome negatively affects total fungal abundance. Mol Ecol 23:1584–1593. https://doi.org/10.1111/mec.12451.
124. Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc Natl Acad Sci U S A 112:10967–10972. https://doi.org/10.1073/pnas.1508382112.
125. Beauregard MS, Hamel C, Atul-Nayyar, St-Arnaud M. 2010. Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. Microb Ecol 59:379–389. https://doi.org/10.1007/s00248-009-9583-z.
126. Dai Z, Su W, Chen H, Barberán A, Zhao H, Yu M, Yu L, Brookes PC, Schadt CW, Chang SX, Xu J. 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. Glob Chang Biol 24:3452–3461. https://doi.org/10.1111/gcb.14163.
127. Degrune F, Theodorakopoulos N, Colinet G, Hiel M-P, Bodson B, Taminiau B, Daube G, Vandenbol M, Hartmann M. 2017. Temporal dynamics of soil microbial communities below the seedbed under two contrasting tillage regimes. Front Microbiol 8:1127. https://doi.org/10.3389/fmicb.2017.01127.
128. Hussain I, Olson KR, Ebelhar SA. 1999. Long-term tillage effects on soil chemical properties and organic matter fractions. Soil Sci Soc Am J 63:1335–1341. https://doi.org/10.2136/sssaj1999.6351335x.
129. Veenstra J, Horwath W, Mitchell J, Munk D, Others. 2006. Conservation tillage and cover cropping influence soil properties in San Joaquin Valley cotton-tomato crop. Calif Agric (Berkeley) 60:146–153. https://doi.org/10.3733/ca.v060n03p146.
130. Minoshima H, Jackson LE, Cavagnaro TR, Sánchez-Moreno S, Ferris H, Temple SR, Goyal S, Mitchell JP. 2007. Soil food webs and carbon dynamics in response to conservation tillage in California. Soil Sci Soc Am J 71:952–963. https://doi.org/10.2136/sssaj2006.0174.
131. Baker JM, Ochsner TE, Venterea RT, Griffis TJ. 2007. Tillage and soil carbon sequestration: what do we really know? Agric Ecosyst Environ 118:1–5. https://doi.org/10.1016/j.agee.2006.05.014.
132. Green VS, Stott DE, Cruz JC, Curi N. 2007. Tillage impacts on soil biological activity and aggregation in a Brazilian Cerrado Oxisol. Soil Tillage Res 92:114–121. https://doi.org/10.1016/j.still.2006.01.004.
133. Garbeva P, van Veen JA, van Elsas JD. 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270. https://doi.org/10.1146/annurev.phyto.42.012604.135455.
134. Jones DL, Cooledge EC, Hoyle FC, Griffiths RI, Murphy DV. 2019. pH and exchangeable aluminum are major regulators of microbial energy flow and carbon use efficiency in soil microbial communities. Soil Biol Biochem 138:107584. https://doi.org/10.1016/j.soilbio.2019.107584.
135. Ontario Ministry of Agriculture. 1997. Best management practices, no-till: making it work. Ontario Ministry of Agriculture, Guelph, Ontario, Canada.
136. Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. 2014. Development of a prokaryotic universal primer for simultaneous analysis of *Bacteria* and *Archaea* using next-generation sequencing. PLoS One 9: e105592. https://doi.org/10.1371/journal.pone.0105592.
137. Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T. 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. Appl Environ Microbiol 82:7217–7226. https://doi.org/10.1128/AEM.02576-16.
138. Simmons T, Caddell DF, Deng S, Coleman-Derr D. 2018. Exploring the root microbiome: extracting bacterial community data from the soil, rhizosphere, and root endosphere. J Vis Exp 135:e57561. https://doi.org/10.3791/57561.
139. Gao C, Montoya L, Xu L, Madera M, Hollingsworth J, Purdom E, Hutmacher RB, Dahlberg JA, Coleman-Derr D, Lemaux PG, Taylor JW. 2019. Strong succession in arbuscular mycorrhizal fungal communities. ISME J 13:214–226. https://doi.org/10.1038/s41396-018-0264-0.
140. Deng S-L, Wipf HM, Pierroz G, Raab TK, Khanna R, Coleman-Derr D. 2019. A plant growth-promoting microbial soil amendment dynamically alters the strawberry root bacterial microbiome. Sci Rep 9:1–15. https://doi.org/10.1038/s41598-019-53623-2.
141. Xu L, Naylor D, Dong Z, Simmons T, Pierroz G, Hixson KK, Kim Y-M, Zink EM, Engbrecht KM, Wang Y, Gao C, DeGraaf S, Madera MA, Sievert JA, Hollingsworth J, Birdseye D, Scheller HV, Hutmacher R, Dahlberg J, Jansson C, Taylor JW, Lemaux PG, Coleman-Derr D. 2018. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. Proc Natl Acad Sci U S A 115:E4284–E4293. https://doi.org/10.1073/pnas.1717308115.
142. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10. https://doi.org/10.14806/ej.17.1.200.
143. Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Tennessen K, Palaniappan K, Szeto E, Pillay M, Chen I-MA, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2016. The standard operating procedure of the DOE-JGI Metagenome Annotation Pipeline (MAP v.4). Stand Genomic Sci 11:17. https://doi.org/10.1186/s40793-016-0138-x.
144. Lukashin AV, Borodovsky M. 1998. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res 26:1107–1115. https://doi.org/10.1093/nar/26.4.1107.
145. Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res 15:387–396. https://doi.org/10.1093/dnares/dsn027.
146. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471-2105-11-119.
147. Rho M, Tang H, Ye Y. 2010. FragGeneScan: predicting genes in short and error-prone reads. Nucleic Acids Res 38:e191. https://doi.org/10.1093/nar/gkq747.
148. Bray NL, Pimentel H, Melsted P, Pachter L. 2016. Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol 34:525–527. https://doi.org/10.1038/nbt.3519.
149. Robinson MD, Oshlack A. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 11:R25. https://doi.org/10.1186/gb-2010-11-3-r25.
150. Nakayasu ES, Nicora CD, Sims AC, Burnum-Johnson KE, Kim Y-M, Kyle JE, Matzke MM, Shukla AK, Chu RK, Schepmoes AA, Jacobs JM, Baric RS, Webb-Robertson B-J, Smith RD, Metz TO. 2016. MPLEx: a robust and universal protocol for single-sample integrative proteomic, metabolomic, and lipidomic analyses. mSystems 1:e00043-16. https://doi.org/10.1128/mSystems.00043-16.