Tillage homogenizes soil bacterial communities in microaggregate fractions by facilitating dispersal

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Abstract

Soil aggregation physically protects soil organic matter and promotes soil carbon persistence through microaggregate formation and organo-mineral associations. Tillage is a ubiquitous disturbance to arable soil that disrupts aggregation, thus affecting microbial resource availability, soil microhabitat conditions, and microbial interactions. We investigated how tillage affects bacterial community composition of soil microaggregate fractions (53–250 µm), specifically the free microaggregate fraction in bulk soil and the occluded microaggregate fraction within macroaggregates, using two long-term tillage vs. no-tillage experiments in southern WI, U.S., that represent two different silt loam soils (Alfisol and Mollisol). We applied 16S rRNA gene amplicon sequencing to characterize the effects of tillage on microaggregate bacterial communities by relating compositional changes and ecological community assembly patterns to various tillage-driven changes in the soil environment, including aggregate size distribution and carbon content. Tillage homogenized soil bacterial communities, as quantified by increased compositional similarity at both within-plot and between-plot scales, and community assembly was increasingly influenced by homogenizing dispersal with tillage. We did not identify major distinctions between bacterial communities of the free and occluded microaggregate fractions, thus highlighting how soil microaggregates readily shift between these operationally defined fractions in temperate annual cropping systems, where the soil environment is subject to drastic seasonal changes that are exacerbated by tillage. By identifying influential community assembly processes and analyzing communities in microaggregate fractions, we improve our understanding of the microbial response to soil disturbance, and thus the potential mechanisms through which disturbances like tillage affect soil carbon persistence.

Keywords:
microaggregates; tillage; aggregate size fractionation; community assembly; soil microbial communities; soil carbon

Abbreviations:
soil organic matter (SOM); soil organic carbon (SOC); particulate organic matter (POM)

1. **Introduction**

Agricultural tillage, employed on over 60% of U.S. farmland (Zulauf and Brown, 2019), disrupts the fine roots and fungal hyphae that stabilize macroaggregates (Tisdall and Oades, 1982; Elliott, 1986; Six et al., 1998), thus decreasing mean aggregate size by over 35% and/or proportion of aggregated soil by over 20% (Frey et al., 1999; Six et al., 1999; Al-Kaisi et al., 2014; Zheng et al., 2018). This disturbance reduces aggregate-associated physical protection of soil organic matter (SOM) while potentially increasing microbial mineralization of soil organic carbon (SOC) (Elliott, 1986; Paustian et al., 1997; Six et al., 1998; Schimel and Schaeffer, 2012). Improved understanding of the ecological factors that relate mixing disturbance-related changes in aggregation to microbial community composition and SOC persistence will populate a key knowledge gap in soil microbial ecology (Wilpiszeski et al., 2019). These relationships may be pertinent in the highly protected microenvironments of soil microaggregates (< 250 μm in diameter), which are more stable than macroaggregates (250–2000 μm in diameter) (DeGryze et al., 2006; Davinic et al., 2012; Totsche et al., 2018), and further inhibit microbial activity due to nutrient and oxygen limitation (Sexstone et al., 1985; Ranjard and Richaume, 2001). These same microhabitats that protect SOM from microbial decomposition are also disproportionately high in microbial abundance; an estimated 70% of soil bacteria live within microaggregates (Ranjard et al., 2000), despite the microaggregate fraction comprising perhaps 30–50% of arable soil by mass (Sheehy et al., 2015; Cates et al., 2016). Overall, the mechanisms that balance microbially mediated SOC persistence with carbon-consuming microbial activity in microaggregates (i.e., "microbial hotspots", *sensu* Kuzyakov and Blagodatskaya, 2015) are not well-understood (Six et al., 2004; Wilpiszeski et al., 2019).

Microaggregates are found both unprotected in the bulk soil, and occluded in macroaggregate structures (Oades, 1984; Totsche et al., 2018). With tillage-related macroaggregate instability, the potential for occluded microaggregate development within protective macroaggregate structures is reduced (Six et al., 2000a). Further, as macroaggregates destabilize, existing occluded microaggregates become more freely connected to the bulk soil environment, increasing resource diffusion (e.g., oxygen and extracellular enzymes) and decomposer pressure (Six et al., 1999; Garland et al., 2018; Piazza et al., 2020). Through these mechanisms, tillage has been associated with decreases in total SOM content (Elliott, 1986), SOM residence time (Paustian et al., 2000), SOC content (Paustian et al., 1997; Al-Kaisi et al., 2014; Zheng et al., 2018), aggregate-occluded particulate organic matter (POM) (Six et al., 1999), microbial biomass (Zuber and Villamil, 2016), and microbial necromass accumulation (Simpson et al., 2004). While these effects are well-documented, they are typically noted only in the top 5 or 10 cm of soil (Frey et al., 1999; Six et al., 1999; Simpson et al., 2004; Zheng et al., 2018), and some work suggests that tillage does not decrease total C stocks of the plow layer plus subsoil (Powlson et al., 2014; Ogle et al., 2019). There is also evidence that minimum tillage practices can be equally beneficial as no-tillage regarding SOC and microbial necromass accumulation, by incorporating nutrients and alleviating compaction (Sae-Tun et al., 2022).

A recent meta-analysis demonstrated that the occluded microaggregate fraction preferentially accumulates SOC at a higher rate than the free microaggregate and other soil fractions (King et al., 2019). One study found over 90% of the increase in SOC content in no-tillage as compared to conventional tillage systems was attributable to the occluded microaggregate fraction, across soils of various clay mineralogies (Denef et al., 2004), while another study found that the occluded microaggregate fraction contributed 49–112% of the increase in SOC following a shift to no-tillage across the U.S. (Six and Paustian, 2014). Together, this indicates a higher capacity for SOC persistence in the occluded microaggregate fraction as compared to the free microaggregate fraction.

In the limited number of studies that have applied high-throughput sequencing to aggregate fractions, distinct and more diverse bacterial communities are supported by the free microaggregate fraction than the macroaggregate fraction (Trivedi et al., 2017; Bach et al., 2018; Upton et al., 2019). One study that specifically assayed communities of the free vs. occluded microaggregate fractions found both to harbor similar community compositions, yet suggested that copiotrophic bacteria live in association with free microaggregates whereas oligotrophic bacteria are characteristic of occluded microaggregates (Biesgen et al., 2020). This assessment is consistent with the idea that free microaggregates have higher resource availability, notably C and oxygen, that may support copiotrophic microorganisms, whereas occluded microaggregates may be more insulated from perturbation, resource fluxes, and decomposers, as evidenced by increased SOC persistence (King et al., 2019). The effects of tillage on soil microenvironments (e.g., aggregate size and porosity), and the resulting redistribution of resources (e.g., oxygen, water, biomass), suggests that tillage also alters soil microbial community composition and function (Bhattacharyya et al., 2021). Tillage-driven decreases in aggregate size may select for more oligotrophic communities due to lower substrate and oxygen availability (Trivedi et al., 2017), though some have found fast growing, copiotrophic competitors to dominate soil communities under tillage or disturbance (Srour et al., 2020; West and Whitman, 2022). These results suggest that tillage-related impacts on macroaggregate formation and turnover (and, thus, occluded microaggregates) extend to microbial community composition (Six et al., 2004), and understanding changes in microbial communities under a given management practice, such as tillage, is essential for improving predictions of SOC persistence and storage.

Tillage disperses soil and its inhabitants, and can impact abiotic soil conditions and biotic interactions through changes to soil structure. These effects can be quantified by estimating the influence of ecological community assembly processes (Vellend, 2010), which are as follows: Dispersal describes the generally stochastic movement and establishment of organisms in space, and may occur in soil via physical disturbance or mass flow of pore water (Zhou and Ning, 2017). Homogenizing dispersal increases compositional similarity between communities, whereas dispersal limitation increases compositional differences between communities, which may allow for stochastic demographic changes in community composition — termed ‘drift’ (Stegen et al., 2013). Selection refers to deterministic or niche-based processes dictated by biotic factors, such as inter-taxa fitness differences, and abiotic factors, such as environmental filters (Hutchinson, 1957). Homogeneous selection decreases phylogenetic differences between communities due to community assembly under similar conditions or filters (Dini-Andreote et al., 2015). Variable selection increases phylogenetic differences between communities due to variable conditions (Stegen et al., 2015). When neither dispersal nor selection are identified, community assembly is considered undominated by any particular process, which may reflect stochastic drift, or potentially multiple community assembly processes interacting to obscure a singular process signal (Ning et al., 2020). To statistically infer the relative influences of these community assembly processes in soil microbial communities, Stegen et al. (2012, 2013, 2015) developed a null modeling approach that compares observed phylogenetic distances and dissimilarity metrics between communities to null models of stochastically assembled communities. A more recent approach separately assessed community assembly processes within phylogenetically related 'bins' of OTUs, thus enabling representation of various assembly processes that may influence subsets of community members (Ning et al., 2020). To our knowledge, these approaches have not yet been used to directly compare the effects of tillage on community assembly, let alone at the microaggregate fraction scale.

We sought to better understand how bacterial communities are affected by tillage, as modulated through soil aggregation. We collected soil samples in no-tillage and chisel-plowed tillage plots from two long-term studies in southern Wisconsin, U.S., and related soil properties to tillage-driven differences in bacterial community composition, diversity, and community assembly processes of the bulk soil, free microaggregate, and occluded microaggregate fractions, using 16S rRNA gene amplicon sequencing. In addition to expecting standard responses to tillage including decreased SOC and aggregation, we hypothesized that the free microaggregate and occluded microaggregate fractions would support distinct bacterial communities, and demonstrate differences due to tillage treatments. Specific hypotheses included: H1) With tillage, community assembly would be driven by the influence of homogenizing dispersal and homogeneous selection, whereas in the no-tillage system, community assembly would be driven by dispersal limitation and variable selection, as determined via null model-based estimates of community assembly processes. H2) The communities of the free and occluded microaggregate fractions would be distinct from each other, and the occluded microaggregate fraction would demonstrate stronger evidence for dispersal limitation, whereas the free microaggregate fraction would demonstrate stronger evidence for homogeneous selection. H3) Tillage would increase sample-to-sample similarity in community composition (i.e., lower beta diversity). Better understanding microbial community composition and assembly in microaggregate environments will improve our understanding of mechanisms of SOC persistence, thus contributing to climate resilience (Paustian et al., 2000), ecosystem services, and crop productivity (Janzen, 2006).

2. Methods

2.1 Soil collection

Soil was sampled from two separate long-term tillage studies located at 1) the University of Wisconsin (UW) Arlington Agricultural Research Station in Arlington, WI, U.S., (43°17’56”N, 89°21’11”W, 314 m a.s.l.) on a Plano silt loam soil (fine-silty, mixed, superactive, mesic Typic Argiudoll), under a corn (*Zea mays* L.) – soybean (*Glycine max* L.) rotation; and, 2) the UW Lancaster Agricultural Research Station in Lancaster, WI, U.S., (42°49’53”N, 90°47’35”W, 313 m a.s.l.) on a Fayette silt loam soil (Fine-silty, mixed, superactive, mesic Typic Hapludalfs), under a continuous corn rotation. The tillage study at Arlington, WI was established in 1987 with a no-tillage treatment, in which crops are planted directly into the undisturbed residue of the previous crop, and a tillage treatment, which consists of fall chisel plow (~ 20 cm depth) followed by two spring field cultivator passes prior to planting. Further details regarding management practices and agronomic findings have been reported (Pedersen and Lauer, 2003; Chamberlain et al., 2021). The tillage plots at Lancaster, WI were established in 1993, consisting of no-tillage and tillage treatments, the latter of which consists of fall chisel plow and a spring field cultivator pass prior to corn planting. The Lancaster plots have been used for various research projects over the years (e.g., Gupta et al., 2004; Dolliver and Gupta, 2008), which sometimes included manure application treatments (1993–1997, 2003–2005, 2014) or corn fungicide treatments (2008–2010); best efforts were made to avoid split plot areas that received manure. Soil was sampled once in each location (23 October 2021 at Arlington, WI and 6 November 2021 at Lancaster, WI), following corn grain harvest and prior to fall tillage to capture relatively static soil conditions and communities. At Arlington, three plots were sampled for each treatment, collecting five intact cores per plot for a total of 15 cores per treatment. Due to our interest in discerning dispersal processes, our sampling design focused on ensuring relatively high spatial proximity of individual cores within a given plot. Soil cores were 7.9 cm dia, evenly spaced just within the perimeter of a 48 cm dia circle; distance between adjacent cores was approximately 15 cm. As detailed below, the top 5 cm was analyzed to target soil under the greatest intensity of tillage disturbance. Lancaster was sampled in the same fashion, but only two plots per treatment were used for analysis (see section 2.7), for a total of ten cores per treatment. Intact cores were temporarily kept in a cooler, and then held at 4 °C for up to ten days until sample processing. Plots were within 300 m proximity at both sites.

2.2 Aggregate size fractionation and sample processing

To assess variability in community composition and community assembly at a relatively small spatial scale, each core was processed separately. The top 5 cm of each field-moist soil core were gently passed through a 2 mm sieve (henceforth referred to as “bulk” soil). Then, 80 g of this field-moist bulk soil was subjected to aggregate size fractionation via wet sieving (Elliott, 1986) to isolate the macroaggregate fraction (250 μm–2000 μm), free microaggregate fraction (53 μm–250 μm), and the silt + clay-sized fraction (< 53 μm) (Fig. 1). Then, 20 g of the moist macroaggregate fraction was separated into occluded fractions via rapid shaking with glass beads in water to break up the macroaggregates, as previously described (Six et al., 2000a, 2002); macroaggregate-occluded fractions included the occluded microaggregate fraction (53 μm–250 μm), occluded silt + clay-sized fraction (< 53 μm), and occluded coarse POM + coarse sand-sized fraction (250 μm – 2000 μm). Modifications to the cited wet sieving methods included a slaking for two minutes prior to the first wet sieving step (Arlington samples only) and draining each wet sieved fraction for two minutes prior to subsampling as described below. The largely unaggregated Lancaster soil samples did not undergo slaking, and required wet sieving of an additional 80 g of bulk soil to obtain enough macroaggregate fraction for the occluded fraction separation step. The primary objective of fractionation was to isolate the free and occluded microaggregate fractions, but the relative dry mass of each size fraction was also determined. As such, sand content correction of aggregate fractions was not performed and thus all size fractions also include primary mineral particles of that size. Sieves and utensils were cleaned with ethanol between samples.

Gravimetric moisture content was estimated for bulk soil, the wet-sieved macroaggregate fraction, the free microaggregate fraction, and the occluded microaggregate fraction by drying subsamples in a 60 °C oven for 24 hours. Field moist bulk, free microaggregate, and occluded microaggregate soil was subsampled for DNA extraction (see section 2.4), and bulk soil was also subsampled to measure soil respiration (see section 2.3). The remaining wet-sieved soil was washed from each sieve (or washbasin) into aluminum pans to determine the mass of each fraction, dried to 100 °C. Overall recovery (macroaggregate + free microaggregate + [silt + clay] fractions) was 99% for both treatments at both sites, and macroaggregate recovery (occluded microaggregate + [occluded silt + clay] + occluded coarse POM) was 101% for tillage treatments, and 96–97% for no-tillage treatments.

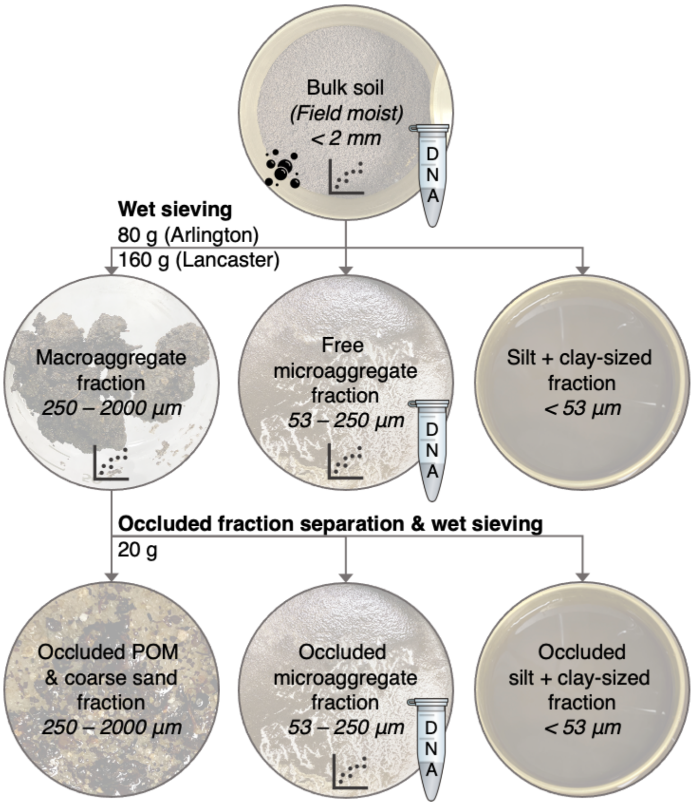

Figure 1. Aggregate size fractionation schematic. Bulk soil (80 g or 160 g) was subjected to wet sieving to separate macroaggregate (250–2000 μm), free microaggregate (53–250 μm), and silt + clay-sized (< 53 μm) fractions. A 20 g (wet) subsample of the macroaggregate fraction was then further separated into occluded microaggregate (53–250 μm), occluded silt + clay-sized (< 50 μm), and occluded POM + coarse sand fractions (250–2000 μm). The "DNA" tube indicates that subsamples were retained for 16S rRNA gene amplicon sequencing. The graph icon indicates that subsamples were collected to measure total carbon and total nitrogen. The bubble icon indicates that soil respiration was measured on bulk soil.

2.3 Soil analysis

The bulk soil (sieved to < 2 mm), macroaggregate, free microaggregate, and occluded microaggregate fraction subsamples that were retained for dry mass conversion were ground to a powder using 2.4 mm ball bearings and a FastPrep-24 (MP Biomedical, Santa Ana, CA, U.S.), and used to quantify total soil carbon and nitrogen by flash combustion with a Flash EA 1112 CHN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy) and soil pH (soil pH method and results can be found in the Supplementary Information). For routine soil analysis, a composite soil sample representing each treatment was comprised of an equal mass of bulk soil from each plot, and samples were submitted to the UW Soil and Forage Analysis Lab (Madison, WI, U.S.) to determine soil texture, organic matter content, pH, and plant-available P, K, Ca, and Mg, as reported in Table S1.

Soil respiration (CO_2 evolution) from fresh sieved soil was measured using the MicroResp system (James Hutton Ltd., Aberdeen, Scotland), following general instructions for use and calculation of CO_2 evolution, without added substrate (Campbell et al., 2003), per the MicroResp manual (version 4). At the time of aggregate fractionation, 300 mg of freshly sieved (< 2 mm), field-moist soil from each soil core was placed into each of six wells of a deep-well plate, covered and stored at 4 °C for up to six hours. Each deep-well plate, containing soil from up to ten different cores, was covered in parafilm and firmly tapped on the benchtop 20 times to repack soil and minimize large air pockets. The deep-well plate was then incubated at 25 °C in a dark CO_2-free environment for approximately 16 hours to help deplete CO_2 from the well headspace and soil air. Then, a colorimetric detection plate was read at absorbance wavelength 570 nm using a BioTek Synergy 2 spectrophotometer microplate reader. After confirming that all detection plate wells had similar readings (< 5 % coefficient of variance), the detection plate was inverted over the deep-well plate, connected by the 96-well seal, and clamped together. After six hours of incubation at 25 °C, the colorimetric plate was read to determine CO_2 evolution.

2.4 DNA extraction and 16S rRNA gene sequencing

Total genomic DNA was extracted from bulk soil, free microaggregate, and occluded microaggregate soil fractions using the DNeasy PowerLyzer PowerSoil Kit (Catalog No. 12855, Qiagen, Germantown, MD), following manufacturer's instructions. We used 250 mg samples of field-moist bulk soil for DNA extraction, but, due to the wetness of the microaggregate fractions following wet sieving, we used 450 mg samples of these fractions to capture the same dry-mass equivalent of 250 mg of field-moist bulk soil, based on preliminary measurements of gravimetric water content. The microaggregate samples were transferred directly from the drained soil sieves into the DNA extraction tubes, which were immediately frozen at −20 °C, and stored at −80 °C for up to three months prior to DNA extraction. Complete library preparation details can be found in the Supplementary Information. Briefly, the 16S rRNA genes of extracted DNA were amplified in triplicate using PCR. Variable region V4 of the 16S rRNA gene was targeted using forward primer 515f and reverse primer 806r (Walters et al., 2016). Primers also contained barcodes and Illumina sequencing adapters (Kozich et al., 2013). The following reagents comprised each 25 µL PCR reaction: 1 µL DNA extract, 12.5 µL Q5 Hot Start High-Fidelity 2X Master mix (Catalog No. M0494, New England BioLabs, Ipswich, MA), 1.25 µL 515f forward primer (10 mM), 1.25 µL 806r reverse primer (10 mM), 1.25 µL Bovine Serum Albumin (20 mg/mL; Catalog No. 97064-342, VWR International, Radnor, PA), and 7.75 µL PCR-grade water. The plate was centrifuged prior to 30 PCR cycles on an Eppendorf Mastercycler nexus gradient thermal cycler (Hamburg, Germany) using the following parameters: 98 °C for 2 min + 30 × (98 °C for 10 seconds + 58 °C for 15 seconds + 72 °C for 10 seconds) + 72 °C for 2 min and 4 °C hold. Amplified DNA was confirmed via gel electrophoresis, then normalized and purified (as detailed in the Supplementary Information), prior to paired-end 250 base pair sequencing on an Illumina MiSeq sequencer at the UW–Madison Biotech Center. To obtain high coverage, the same library was sequenced twice under identical conditions, and total reads were pooled for each sample after processing as described next. Sequencing data were processed using a QIIME2 (Bolyen et al., 2019) pipeline, with DADA2 (Callahan et al., 2016) as the operational taxonomic unit (OTU, or amplicon sequence variant)-picking algorithm, and taxonomy was assigned using the SILVA 132 reference database (Quast et al., 2013; Yilmaz et al., 2013). This yielded 10,102,355 demultiplexed sequences, which was reduced to 6,307,452 after denoising, with a mean length of 227 base pairs (SD = 2.2). Excluding extraction blanks, a total of 18,180 OTUs were identified. Amplicon sequences are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession

PRJNA977693. Our primers targeted both bacteria and archaea, but because our reads were dominated by bacteria (94.5% of total reads), we will simply refer to bacteria in this manuscript. Over 99% of archaeal reads represented the phylum *Crenarchaeota*.

2.5 Data analysis

Data analysis was performed in R (R-Core-Team, 2018), using *ggplot2* (Wickham, 2016) for data visualization. The R code used to perform these analyses and to create the following figures is available at https://github.com/jaimiewest/Soil-Disturbance-Tillage. To test for a significant effect of tillage treatment on proportion of soil in each fraction, C content of each fraction, and respiration, we used ANOVA followed by Tukey's HSD *post-hoc* comparison for significant results ($p < 0.05$). To test for a significant effect of tillage treatment, soil fraction, or interaction of these factors on soil C content, soil N content, and soil C:N ratio, we performed ANOVA as described above. Unless otherwise noted, reported p values refer to ANOVA tests.

Community composition was visualized using principal coordinates analysis (PCoA) created with the *ordinate* function in the *phyloseq* package (*phyloseq::ordinate*) (McMurdie and Holmes, 2013) using Bray-Curtis dissimilarities (Bray and Curtis, 1957) of Hellinger-transformed relative abundance data (Legendre and Gallagher, 2001). To test for a significant effect of tillage treatment, soil fraction, or interaction of these factors on community composition, we used permutational multivariate analysis of variance (PERMANOVA) to partition Bray-Curtis dissimilarity matrices among sources of variation (*vegan::adonis2*) (Anderson, 2001). A significant result ($p < 0.05$) was subjected to *post-hoc* pairwise factor comparisons, adjusting p-values using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) to identify significant differences. To compare differences in community composition due to tillage treatment or soil fractions, we tested for homogeneity of multivariate dispersions (PERMDISP; *vegan::betadisper*) (Anderson, 2006), using ANOVA to test the distances to group spatial median. Further, we also evaluated the effect of tillage treatment on dispersion of free and occluded microaggregate fraction communities within each soil core. To describe richness, we used the weighted linear regression model of OTU richness estimates, which weights observations based on variance (*breakaway::betta*) (Willis et al., 2017). We also calculated Faith's phylogenetic diversity (PD) (Faith, 1992; Pérez-Valera et al., 2015) to assess differences in phylogenetic distance (i.e., sample branch length) using *picante::pd* (Kembel et al., 2010).

To further understand changes in community composition, we calculated the weighted mean predicted 16S rRNA gene copy number (Nemergut et al., 2016), which has been shown to correlate with potential growth rate (Klappenbach et al., 2000) and disturbance (Whitman et al., 2019; West and Whitman, 2022), and compared tillage treatments and soil fractions using ANOVA and *post-hoc* testing as described above. 16S rRNA gene copy numbers were predicted using the ribosomal RNA operon database (rrnDB) (Stoddard et al., 2015).

After evaluating our key questions, we used differential abundance to identify significant treatment-driven shifts in relative abundances of individual taxa as well as phyla. For this analysis, we compared the tillage treatments to each other (excluding taxa with mean relative abundance < 0.00001) and subjected those data to a beta-binomial regression model and "Wald" hypothesis test (*corncob::differentialTest*) (Martin et al., 2021), which controls for the effect of the treatment on dispersion. We report the μ value, which is the coefficient used to estimate relative abundance in the *corncob* model, and is proportional to the fold-change in relative abundance between the treatment and control. We also assessed differential abundances of taxa in the microaggregate fractions as compared to the bulk soil communities.

2.6 Community assembly process assignment

In order to determine the influence of community assembly processes characteristic of each treatment and fraction (including bulk soil), we compared sample pairs of interest (i.e., each possible pair of samples from the same site, tillage treatment, and fraction) to stochastically-assembled null models in order to determine the relative influence of selection (based on phylogenetic distances), or dispersal (based on compositional dissimilarities), as detailed below. In order to capture the various community assembly processes of the microbial subcommunities within each soil sample, community assembly processes were assigned separately to phylogenetically-related bins of OTUs (*iCAMP::pdist.big* and *iCAMP::icamp.big*), as detailed by Ning et al. (2020), and the dominant process was weighted by the relative abundance of the taxa in that bin. This method is based on the full-community (i.e., not binned) compositional assessment developed by Stegen et al. (2012, 2013, 2015); our full-community assessment is reported in the Supplementary Information and in Figure S9.

The influence of selection was first tested using the abundance-weighted beta-mean nearest taxon distance (βMNTD; the mean phylogenetic distance between each OTU in one community and its closest relative in another community) (Kembel et al., 2010). Homogeneous selection was identified in comparisons for which βMNTD was more than 2 standard deviations below the mean of the null distribution, indicating lower mean phylogenetic distance between pairwise communities than observed in the null. Variable selection was identified in comparisons for which βMNTD was more than 2 standard deviations above the mean of the null distribution, indicating higher mean phylogenetic distance between pairwise communities than observed in the null. Comparisons that fell within 2 standard deviations of the null mean were considered to lack a dominant influence of selection, and were subsequently tested for the influence of dispersal using the modified Raup-Crick metric based on Bray–Curtis dissimilarities (RC_{Bray}) (Chase et al., 2011). Homogenizing dispersal was identified in comparisons for which RC_{Bray} was significantly lower than the mean of the null distribution, indicating a higher level of similarity between community compositions than was observed in the null condition; and dispersal limitation was identified in comparisons for which RC_{Bray} was significantly higher than the null mean, indicating lower similarity. Comparisons that were similar to the null mean for both metrics were considered undominated by any particular community assembly process, which may reflect stochastic assembly or a lack of a singular signal due to multiple community assembly processes within a subcommunity.

We selected bins and ran the analysis using the default parameters as detailed in Ning et al. (2020) and the associated R documentation (i.e., minimum of 24 OTUs per bin, confirmed by phylogenetic signal testing using *iCAMP::dniche* and *iCAMP::ps.bin*; phylogenetic null model randomization within bins; taxonomic null model randomization across all bins), with the exception of the phylogenetic distance metric, for which we used βMNTD so that results would be more comparable to the full-community scale assessment based on the method by Stegen et al. (2012, 2013, 2015). To test for a significant effect of tillage treatment on the influence of community assembly processes that had > 5% influence, we performed ANOVA as described above. Community relative abundance data was Hellinger-transformed for all community assembly assessments (Legendre and Gallagher, 2001).

2.7 Exclusion of plots from analysis

At the Lancaster site, one no-tillage plot and one tillage plot were excluded from analysis. Though this field has been under long-term tillage treatments since the early 1990's, there are strong indicators in the dataset that the two plots in question were subjected to treatments or conditions that differentiate them from the other plots, likely due to the split-plot use of manure and/or corn fungicide treatments, or the disruptive installation of large pan lysimeters. Though we made every attempt to avoid areas where manure was applied or lysimeters were installed, it was challenging to confirm the precise boundaries of historic split-plots, and the history of fungicide application (unpublished) was unbeknownst to us prior to sampling. The microbial community compositions of the excluded plots are clearly differentiated in the PCoA (Fig. S1).

3. Results

3.1 Tillage generally decreased aggregation; responses differed by site

At Arlington, over 60% of bulk soil (dry mass basis) was in water-stable aggregate fractions (macroaggregate plus microaggregate fractions), with over 50% of soil in the macroaggregate fraction (Fig. 2A). There were no significant differences in proportion of soil in macroaggregate, free microaggregate, or silt + clay fractions due to tillage treatment, but within the macroaggregate fraction (Fig. 2C) there was a significant decrease in proportion of soil in the occluded microaggregate fraction ($p < 0.001$; Fig. 2C) from 28% in no-tillage to 22% with tillage, with a complementary increase in the occluded silt + clay fraction ($p < 0.001$).

At Lancaster, soil was largely unaggregated, with 25% and 14% of bulk soil in water-stable aggregate fractions in the no-tillage and tillage treatments, respectively (Fig. 2B). In particular, the tillage treatment had a significantly lower proportion of soil in the macroaggregate fraction, with 6% as compared to 14% in the no-tillage treatment ($p < 0.001$). This was complemented by a significantly higher proportion in the silt + clay fraction ($p < 0.001$). Within the macroaggregate fraction at Lancaster (Fig. 2D), the proportions of the occluded microaggregate fraction and occluded silt + clay fractions were both significantly lower with tillage as compared to no-tillage ($p < 0.001$ and $p < 0.05$, respectively), with 17% of macroaggregate soil in the occluded microaggregate fraction in no-tillage, down to 11% with tillage. There was also a significant increase in occluded POM ($p < 0.001$), from 9% in the no-tillage treatment to 21% with tillage.

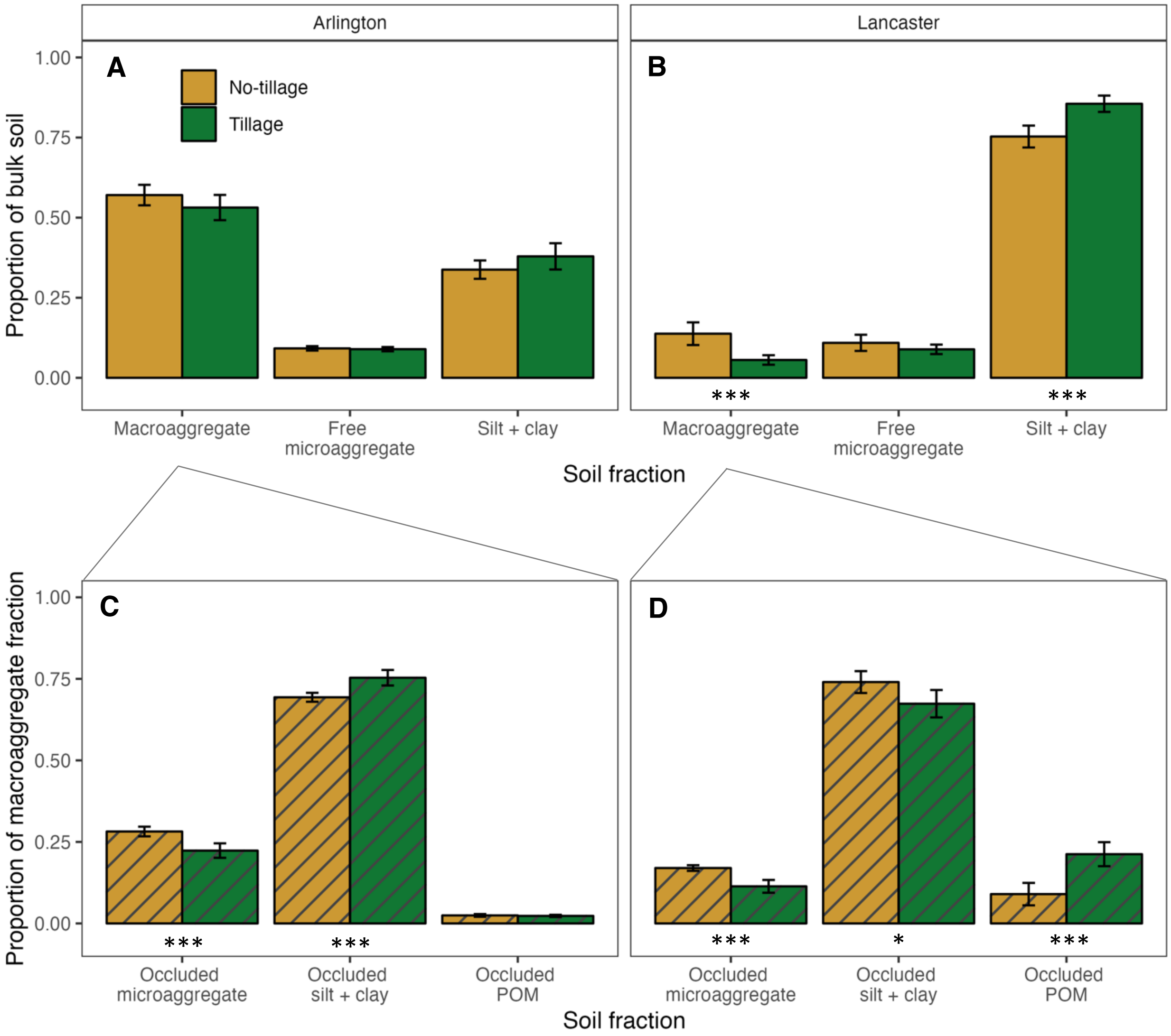

Figure 2. Distribution of bulk soil in various fractions at Arlington, WI (**A**) and Lancaster, WI (**B**), on a dry soil basis. Lower panels show distribution of macroaggregate soil in the occluded fractions (**C** and **D**). Macroaggregate = macroaggregate fraction, 250–2000 µm; Free microaggregate = microaggregate fraction from bulk soil, 53–250 µm; Silt + clay = silt and clay-sized fraction from bulk soil, < 53 µm; Occluded microaggregate = microaggregate fraction occluded in macroaggregate fraction, 53–250 µm; Occluded silt + clay = silt and clay-sized fraction occluded in macroaggregate fraction, < 53 µm; Occluded POM = particulate organic matter and sand occluded in macroaggregate fraction, 250–2000 µm. Error bars represent ± 1.96 SE (95% CI). Asterisks indicate significant tillage treatment differences within soil fraction: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. Striped bars denote occluded fractions.

3.2 Tillage reduced total soil carbon

Tillage decreased total carbon content in all measured fractions at both sites, reported here on a per unit of bulk soil basis (Fig. 3; $p < 0.001$ for each fraction at Arlington, $p < 0.05$ for each fraction at Lancaster). At Arlington, C concentrations of both free and occluded microaggregate fractions were greater than those of the bulk soil and macroaggregate fractions ($p < 0.05$, Tukey's HSD) in both treatments, and C concentration of the occluded microaggregate fraction was greater than the free microaggregate fraction in no-tillage only ($p < 0.01$, Tukey's HSD) (Table S2). At Lancaster, the C concentrations of both free and occluded microaggregate fractions were greater than C concentrations in the macroaggregate fraction and the bulk soil in the no-tillage treatment only ($p < 0.001$, each comparison, Tukey's HSD), and C concentration of the free microaggregate fraction was greater in no-tillage as compared to tillage ($p < 0.001$, Tukey's HSD). See the Supplementary Information for more detailed soil C and soil N results.

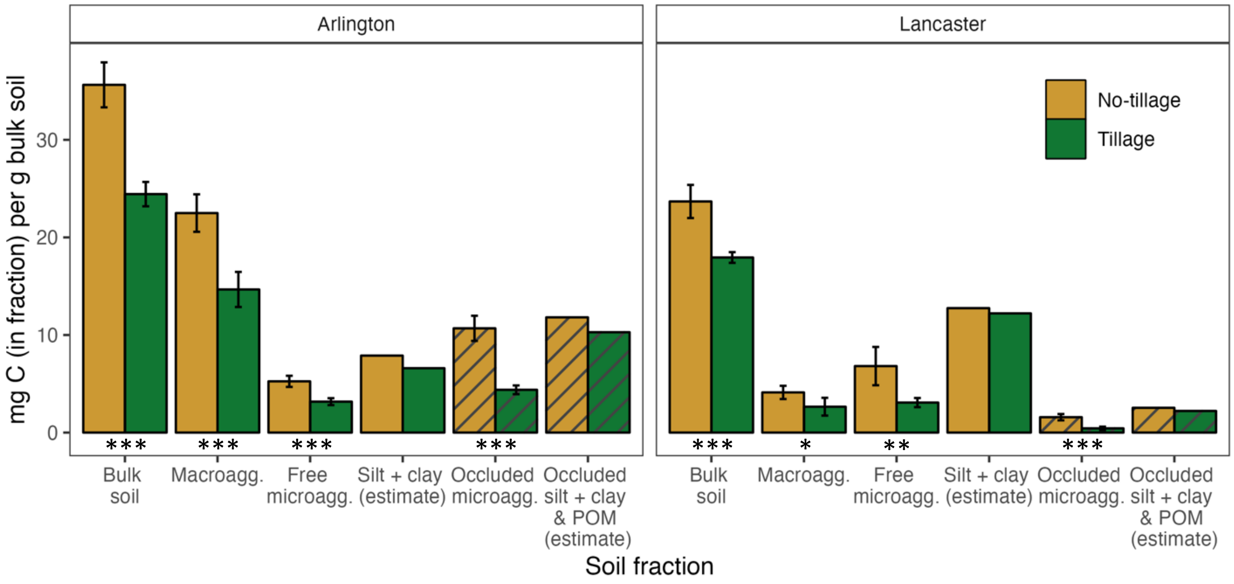

Figure 3. Carbon content of each soil fraction, on a per unit bulk soil basis. Bulk soil = whole soil; Macroagg. = macroaggregate fraction, 250–2000 µm; Free microagg. = microaggregate fraction from bulk soil, 53–250 µm; Silt + clay (estimate) = Carbon content in the < 53 µm fraction, estimated as Bulk soil – (Macroagg. + Free microagg.); Occluded microagg. = microaggregate fraction occluded in macroaggregate fraction, 53–250 µm; Occluded silt + clay & POM (estimate) = Carbon content in the < 53 µm fraction occluded in the macroaggregate fraction, estimated as Macroagg. – Occluded microagg. Error bars represent ± 1.96 SE (95% CI). Asterisks indicate significant treatment differences within soil fraction: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. The estimated silt + clay carbon contents do not have associated error bars or statistics. Striped bars denote occluded fractions.

The soil C:N ratio demonstrated significant effects of tillage treatment ($p < 0.001$) and soil fraction ($p < 0.001$) at both sites (Table S2 and Fig. S4). At Arlington, C:N ratio was greater in no-tillage compared to tillage ($p < 0.001$, Tukey's HSD), and greater in free and occluded microaggregate fractions compared to the macroaggregate fraction or bulk soil ($p < 0.001$, Tukey's HSD). At Lancaster, there was a significant interaction effect of tillage and soil fraction ($p < 0.001$), with a significantly higher C:N ratio in the macroaggregate fraction with tillage.

Tillage decreased respiration (CO_2 evolution from sieved, field-moist bulk soil) by 50% at Arlington ($p < 0.01$; Fig. S5) on a *per unit soil* basis, was though this difference was not significant on a *per unit soil C* basis ($p = 0.106$). Tillage did not have a significant effect on respiration at Lancaster. No-tillage plot samples averaged 23% gravimetric soil moisture at both sites, whereas tillage plots averaged 19–20% soil moisture; no adjustments to soil moisture were made prior to respiration measurements.

3.3 Tillage affected bacterial community composition

Bacterial community composition was significantly affected by tillage treatment at both sites (Fig. 4; $R^2 = 0.30$ and $p < 0.001$ at Arlington, $R^2 = 0.22$ and $p < 0.001$ at Lancaster; PERMANOVA). The homogeneity of variance test (BETADISPER) was also significant for tillage treatment at Arlington and Lancaster ($p < 0.001$, $p < 0.05$, respectively), which indicates that there are treatment differences in sample dispersion, and that the assumptions of the PERMANOVA were not met.

Tillage decreased dispersion of sample community composition by 14% and 6% relative to the no-tillage treatment at Arlington and Lancaster, respectively, as quantified by between-plot mean distance to spatial median (Fig. 5A and D; $p < 0.001$ for Arlington and $p < 0.01$ for Lancaster). This trend, which indicates higher dissimilarity of samples within the no-tillage treatment, was also apparent at the plot scale, where tillage decreased sample dispersion within plots by 13% and 5% relative to no-tillage at Arlington and Lancaster, respectively (Fig. 5B and E; $p < 0.001$ for Arlington and $p < 0.05$ for Lancaster). The dispersion of the free microaggregate and occluded microaggregate communities within each soil core did not significantly differ between tillage treatments, though there was a trend towards decreased dispersion with tillage at Lancaster ($p < 0.1$; Fig. 5C and F). These decreases in beta diversity with tillage were not apparently driven by decreases in alpha diversity since there were no significant differences in richness estimates (Fig. S7) or Faith's PD (Fig. S8) attributable to tillage at either site.

3.4 Community composition of free and occluded microaggregate fractions only differed slightly

There was a significant effect of soil fraction on bacterial community composition at both sites ($R^2 = 0.03$ and $p < 0.05$ for Arlington and $R^2 = 0.09$ and $p < 0.001$ for Lancaster, PERMANOVA; Fig. 4). Pairwise testing demonstrated significant differences at Lancaster only between bulk soil and the free microaggregate fraction, and between bulk soil and the occluded microaggregate fraction ($p < 0.01$), whereas pairwise testing amongst soil fractions was not significant at Arlington. Dispersion of sample community composition was homogeneous (i.e., beta diversity was similar) across soil fractions at both treatment and plot scales at both sites. There was no interaction effect of tillage treatment × soil fraction on community composition at either site.

Richness estimates demonstrated a significant effect of soil fraction ($p < 0.05$, Fig. S7) at Lancaster only; the richness estimate for the occluded microaggregate fraction was 8% lower than that of the bulk soil ($p < 0.05$, Tukey's HSD). Faith's PD was also affected by fraction ($p < 0.05$, Arlington, and $p < 0.001$, Lancaster; Fig. S8) by which the occluded microaggregate fraction was significantly lower than bulk soil at both sites ($p < 0.05$ and $p < 0.001$, respectively, Tukey's HSD), and the free microaggregate fraction was also lower than bulk soil at Lancaster ($p < 0.01$, Tukey's HSD).

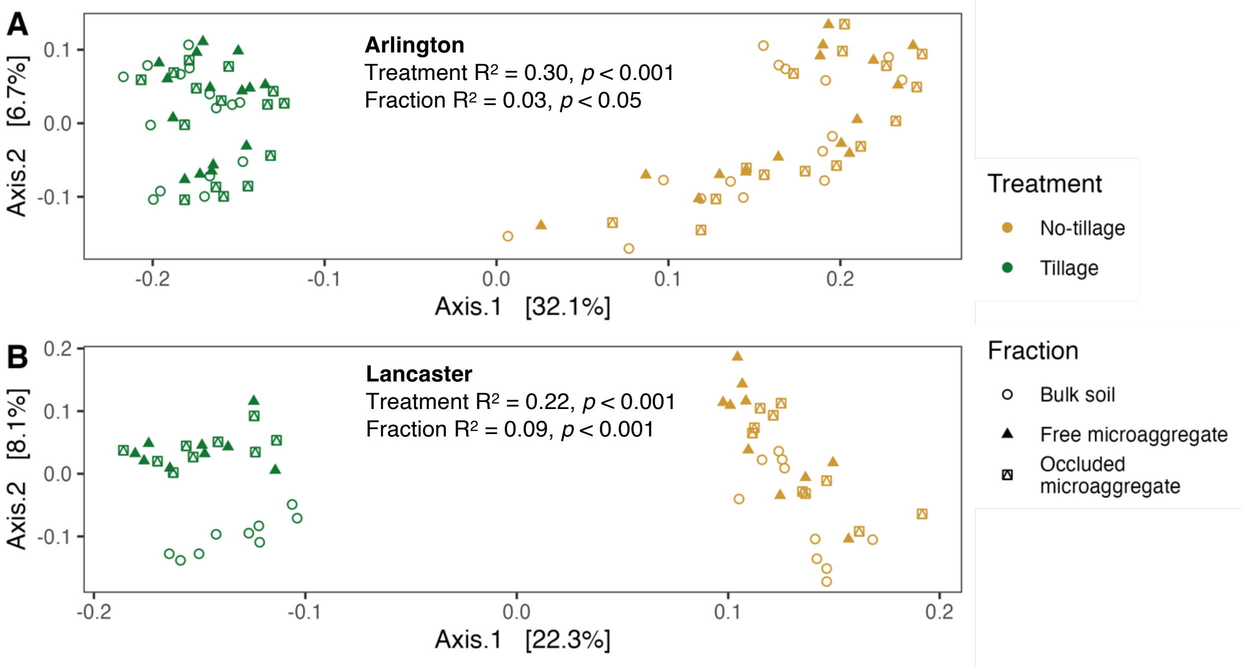

Figure 4. Principal coordinates analysis of Bray-Curtis dissimilarities of Hellinger-transformed community relative abundances, by tillage treatment for Arlington, WI (**A**), and Lancaster, WI (**B**). Each point represents the community of one sample-fraction. Soil fractions are as follows: Bulk soil = whole soil; Free microaggregate = microaggregate fraction from bulk soil, 53–250 µm; Occluded microaggregate = microaggregate fraction occluded in macroaggregate fraction, 53–250 µm. Displayed statistics are from PERMANOVA.

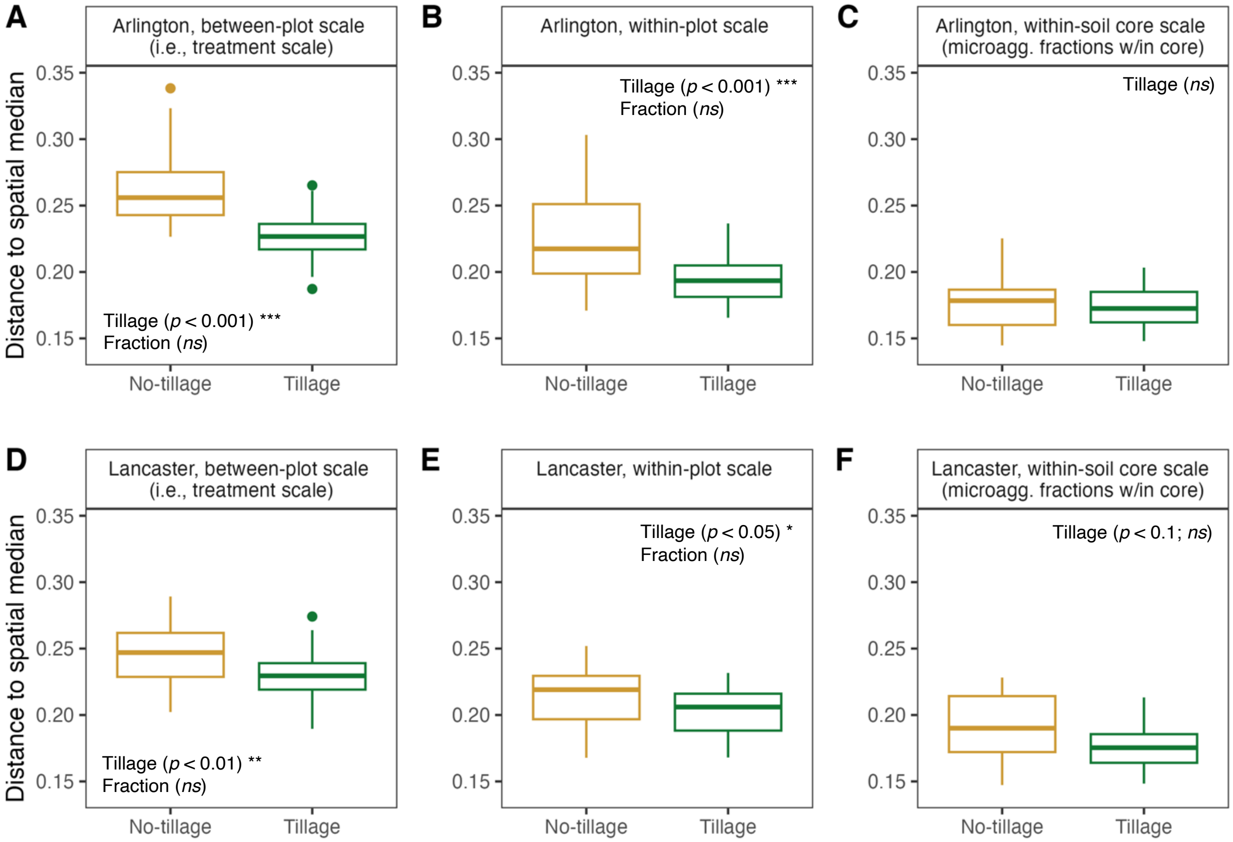

Figure 5. Dispersion of sample community composition as Bray-Curtis dissimilarities, represented here as distance to spatial median (which can be conceptualized as distance to the center [median] of a cloud of points in an ordination plot) at the between-plot scale (i.e., treatment scale; **A** and **D**); within-plot scale (**B** and **E**); and soil core scale (free vs. occluded microaggregate fraction samples within each soil core; **C** and **F**) at Arlington, WI (**A**, **B**, and **C**) and Lancaster, WI (**D**, **E**, and **F**). Data presented in A, B, D, and E represent bulk soil and both microaggregate fractions together.

3.5 Weighted mean predicted 16S rRNA gene copy number increased with tillage

At Arlington, there was small but statistically significant 7% increase in the weighted mean predicted 16S rRNA gene copy number with tillage ($p < 0.001$; Fig. 6). Fraction was also significant ($p < 0.001$), and there was a significant interaction effect of tillage and fraction

($p < 0.05$). The weighted mean predicted 16S gene copy number was lower in the occluded microaggregate fraction relative to the bulk soil or free microaggregate fraction in the tillage treatment, whereas weighted mean predicted 16S gene copy number was similar across fractions of the no-tillage treatment. At Lancaster, there was a significant 10% increase in the weighted mean predicted 16S gene copy number with tillage ($p < 0.001$), and no significant effect of fraction or interaction effect.

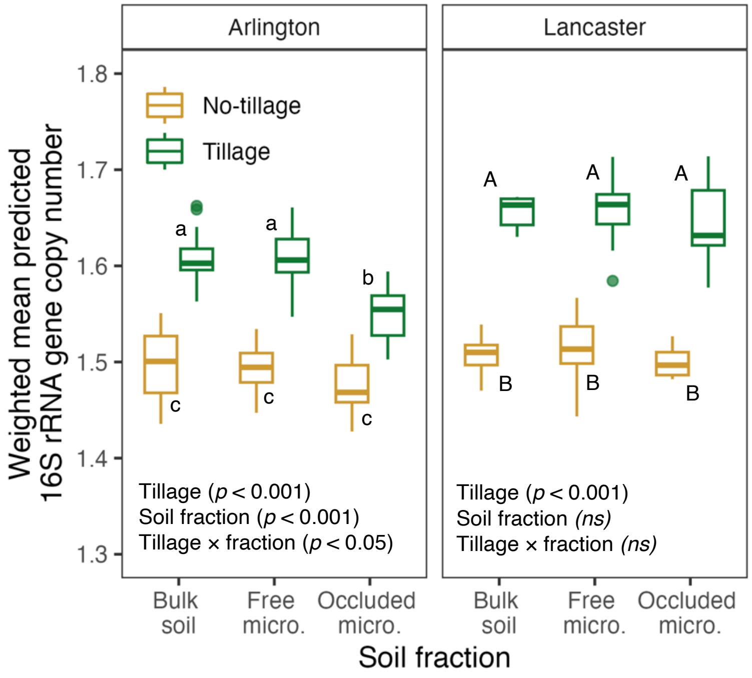

Figure 6. Weighted mean predicted 16S rRNA gene copy number. These data represent taxa for which a gene copy number was available in the rrnDB (Stoddard et al., 2015). Bulk soil = whole soil; Free micro. = microaggregate fraction from bulk soil, 53–250 µm; Occluded micro. = microaggregate fraction occluded in macroaggregate fraction, 53–250 µm. Boxplots with the same letter (within site) are not statistically different.

3.6 Influence of homogenizing dispersal increases with tillage

The OTU binning-based approach for assessing influential community assembly processes (*sensu* Ning et al., 2020) indicated that homogeneous selection had a ~14% relative influence across treatments and fractions, for both within-plot and between-plot comparisons at Arlington (Fig. 7A and B), and the between-plot comparisons demonstrated a significant decrease in homogeneous selection under tillage relative to no-tillage ($p < 0.05$). The influence of homogenizing dispersal significantly increased under tillage ($p < 0.001$), from 25% to 46% in bulk soil for within-plot comparisons; and from 12% to 28% for between-plot comparisons. There was also a large proportion of undominated comparisons— 30–60% at the within-plot scale and 50–70% at the between-plot scale.

At Lancaster (Fig. 7C and D), the within-plot comparisons demonstrated trends similar to those at Arlington regarding influences of homogeneous selection (~15%), homogenizing dispersal (~40%), and undominated (~45%). Unlike Arlington, there was no significant effect of tillage on the influence of homogenizing dispersal at the within-plot scale, whereas homogeneous selection significantly increased from 10% to 15% of relative influence in the bulk soil ($p < 0.05$). In the between-plot comparisons, the influence of homogenizing dispersal significantly increased under tillage in the bulk soil and the occluded microaggregate fraction ($p < 0.001$), whereas homogeneous selection experienced a small decrease with tillage in the free microaggregate fraction, and a small increase with tillage in the occluded microaggregate fraction ($p < 0.05$, and $p < 0.01$, respectively). For the between-plot comparisons, most were undominated— 60–80%.

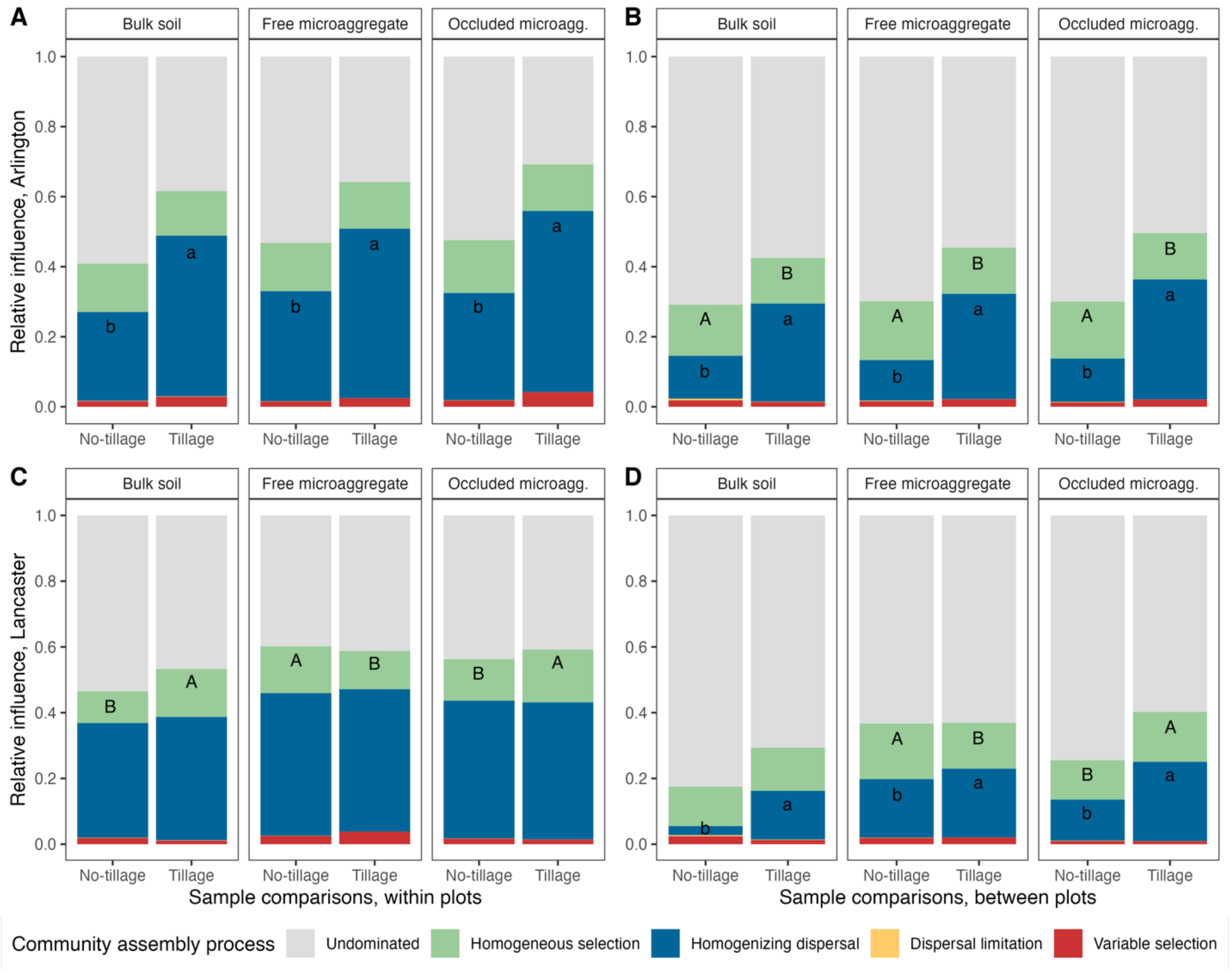

Figure 7. The relative influences of community assembly processes, by tillage treatment, within bulk soil, free microaggregate, and occluded microaggregate fractions at Arlington, WI (**A** and **B**); and Lancaster, WI (**C** and **D**). Sample comparisons were made within-plot (**A** and **C**) or between-plot (**B** and **D**). Community assembly processes were assigned within phylogenetically related bins of OTUs for pairwise comparisons of samples using a null modeling approach, and weighted by the relative abundance of OTUs in that bin (Ning et al., 2020). As detailed in the text, first the influence of selection was determined using the β-mean nearest taxon distance, and then the influence of dispersal was determined using the modified Raup-Crick metric based on Bray-Curtis dissimilarity. For homogeneous selection and homogenizing dispersal (the processes with > 5% influence), different letters signify a statistically significant difference in the influence of that process due to tillage (within site and fraction).

3.7 Taxonomic differences attributable to tillage

The most common phyla across the sites were *Actinobacteria*, *Acidobacteria*, and *Proteobacteria*, which together comprised about 60% of all reads at each site in both tillage treatments. At Arlington, there was a generally consistent phylum-level response across the bulk soil, free microaggregate, and occluded microaggregate fractions by which tillage resulted in significant increases in relative abundances of *Actinobacteria*, *Armatimonadota*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *Gemmatimonadetes*, and *Methylomirabilota*, and significant decreases in relative abundances of *Acidobacteria*, *Myxococcota*, *Proteobacteria*, and *Verrucomicrobia* (see Fig. S10 for relative abundances and *p* values). At Lancaster, tillage resulted in significant increases in relative abundances of *Chloroflexi*, *Cyanobacteria*, and *Gemmatimonadetes*, and significant decreases in relative abundances of *Crenarchaeota* and *Verrucomicrobia* (see Fig. S11 for relative abundances and *p* values).

We also identified key taxa associated with no-tillage and tillage treatments, based on differential abundance. Across both sites, we identified a total of 1658 taxa that were enriched with tillage (relative to the no-tillage treatment), and 1602 taxa that were enriched in no-tillage (relative to tillage). See Supplementary Table S3 for a complete list of enriched taxa, with coefficients of differential abundance (μ) and sequences. For tractability, we focused on the taxa with the biggest responses ($\mu > 1.0$), and only considered enriched taxa with mean relative abundances greater than 0.001 (0.1%), which resulted in 15 and 9 focal taxa enriched under tillage and no-tillage, respectively, at Arlington, and 9 and 4 focal taxa enriched under tillage and no-tillage, respectively, at Lancaster (Figs. S12 and S13). Though some taxa were unique responders within a soil fraction, numerous taxa were similarly enriched across bulk soil, free microaggregate, and occluded microaggregate fractions.

3.8 Taxonomic differences between microaggregate fractions

We also identified a total of 382 taxa across both sites taxa that were enriched in the free or occluded microaggregate fractions relative to the bulk soil, using differential abundance. See Supplementary Table S4 for a complete list of enriched taxa. Narrowing our focus on taxa with the biggest responses, as described above, there were 8 and 10 taxa enriched in the free microaggregate and occluded microaggregate fractions, respectively, at Lancaster, most of which were in the tillage treatment (Fig. S14), and no taxa fitting those parameters at Arlington. There were several *Chloroflexi* OTUs representing the class *Anaerolineae*, and several *Cyanobacteria* OTUs that were relatively enriched in the occluded microaggregate fraction. We did not assess taxa that were depleted in microaggregate fractions relative to bulk soil because the former is inherently a subset of the latter.

4. Discussion

We examined the effects of tillage on soil bacterial community composition and assembly, specifically in the free and occluded microaggregate fractions, and will discuss these findings with respect to soil carbon protection as modulated through changes to soil aggregation. Findings generally supported our hypotheses that tillage would homogenize bacterial communities, with community assembly driven by homogenizing dispersal. While fairly intuitive, this link between physical soil disturbance in an applied setting (i.e., tillage) and community assembly has not been previously established that we are aware of. On the other hand, our findings only weakly supported hypothesized distinctions between the free and occluded microaggregate communities, despite differences in soil C and a significant body of literature indicating various other differences between these fractions. Overall, we found decreased aggregation, soil C, and soil N with tillage (Figs. 2 and 3, Table S2), which agrees with previous work (Frey et al., 1999; Six et al., 1999; Al-Kaisi et al., 2014; Zheng et al., 2018). Overall, this supports the paradigm that tillage increases macroaggregate turnover, thus derailing occluded microaggregate formation, and decreasing soil C content through enhanced decomposition and weakened long-term protection (Six et al., 1999; King et al., 2019).

4.1 Tillage decreased soil aggregation and soil carbon

Our work provides further evidence supporting the relationship between soil aggregation and SOC content, while reiterating that tillage reduces aggregation and SOC in surface soil. We found that 90% of the increase in SOC under no-tillage relative to tillage was in aggregate fractions, with the majority (> 75%) of this increase specifically in the microaggregate fractions (Figs. 3 and S2). At Arlington, most of the increase in C under no-tillage was attributed to the occluded microaggregate fraction, which is consistent with previous work (Denef et al., 2004; Six and Paustian, 2014). However, at Lancaster, most of the increase in C was in the free microaggregate fraction, which could reflect post-season sample timing with respect to macroaggregate seasonal dynamics. As roots and hyphae die following crop plant senescence, macroaggregates rapidly destabilize, liberating previously occluded microaggregates into the free microaggregate pool (Perfect et al., 1990; Oades and Waters, 1991) (see Section 4.6).

As with the difference in SOC accumulation in the free vs. occluded microaggregate fractions, the two sites continue to tell somewhat different stories of aggregation and SOC distribution. Arlington exemplifies the "cultivation loop" (*sensu* Six et al., 1999), by which tillage stimulates decomposition and macroaggregate turnover, thus precluding SOM enrichment and resulting in older, C-depleted microaggregate fractions (Table S2). Alternatively, under no-tillage, undisturbed macroaggregates foster development of new occluded microaggregates, as indicated by higher C concentrations and wider C:N ratios in the microaggregate fractions (Table S2, Figs. S3 & S4).

On the contrary, at Lancaster, the macroaggregate fraction *under tillage* had a high C concentration, wide C:N ratio, and increased proportion of macroaggregate-occluded POM relative to no-tillage (Table S2, Figs. 2 and S4), indicative of largely undecomposed residue. Substantial residue at Lancaster is a testament to the continuous corn rotation—he residue from the previous crop (corn) was potentially double that of Arlington (where the previous crop was soybean), and of a higher C:N ratio (Ordóñez et al., 2020). Tillage breaks down and incorporates crop residue, bringing it into direct contact with mineral particles and soil microbiota to nucleate new macroaggregates, which could have enhanced C and POM concentration in the macroaggregate fraction, despite the overall tillage-driven decrease in proportion of macroaggregates. Though counter to how we typically characterize macroaggregates under tillage (e.g., low soil C and POM concentrations), this evidently less processed SOM in the macroaggregate fraction supports the overall narrative of a shorter mean macroaggregate lifespan under higher turnover with tillage (Elliott, 1986). In contrast, the corn-soy rotation at Arlington resulted in more straightforward soil C trends (e.g., C concentration in no-tillage > tillage; C concentration in microaggregates > macroaggregates and bulk soil; Table S2 and Fig S3). It would be relevant to repeat these measurements shortly after a fall tillage event to assess if tillage accelerates the decomposition of occluded POM and decreases SOC in the macroaggregate fraction, particularly in a system such as Lancaster where these metrics were high just prior to a fall tillage event.

The overall weak aggregation at Lancaster (Fig. 2), with less than 15% of soil in aggregates, lends support to a recently proposed paradigm shift that suggests soils under tillage may not be a relevant application of the physicogenic aggregate, but instead represent engineered, loosely arranged soil fragments that largely lack natural biopore networks (Or et al., 2021).

4.2 Tillage homogenized bacterial communities via dispersal

Tillage had a significant effect on bacterial community composition at both sites (Fig. 4), as observed by others (Srour et al., 2020; Bhattacharyya et al., 2021), which resulted in more homogeneous communities at both within-plot and between-plot scales, confirming hypothesis H3 (Figs. 4 and 5). At the within-plot scale, decreased compositional differences with tillage (Fig. 5) may be driven by homogenizing dispersal at Arlington (Fig. 7A), partially confirming hypothesis H1. At Lancaster, the relatively smaller effect of tillage on community composition (Fig. 4) and compositional dispersion (Fig. 5) may be attributable to the lack of increased influence of homogenizing dispersal, and only small increases in homogeneous selection (Fig. 7).

At the between-plot scale, we might have expected to see an increase influence of homogeneous selection and perhaps dispersal limitation with tillage, because management of these plots is similar, yet they are spatially separated. However, like findings at the within-plot scale, tillage also increased the influence of homogenizing dispersal at the between-plot scale at both sites. Therefore, another tillage-driven mechanism increased the compositional similarity amongst these spatially distinct plots, barring direct organismal dispersal, without increasing phylogenetic similarity (which would have increased the influence of homogeneous selection). For example, tillage systematically preserves the most stable, potentially older microaggregates, and therefore we may be observing founder effects that manifest as compositional similarity between plots in a field (Rillig et al., 2017). Despite some significant shifts in selection and dispersal, community assembly is largely undominated at the between-plot scale, demonstrating a high level of stochasticity, and potential for ecological drift.

Despite homogenizing community composition, tillage did not have a significant effect on bacterial richness (Fig. S7). Previous work has found tillage to have both neutral and negative effects on richness (Constancias et al., 2013; Smith et al., 2016). The tillage practices used at these sites (fall chisel plow plus spring cultivation) are perhaps too infrequent or mild to affect richness estimates, as previous work has found that richness significantly decreased only in soil disturbed at least biweekly (West and Whitman, 2022). It is also possible that sequencing efforts poorly represented the relative richness of these systems and soil fractions (Bach et al., 2018), though the *betta* model that we used for richness estimation is specifically designed to account for unobserved taxa (Willis et al., 2017).

We did not observe strong influences of dispersal limitation or variable selection under no-tillage, as was hypothesized (H1). This may be attributed to the largely uniform, homogeneous soil environment that is characteristic of intensively managed monocrop systems, regardless of tillage practices.

4.3 Tillage favors potential for fast growth

Increased weighted mean predicted 16S gene copy number under tillage (Fig. 6) was also noted in a recent global metanalysis (Wilhelm et al., 2023), and is consistent with the idea that pulses of resources (e.g., C liberation or residue incorporation via tillage) select for bacterial competitors with fast growth potential (Schmidt et al., 2018). These studies also found that larger mean estimated genome size correlated with lower soil health ratings and tillage, indicating a need for higher metabolic and regulatory capabilities under environmental instability (Schmidt et al., 2018; Wilhelm et al., 2023). However, the fairly uniform effect on weighted mean predicted 16S gene copy number across soil fractions (Fig. 6), which do differ in chemical composition (Table S2), indicate that physical disturbance may also influence fitness as it relates to other aspects of life history strategy, such as chemical signaling, community goods, or secondary metabolites. For example, this could point to a scenario by which oligotrophic organisms, which invest heavily in extracellular enzymes, are at a disadvantage when proximity to these metabolites is disrupted by physical disturbance (Junkins et al., 2022), as compared to copiotrophic generalists, which are less reliant on proximity-based life strategies.

Tillage-driven increases in weighted mean predicted 16S gene copy number (Fig. 6) may be expected to be accompanied by increases in soil respiration (on a per gram C basis), due to lower carbon use efficiency (Roller et al., 2016). However, C respiration was similar across tillage treatments at both sites (Fig. S5B), which implies that the no-tillage and tillage communities processed C similarly, and/or the small, yet significant, increase in weighted mean predicted 16S gene copy number was not biologically relevant for C mineralization. Further, sequencing-based estimates of 16S gene copy numbers are limited due to the presence of relic DNA (Carini et al., 2016) or dormant organisms (Lennon and Jones, 2011).

Soil respiration—on a per gram soil basis—did decrease under tillage at Arlington (Fig. S5A), as a function of decreased bulk soil C concentration (Table S2). The no-tillage samples averaged slightly higher gravimetric soil moisture, which also may help explain relative increases in respiration (Moyano et al., 2013). Though our measurements of CO_2 evolution from sieved soil may not accurately represent an intact soil (Vogel et al., 2022), this analysis indicates that the C mineralization potential of these soil communities may not be limited by tillage-driven compositional changes.

4.4 Evidence for fluidity between the free and occluded microaggregate fractions

Within either tillage or no-tillage treatment, the fairly indistinct bacterial community compositions and community assembly patterns of the free and occluded microaggregate fractions (Figs. 4 and 7) ran contrary to hypothesis H2, in which we suggested that the communities of the free and occluded microaggregate fractions would be distinct from each other, driven by different community assembly processes. Despite differences in soil C and N (Table S2), community similarity indicates that these operationally defined fractions likely have substantial overlap, which may be attributable to wholesale shifts of occluded microaggregates to the free fraction at the end of the temperate annual cropping season, when macroaggregates rapidly degrade with root senescence (Oades and Waters, 1991), as detailed below in Section 4.6. Further, the sample dispersion of community composition (i.e., the distance on a PCoA) of free and occluded microaggregate fractions from within each soil core was unaffected by tillage treatment (Fig. 5C and F), which indicates that the overlap or fluidity between the free and occluded microaggregate fractions may not be particularly responsive to tillage. Since we did not identify bacterial drivers that explain enhanced SOC persistence specific to the occluded microaggregate fraction, future work could instead focus on the physical and chemical drivers of C storage and persistence in microaggregate fractions (Bailey et al., 2019; Kravchenko et al., 2019), or fungal community drivers (Lehmann and Rillig, 2015).

Generally speaking, the high level of similarity in microbial communities of the microaggregate fractions and the bulk soil (Figs. 4, 6, & 7) supports previous work suggesting that the majority of soil bacteria live in association with microaggregate structures (Ranjard et al., 2000), which only comprised 25% and 15% of the bulk soil at Arlington and Lancaster, respectively (Fig. 2).

4.5 Taxonomic differences due to tillage

Some broad, phylum-level compositional differences follow archetypical expectations under tillage: *Firmicutes*, generally thought to include fast-growing copiotrophs, increased in relative abundance with tillage, as was previously noted (Schmidt et al., 2018), whereas *Verrucomicrobia* include numerous oligotrophic taxa (Bergmann et al., 2011), and decreased under tillage (Figs. S10 and S11). *Firmicutes* also had higher mean relative abundances in the bulk soil compared to the microaggregate fractions. There were several taxa that responded to tillage representing the genus *Nocardioides* (*Actinobacteria*), an observed responder to frequent soil disturbance (West and Whitman, 2022) that has been negatively correlated with soil health (Wilhelm et al., 2023). We also found relative enrichment of *Sphingomonas* and *Geodermatophilus* under tillage, both of which have been identified as key tillage responders (Wilhelm et al., 2023). Under no-tillage, we found enrichment of the genus *Gaiella*, (*Actinobacteria),* which was one of several identified bioindicators of high biological soil health ratings (Wilhelm et al., 2023). We also found that anaerobic taxa (e.g., *Anaerolineae*) (Yamada and Sekiguchi, 2020) were enriched in microaggregates (Fig. S14), which have anoxic microsites (Sexstone et al., 1985).

The enrichment of *Cyanobacteria* in microaggregate fractions, where we would not expect photosynthetic organisms to survive or thrive, may reflect the presence of relic DNA (Carini et al., 2016) or dormant organisms (Lennon and Jones, 2011), integrated into the soil matrix via tillage, and under microaggregate protection. The specific *Cyanobacteria* taxa enriched in our study (*Microcoleus* PCC-7113 and *Tychonema* CCAP 1459-11BA) were both previously found in soil under frequent disturbance (Santoni et al., 2022).

4.6 Factors that may have moderated the measured impact of tillage

We will briefly consider several nuanced factors in this study. The tillage treatment at both sites included a fall chisel plowing, which is sometimes considered a reduced or even conservation tillage approach because it is shallower and more moderate compared to moldboard or disk plowing, and does not invert the soil (e.g., Zuber and Villamil, 2016). Some previous work has found chisel plow tillage to have the same effect as no-tillage on aggregate stability and microbial biomass (Al-Kaisi et al., 2014; Zuber and Villamil, 2016). Several other factors may obscure or diminish the relative impacts of tillage in this study, including crop-related seasonal macroaggregate dynamics, wet-dry or freeze-thaw cycles, and clay mineralogy.

As noted above, macroaggregates rapidly destabilize following crop senescence—which begins four to eight weeks prior to grain harvest—thus potentially diminishing tillage-driven differences in soil aggregation measured post-harvest (Fig. 2) and liberating occluded microaggregates into the free microaggregate pool (Perfect et al., 1990; Oades and Waters, 1991). Similar aggregation patterns across tillage treatments were previously observed by Huang et al. (2010), in which sampling occurred months after corn harvest. Tillage differences may be further diminished by the physically disruptive effects of freeze-thaw and wet-dry cycles at the soil surface, which would impact aggregate stability of otherwise undisturbed soil under no-tillage (LeGuillou et al., 2012; Bailey et al., 2019). Further, these effects are likely variable in tillage vs. no-tillage treatments, given differences in protective surface residues and roughness (Cruse et al., 2001).

Another factor potentially contributing to differences in aggregation and C concentration between sites may be variable mineralogy (Denef et al., 2004). Mollisols, such as at Arlington, are generally recognized to promote organo-mineral complexes. The clay mineralogy of the Plano silt loam at Arlington is interstratified smectite-illite (Liu et al., 1997); the high specific surface area of illite may promote SOC retention, and the expansible nature of smectite may physically protect organic matter (Sarkar et al., 2018). The Fayette silt loam at Lancaster (Alfisol) has been mineralogically characterized as predominantly montmorillonite clay minerals—an expansible layer phyllosilicate (Caldwell et al., 1955). However, the low activity clay (1:1) may explain the largely unaggregated soil even under no-tillage (Fig. 2), as was previously noted for a mixed-mineralogy clay (Six et al., 2000b). These literature-based suppositions are supported by lower concentrations of base cations and lower overall cation exchange capacity measured at Lancaster (Table S1). The mineralogical differences may explain the higher proportion of aggregated soil at Arlington, and higher SOC and SOM concentrations relative to Lancaster, despite similar texture (silt loam) and corn-based cropping systems.

5. Conclusions

This study both demonstrates that tillage homogenizes soil bacterial communities and links this disturbance to the ecological process of homogenizing dispersal, while supporting previous conclusions that tillage disrupts aggregation and decreases carbon at the soil surface. Counter to one of our hypotheses, the bacterial communities of the free and occluded microaggregate fractions are highly similar, indicating that microaggregates may readily shift between these operationally defined soil fractions. Tillage may accentuate seasonal changes characteristic of temperate annual cropping systems (e.g., crop senescence, freeze-thaw, and wet-dry cycles), which together challenge the strength and longevity of macroaggregates in which occluded microaggregates form and soil carbon is protected. Thus, while our findings reiterate the importance of the occluded microaggregate fraction for soil C persistence, we also suggest that this occluded microaggregate C is subject to an increased rate of turnover when the previously occluded fraction becomes part of the free microaggregate fraction upon macroaggregate turnover. Conceptually, this underscores how aggregate microhabitats develop and devolve throughout the soil matrix, in concert with microbial activity, forming isolated hotspots driven by resource availability in the patchy soil environment.

Supplementary Information

Supplementary Information can be found online.

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Author contributions. JW and TW conceived of the project. JL has maintained the tillage experiment in Arlington, WI since 1994. JW collected soil samples, conducted lab work, analyzed the data, and drafted the manuscript. All authors reviewed and edited the manuscript.

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