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Dredged Sediments Contain Potentially Beneficial Microorganisms for Agriculture and Little Harmful Cyanobacteria

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RESEARCH ARTICLE

Dredged sediments contain potentially beneficial microorganisms for agriculture and little harmful cyanobacteria

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Abstract

Introduction: Soils worldwide are degrading, raising concerns about our ability to feed the growing global population. Soil amendments that can alleviate degradation are gaining attention. The application of sediments dredged from waterways to agricultural fields has increasing promise as a means for improving degraded soils. However, herbaceous plant species may have difficulty establishing on dredged material because of low nutrient availability, inhibitory levels of toxins, unsuitable moisture conditions and lack of microorganisms capable of ameliorating these characteristics. To counteract these issues, we sought to understand if the use of a cover crop would increase the abundance, diversity and function of beneficial soil microorganisms compared to harmful microorganisms in dredged sediments.

Materials and Methods: We collected soil samples from two 100% dredged sediment plots, one where winter cereal rye (*Secale cereal)* was grown as a winter cover crop and one left fallow over the winter, followed by traditional corn (*Zea mays*) planting. We sampled both plots three times during the growing season: before cover crop application, following cover crop application but before corn planting and following final corn harvest. We then used high-throughput sequencing to identify the bacterial and fungal communities present in the samples.

Results: Our data show that cover crop application did not alter the microbial community in these plots. However, sampling time decreased species diversity and altered the composition of both fungal and bacterial communities recovered from these plots. Across both plots, microorganisms associated with carbon cycling were more abundant than those associated with harmful effects, including microcystin-producing cyanobacteria, which were an extremely small portion of the overall community.

Conclusion: Our work suggests that dredged sediments have the potential to improve soil function through the addition of microorganisms associated with nutrient cycling, but a cover crop is not necessary to incur these benefits.

KEYWORDS
arbuscular mycorrhizal fungi, cover crop, saprobes, soil amendment, *Zea mays*

1 | INTRODUCTION

Soil is a dynamic natural system that lies at the interface between earth, air, water and life, providing critical ecosystem services to maintain humanity (Needelman, 2013); however, it is also a limited resource and easily degraded. There is growing recognition that soil erosion, soil nutrient depletion and physical deterioration of soil are widespread and increasing, representing a serious threat to food security on a global scale (Eswaran et al., 2001; Houghton & Charman, 1986; Lal, 2015). The annual loss of 75 billion tons of soil costs the world US$400 billion per year, approximately US$70 per person per year (Eswaran et al., 2001). The restoration and maintenance of soil productivity through the combination of cover crops and soil amendments can aid in the recovery of soil fertility and structure, in part by introducing soil microorganisms that drive changes to soil nutrient cycling (Hallama et al., 2019; Hu et al., 2021). However, the source of the soil amendment is vital for determining the identity and function of the microorganisms (Yu et al., 2018). Here, we investigate the microbial community in dredged sediments, a relatively novel soil amendment for restoring degraded soils. We further evaluate the extent to which microbial identity and function are altered by the use of a cover crop to better understand its potential for restoring degraded soils through the introduction of beneficial microorganisms.

Recent research suggests that the addition of an organic amendment can restore productivity to degraded soils (Larney & Angers, 2012; Martínez-García et al., 2018). Organic amendments can take the form of animal manure, municipal biosolids and manufacturing wastes. More recently, the application of sediments removed from aquatic waterways (dredged sediments) to degraded agricultural lands has received attention for its capability to act as an organic amendment (Sigua, 2005). Such sediments have the potential to increase production in low/nonproducing areas by improving soil characteristics as a result of their high soil fertility, organic matter and water-holding capacity (Darmody & Marlin, 2002; Koropchak et al., 2016; Sigua, 2005). These attributes can improve soil health and lead to increases in overall yield and crop production (Canet et al., 2003; Daniels et al., 2007, Darmody & Diaz, 2017). Despite this potential, herbaceous plant species often have difficulty establishing on dredged materials because of low nutrient availability, inhibitory levels of toxins, unsuitable moisture conditions and a lack of microorganisms capable of ameliorating these characteristics.

The use of a cover crop is a common practice for restoring degraded soils due to its ability to reduce soil erosion and water runoff and improve water infiltration and retention, soil tilth and soil organic carbon (Nakajima et al., 2016; Villamil et al., 2006). Cover crops may also be able to ameliorate some of the negative effects dredged sediments may have on amended soils, including inhibitory levels of toxins and/or metals, unsuitable moisture conditions and a lack of microorganisms capable of ameliorating these characteristics (Koropchak et al., 2016; Sigua 2005). For example, when wild oat (*Avena sativa*) was used as a cover crop, it significantly reduced the amount of copper in the soil and this effect was amplified when inoculated with bacteria (Andreazza et al., 2010).

Microorganisms are a pivotal part of soil health, playing an essential role in nutrient cycling, decomposing organic material and fertilizing soil (Martin et al., 2017; Smith & Read, 2008). In particular, arbuscular mycorrhizal fungi (AMF) are ubiquitous fungal symbionts that associate with over 80% of all land plants and are well-documented to improve plant health by increasing plant absorption of limiting nutrients like phosphorus and water (Smith & Read, 2008). There is a substantial body of literature relating microbial community composition as a driver of soil processes related to soil health (Chen et al., 2020; Van Der Heijden et al., 2008; Metcalfe et al., 2011; Schloter et al., 2018; Sharma et al., 2011; Wagg et al., 2014). For example, microorganisms improve water retention and permeability through the secretion of polysaccharides and mucilage, which aggregate soil particles, making them less likely to crumble when exposed to water (Rillig et al., 2002). Their role within dredged sediments may be particularly important if dredged sediments are to be used as a soil amendment due to their key roles in nutrient cycling, their ability to mitigate soil moisture and their capacity to mitigate harmful toxins and pollutants.

Despite the pivotal role microorganisms play in soil health broadly, and their potential in dredged sediments specifically, there has been little attention paid to their utility in dredged sediments. There is currently only a single published study examining shifts in microbial community structure and function due to amendments with dredged sediments. Using phospholipid fatty acid analysis (PLFA), Kelly et al. (2007) found that dredged sediments increased the biomass of beneficial microorganisms compared to biosolids. However, the use of PLFA to identify microbial identity and function is limited in its ability to identify the species or guilds of microorganisms responsible for this result as the same PLFAs can indicate very different groups (Frostegård et al., 2011). For example, the PLFA $16:1\omega5$ is commonly used to indicate AMF but is also found in bacteria (Frostegård et al., 2011), so studies using this PLFA often misidentify microbial abundances.

Understanding the identity, and not just the functional group, of the microorganisms contained within dredged sediments is especially concerning for sediments from Lake Erie, which regularly experiences microcystin-producing cyanobacterial harmful algal blooms. While the full life cycle of *Microcystis* in Lake Erie is still an area of active research, sediments play an integral role in the annual cycle of *Microcystis* in other freshwater systems by serving as internal reservoirs (Humbert et al., 2005; Reynolds et al., 1981). The full extent to which Lake Erie sediments serve as a reservoir for *Microcystis* has received little attention, but sediment samples collected from Lake Erie in 2004 found the signature genes for *Microcystis* not only on the surface sediments but also 10–12 cm in depth (Rinta-Kanto et al., 2009). Since this is the same spatial extent that sediments are dredged, this suggests the potential for microcystin-producing cyanobacteria to be present in dredged sediments. The presence of these cyanobacteria is concerning because of their ability to produce cyanotoxins that have been shown to induce oxidative stress (Prieto et al., 2011), reduce germination rates (Metcalf et al., 2004) and inhibit growth (Beyer et al., 2009; Vasas et al., 2002) in agriculturally important plants; however, the productivity and nutritional quality of other agricultural

plants can be unaffected or even enhanced by cyanotoxins (Machado et al., 2017). Therefore, understanding the identity and persistence of this and other microorganisms and their associated toxins through standard farming practices is vital to understanding the full extent to which we can use dredged sediments in an agricultural setting.

Here, we will determine if the use of a cover crop will increase the abundance, diversity and function of beneficial soil microorganisms for dredged sediments originating from Lake Erie. We predict that the use of a cover crop will increase microorganisms associated with beneficial outcomes, including AMF fungi and other microorganisms associated with nutrient cycling like saprobes. We further predict that the use of a cover crop will increase the diversity of bacteria and fungi compared to the plot without a cover crop. Finally, we predict that the use of a cover crop will decrease the abundance of microcystin-producing cyanobacteria. Outcomes from this work also provide detailed information on the microbial community in dredged sediments that will be used as an agricultural amendment and how the community changes across a single growing season.

2 | MATERIALS AND METHODS

2.1 | Site description and preparation

To investigate how the use of a cover crop with dredged sediments changes soil microbial communities, we factorially manipulated the use of a cover crop for two plots at the Great Lakes Dredged Material Center for Innovation (GLDMCI), located in Toledo, Ohio (41.670, −83.503). The GLDMCI consists of four 2.3-acre plots, each with 100% dredged sediments sourced from the Toledo Harbour of Lake Erie and the Maumee River in 2016–2017 (Hull & Associates, 2018). Plots are outfitted with traditional Ohio agriculture tile and drainage usage.

Two plots were prepared in November 2018 by mowing the existing weedy vegetation and tilling the vegetation into the dredged material via tractor and discs. One plot was then broadcast seeded with winter cereal rye (*Secale cereale*; The CISCO Companies; 'cover crop'), while the second plot was left fallow over the winter ('control'). On 15 May 2019, soil within both plots was aerated by mechanical tilling and then plots were row-planted via a tractor with corn (*Zea mays*; Wellman Seeds Inc.). Due to a large amount of invasive plant growth obstructing egress through the plots, both plots were sprayed with an herbicide mix consisting of 1.5 qt per acre of glyphosate 53.8% (Buccaneer 5; CommoditAg) and 1.5 lbs per acre of ammonium sulphate 48% (AMS; CommoditAg) into 20 gallons of water per acre on 8 July 2019. The final corn harvest occurred on 12 October 2019.

2.2 | Plant sampling

Corn smut, a plant disease caused by the pathogenic fungi *Ustilago maydis* (=*Ustilago zeae*), was identified visually in both control and cover crop plots. On 31 August 2019, the prevalence in each plot was determined by counting the number of positive plants found along six transects walked lengthwise from North to South in each plot.

To study fungal colonization in roots, a subset of secondary roots from each corn plant were collected and cleaned with normal tap water at the final harvest. Roots were analysed via the grid intersection method (Koske & Gemma, 1989; McGonigle et al., 1990; Rúa et al., 2013). Briefly, roots were soaked in a 10% KOH solution at 80°C for 45 min, rinsed 10 times with deionized water, placed into a 0.25 M HCl solution for acidification for 5 min and then placed into a 0.5% Trypan Blue Dye solution at 80°C for 30 more minutes. Finally, ten 1 cm stained root fragments per plant were affixed to microscope slides with the mountant polyvinyl-lacto-glycerol and viewed under a compound microscope at ×20–40 magnification (Nikon SMZ-1600). We scored 10 random fields from each root fragment under the microscope for signs of fungal colonization, including hyphae, vesicles, arbuscules and spores. In total, a maximum of 100 view fields per plant (10 view fields × 10 root fragments) were scored.

2.3 | Soil sampling

Soil samples from 10 haphazardly chosen locations were collected per plot (total of 20 samples) from the top 6 in. of the plot using a hand trowel. A hand trowel was necessary due to the extremely clay nature of the dredged sediments, which prevented the use of a soil core (Hull & Associates, 2018). Each location was marked for future sampling to control for variability within each plot. We collected soil samples three times throughout the growing season: before planting (12 November 2018), after cover crop/before corn planting (12 April 2019) and following final harvest (12 October 2019). During each sampling period, we collected three 2 ml canonical tubes from each sampling location for a total of 60 samples [10 locations × 3 samples per location × 2 plots (cover crop vs. control)]. Samples were flash frozen with liquid nitrogen in the field and stored at −80°C for up to 3 months until processing. All samples were transferred to the Rúa lab at Wright State University in Dayton, OH within 6 h of collection.

2.4 | DNA extraction and sequencing of bacterial and fungal DNA regions

To identify the microbial community, DNA was extracted in triplicate from each sampling location (10 locations × 3 samples per location per plot × 2 plots) for each collection date (3) for a total of 180 extractions using the Qiagen DNA Powersoil Extraction kit (Qiagen) without modifications. Negative controls (Milli-Q water) were also extracted for each plot and collection date. The three extractions from each sampling site/negative control were pooled for each collection time for a total of 10 unique replicates per sampling period per plot. The resulting 60 samples (20 unique replicates per sampling period × 3 sampling periods) were cleaned with the Qiagen Power Clean-up Pro kit (Qiagen). Final DNA concentrations were obtained using a Qubit 3.0 dsDNA BR assay (Thermo Fisher) and DNA quality

was quantified using a Nanodrop One Microvolume UV–Vis Spectrophotometer (Thermo Fisher). Final DNA extractions were then diluted to 10 µl with Milli-Q water before PCR. No high-quality DNA was extracted from negative controls.

Bacteria were identified using the prokaryotic 16S ribosomal RNA gene (16S rRNA) and fungi were identified via the internal transcribed spacer (ITS). The V3–V4 region of 16S rRNA was amplified using primers 515F and 806R and the ITS1 region of the ITS was amplified using primers ITS1F and ITS2 following the appropriate EMP PCR protocols (Caporaso et al., 2018; Smith et al., 2018). High-throughput sequencing using the MiSeq® Reagent kit v2 (500 cycles) (MiSeq; Illumina) was performed at Argonne National Laboratory.

2.5 | Bioinformatics and statistical analyses

Sequence data were processed using the software package Quantitative Insights into Microbial Ecology (QIIME), version 2 (QIIME2, release 2022.02) (Caporaso et al., 2010). Denoizing was performed using the DADA2 plugin (Callahan et al., 2016) and subjected to error correction, filtration, trimming, chimera removal and grouping into amplicon sequence variants (ASVs, 100% similarity limit). The taxonomic composition of the unique sequences was determined using the pretrained Naive–Bayes classifier against the SILVA version 138 database (Quast et al., 2012) for 16S and UNITE_v2020 database for ITS. The raw sequencing data were deposited in NCBI Sequence Read Archive with an accession number of PRJNA863636.

The resulting feature tables were imported into R version 4.2.0 (R Core Team, 2022), where they were analysed with the *phyloseq* package (McMurdie & Holmes, 2013). First, sequence counts were cleaned so that only those matching bacterial (16S), archaeal (16S) and fungal (ITS) hits were retained. For 16S samples, sequences that matched 'Chloroplast' or 'mitochondria' were also removed, while for ITS samples, sequences from nonfungal taxa (such as Protist or Plantae) were also removed. ASVs appearing only once (singletons) or twice (doubletons) were also removed. Analysis of variance was used to test for effects of sampling time (Fall 2018, Spring 2019 and Fall 2019), treatment (cover crop or control) and their interaction on univariate response variables. Pairwise differences between groups were evaluated with Tukey's honestly significant difference post hoc tests.

Rarefaction curves were created with the *get_rarecurve* and *ggrarecurve* functions from the *MicrobiotaProcess* package (Xu & Yu, 2022). Relative abundance figures were created by merging species at the Class level using the *tax_glom* function from *phyloseq* (McMurdie & Holmes, 2013), the top 50 most abundant taxa were retained using the *get_top_taxa* function from the *fantaxtic* package (Teunisse, 2018) and finally plotted with *ggplot2* (Wickham, 2016). The amount of shared ASVs was calculated with the *get_vennlist* and *get_upset* functions from the *MicrobiotaProcess* package (Xu & Yu, 2022) and visualized using the *ggvenn* (Yan, 2022) and *ComplexUpset* (Krassowski, 2020; Lex et al., 2014) packages.

Species diversity (Shannon, Simpson's) and richness (Chao1, Observed) indices were calculated on untransformed and unfiltered data using the *vegan* package through *phyloseq* (McMurdie & Holmes, 2013; Oksanen et al., 2022). Similarities in microbial community composition were evaluated among all samples with nonmetric multidimensional scaling based on Bray–Curtis distance matrices using the *ordinate* function in the *phyloseq* package. Permutational multivariate analysis of variance using the *adonis2* function in the *vegan* package with 1000 permutations was used to determine significant differences in community composition between treatments and sampling times (Oksanen et al., 2022).

We used the *microeco* package to determine the ecological guilds of the microbial community according to the FAPROTAX v1.2.4 and tax4Fun2 databases for bacteria (Louca et al., 2016; Wemheuer et al., 2020) and FungalTraits for fungi (Põlme et al., 2021). Differential abundance tests for ecological function were then conducted based on the LEfSE (Linear discriminant analysis Effect Size) method (Liu et al., 2021). While using databases to predict ecological function may be limited, functional analyses using such databases are considered standard practice for unravelling mechanisms structuring species co-occurrence patterns and community assembly when using amplicon data (Tedersoo et al., 2022).

To determine whether the odds of infection with corn smut differed by treatment, binary logistic regression on the presence/absence of infection as a function of treatment (cover crop vs. control) was performed with the function *glm* from the *stats* package (R Core Team, 2022). Differences in the presence/absence of belowground fungal colonization by AMF or dark-septate endophyte (DSE) were determined via *glm* with an offset for the number of intersections examined. Results were visualized with the function *ggboxplot* from the library *ggpubr* (Kassambara, 2020).

3 | RESULTS

3.1 | Corn smut

Out of the 4992 plants surveyed, only six had visually confirmed corn smut. The use of a cover crop was not significantly associated with corn smut infection ($p = 0.183$). Plants in the cover crop plot had 0.276 times the odds of infection compared to those from the control (odds ratio [OR]: 0.276; 95% confidence interval [CI]: 0.032–2.366). The causative agent behind corn smut, *U. maydis*, was not recovered in the soil microbiome.

3.2 | Belowground fungal colonization

AM fungal colonization of corn roots did not significantly differ between plants grown in the plot with a cover crop and those grown in a plot without the cover crop ($p = 0.184$; Supporting Information: Figure S1A). The proportion of roots with DSE colonization was 34% greater for corn grown in the cover crop field compared to the plot left fallow ($p < 0.0001$; Supporting Information: Figure S1B).

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3.3 | Microbial communities

3.3.1 | ITS

A total of 3224 distinct fungal ASVs were recovered. Rarefaction plots saturated, indicating that we did not undersample these communities (Supporting Information: Figure S2). Overall, our samples consisted of 27 fungal classes. Our samples were dominated by Ascomycota (52% of total sequences), the majority of which belonged to the classes Sordariomycetes (28% of total sequences) and Dothideomycetes (21% of total sequences; Supporting Information: Figure 1A). The number of ASVs recovered decreased over time
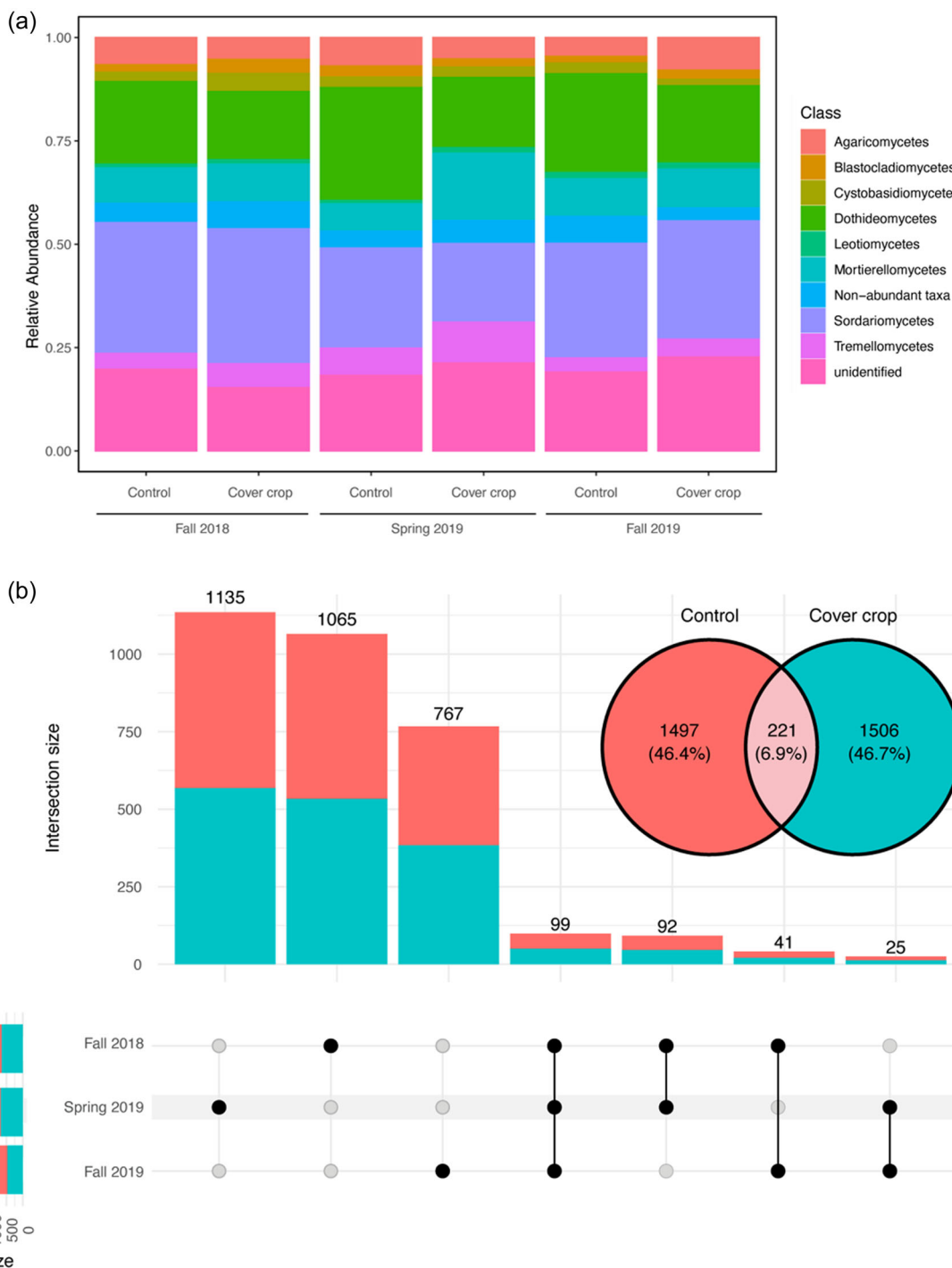

FIGURE 1 Relative abundance of different fungal classes by sampling time and treatment (a) and upset plot of ITS ASVs (b). The upset plot includes the total number of shared ASVs (X-axis) at each time point (Y-axis) as a function of treatment. The inset Venn diagram reflects the shared ITS ASVs by treatment, across time. ASV, amplicon sequence variant; ITS, internal transcribed spacer.

(Supporting Information: Figure 2B) and only 6.9% of fungal ASVs were shared between the two plots (Supporting Information: Figure 1B).

Species diversity was estimated by the Chao1, Simpson's and Shannon indices. No significant differences in diversity were found by treatment over time for the Chao1 index ($F_{2,54} = 44$, $p = 0.9345$), the Shannon index ($F_{2,54} = 0.0106$, $p = 0.9515$) or the Simpson's index ($F_{2,54} = 0.0026$, $p = 0.5499$). However, the Chao1 index did significantly change with time ($F_{2,54} = 11647.4$, $p > 0.0001$) such that the Fall 2019 sampling date following the final harvest was significantly less than both the Fall 2018 sampling date before treatment application and Spring 2019 following cover crop growth (Figure 2).

Fungal community abundances were sorted into an ordination plot according to community similarity (Figure S3). There was significant clustering based on the interaction of treatment and time ($R^2 = 0.04$, $p = 0.022$), but separation was minimal.

3.3.2 | Fungal traits

Guilds were identified for a total of 2427 fungal ASVs. Our samples were dominated by saprotrophs (54% of total sequences), followed by plant pathogens (34% of total sequences; Supporting Information: Figure S4). The interaction of treatment and time significantly affected the frequency of fungi from a number of guilds ($p < 0.0001$). The greatest frequency of saprotrophs and mycoparasites occurred in the cover crop plot in Spring 2019, which was also greater than those recovered from the control plot for the same time period. Conversely, there was a decrease in the frequency of plant pathogens in the cover crop plot in the Spring of 2019. There was no difference for saprotroph, plant pathogen or mycoparasite frequency in any other time period ($p > 0.05$).

Functional traits beyond functional guild were further delineated for 2168 fungal ASVs. Interestingly, by the final collection period, fungi that prefer an aquatic habitat were enriched compared to those that were partly aquatic or nonaquatic for both plots (Figure 3). Also
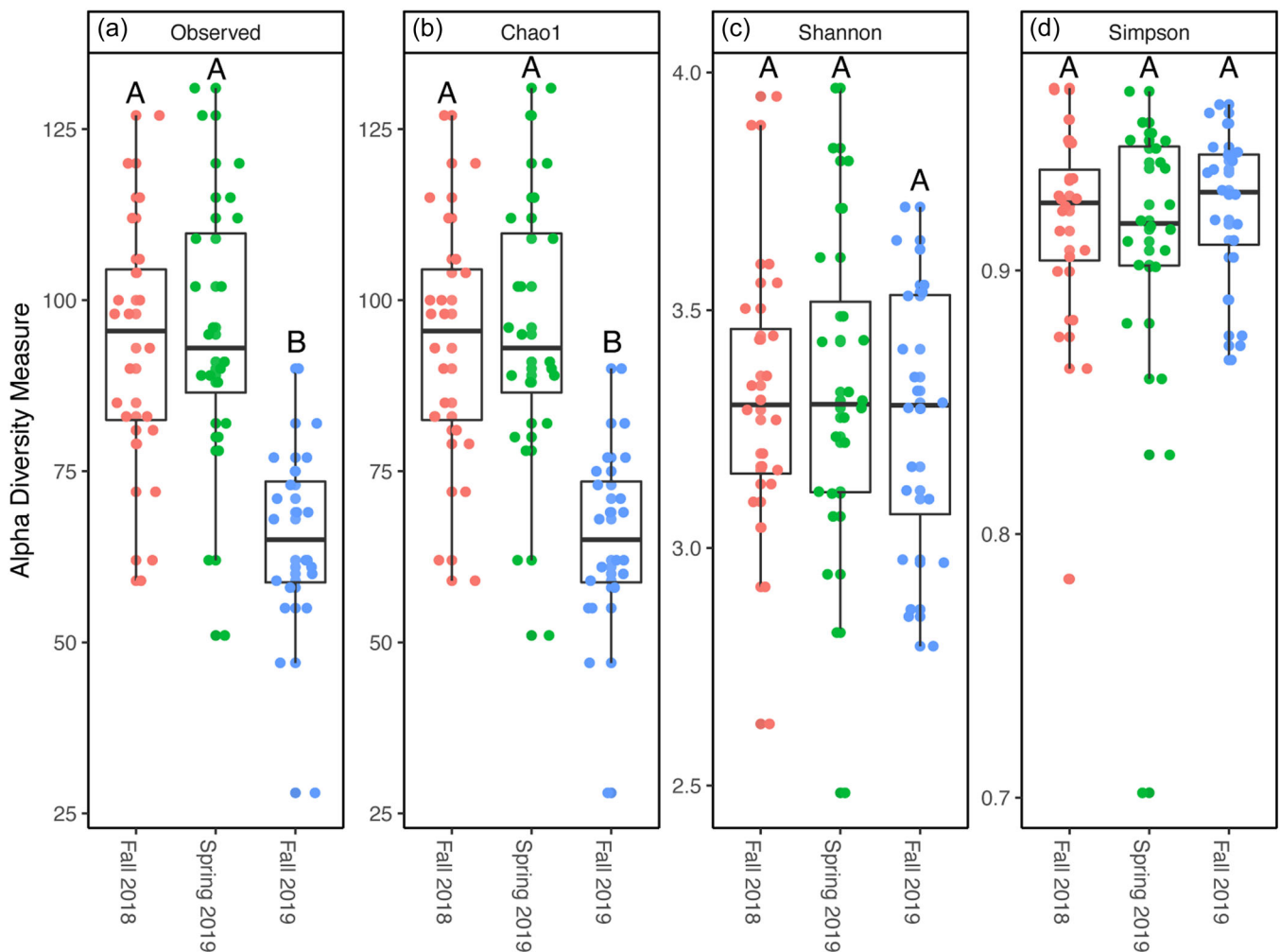

FIGURE 2 Fungal richness of soil samples by collection time according to the (a) Observed richness, (b) Chao1 index, (c) Shannon diversity index and (d) Simpson's diversity index. Letters indicate significant differences among treatment groups.
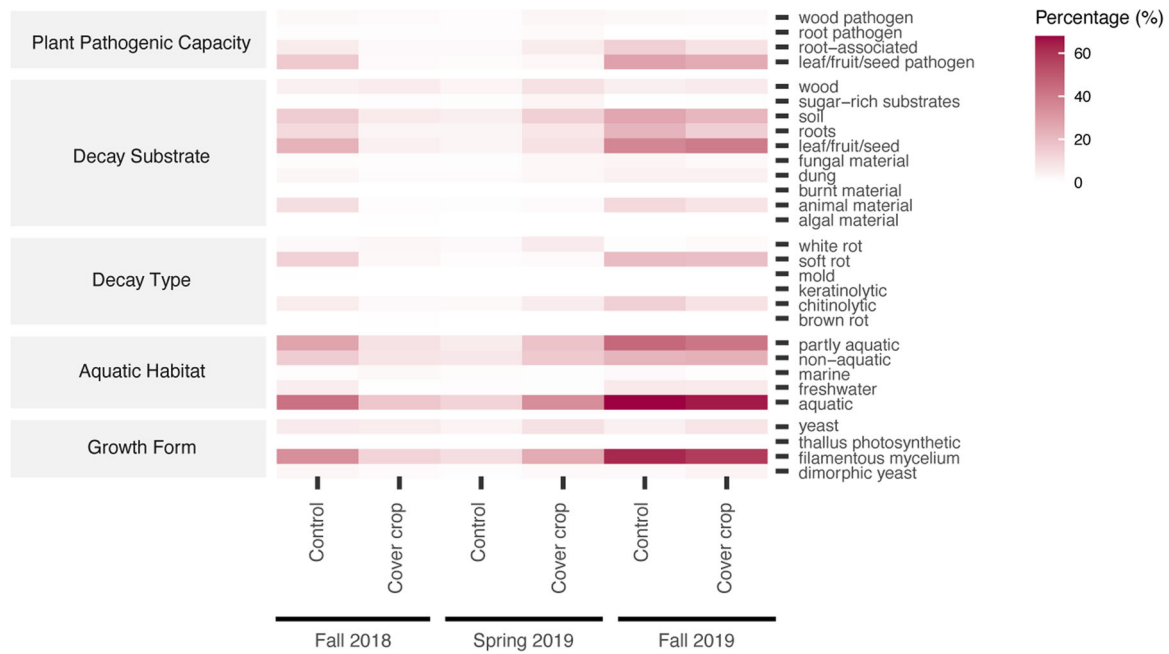
FIGURE 3 Relative abundance of plant pathogenic capacity, decay substrate, decay type, aquatic habitat and growth form by sampling period and treatment

by the final collection period, fungi with filamentous mycelium were the most common growth form for both plots (Figure 3).

3.3.3 | 16S

A total of 25,133 distinct bacterial ASVs were recovered. Rarefaction plots saturated, indicating that we did not under-sample these communities (Supporting Information: Figure S5). Overall, our samples consisted of 216 bacterial classes. Samples were dominated by Proteobacteria (30% of total sequences), the majority of which belonged to the class Gammaproteobacteria (23% of total sequences; Figure 4a). The number of ASVs recovered decreased over time (Figure 4b) and 36.2% of bacterial ASVs were shared between the two plots (Figure 4b).

Species diversity was estimated by Chao1, Simpson's and Shannon indices. No significant differences in diversity were found due to the treatments over time for the Chao1 index ($F_{2,54} = 0.547$, $p = 0.5818$) or the Shannon index ($F_{2,54} = 1.743$, $p = 0.1847$), but they tended to vary by treatment over time for the Simpson's index ($F_{2,54} = 2.977$, $p = 0.0594$). While overall diversity was lower for the final time point (Fall 2019), diversity was higher in the cover crop plot compared to the control plot (Supporting Information: Figure S6). Both the Chao1 ($F_{2,54} = 49.39$, $p < 0.0001$) and Shannon ($F_{2,54} = 29.15$, $p < 0.0001$) indices significantly changed with time such that the Fall 2019 sampling date following final harvest was significantly less than both the Fall 2018 sampling date before treatment application and Spring 2019 following cover crop growth (Figure 5).

Bacterial community abundances were sorted into an ordination plot according to community similarity (Supporting Information: Figure S7). There was significant clustering based on the interaction of treatment and time ($R^2 = 0.06$, $p = 0.004$), but the separation was minimal.

3.3.4 | Bacterial traits

Samples were dominated by aerobic bacteria, but bacteria with functions related to C cycling were also present (Supporting Information: Figures S8 and S9). No pathway was significantly enriched by treatment, time or their interaction ($p > 0.05$).

3.3.5 | Cyanobacteria

Of the 25,133 distinct bacterial ASVs recovered, only 237 come from the phylum Cyanobacteria (0.12% of total sequences). Of these ASVs, our samples were dominated by members of the Nostocaceae (29% of total cyanobacterial sequences) and Sericytichromatia (23% of total cyanobacterial sequences; Figure 6a); however, the abundance of Nostrocaceae changed throughout the course of the experiment in the cover crop plot such that it significantly declined by the final collection period (Figure 6a). Concurrently, the abundance of Cyanobacteria from the family Phormidiaceae increased in the final collection period for the cover crop plot (Figure 6a). The abundance of cyanobacteria from the Microcystaceae does not change over time or with the application of a cover crop ($p > 0.05$).
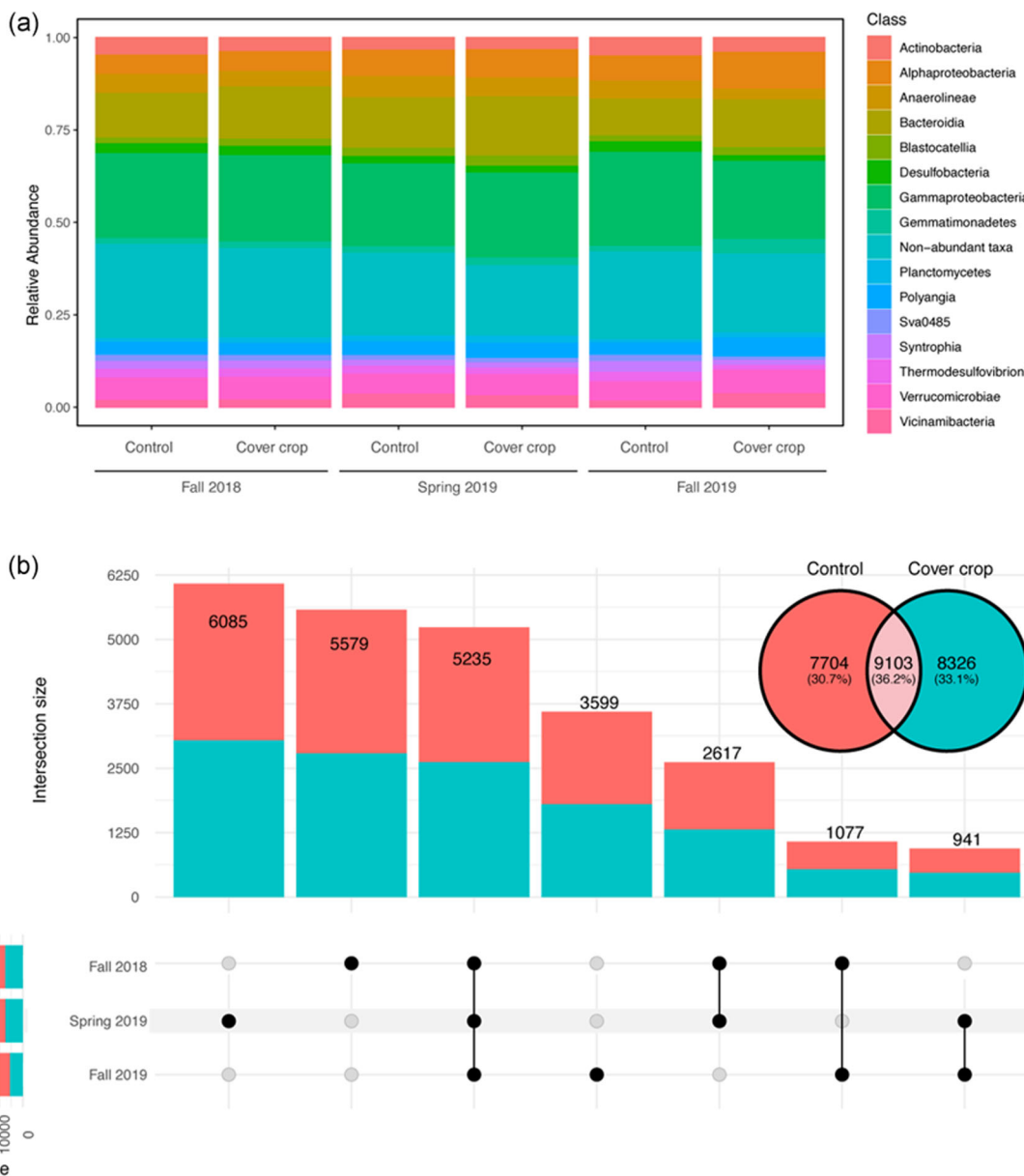
FIGURE 4 Relative abundance of different bacterial classes by sampling time and treatment (a) and upset plot of 16S ASVs (b). The upset plot includes the total number of shared ASVs (*X*-axis) at each time point (*Y*-axis) as a function of treatment. The inset Venn diagram reflects the shared 16S ASVs by treatment, across time. ASV, amplicon sequence variant.

The number of ASVs recovered decreased over time (Figure 6b) and only 27.4% of cyanobacterial ASVs were shared between the two plots across time.

4 | DISCUSSION

Soils worldwide are degraded but soil amendments provide a promising avenue for restoring their ability to support agriculture (Jie et al., 2002; Prăvălie, 2021). Among organic soil amendments, sediments dredged from waterways are receiving increasing attention due to their high soil fertility, organic matter and water-holding capacity (Darmody & Marlin, 2002; Koropchak et al., 2016;

Sigua, 2005), but there is a large concern that these sediments lack the soil microorganisms necessary for plants to establish and survive and/or contain harmful microorganisms (Darmody & Diaz, 2017; Ruiz Diaz et al., 2010; Sigua, 2005). However, the use of a cover crop with these sediments may not only increase the abundance of beneficial microorganisms but also decrease the abundance of harmful microorganisms. Here, we demonstrate that the soil microbial community is diverse and robust. Furthermore, the use of a cover crop, which in many agricultural systems improves the abundance and function of members of the soil microbial community, has little overall effect on the community. Instead, the sampling time is the strongest overall driver of both fungal and bacterial communities.
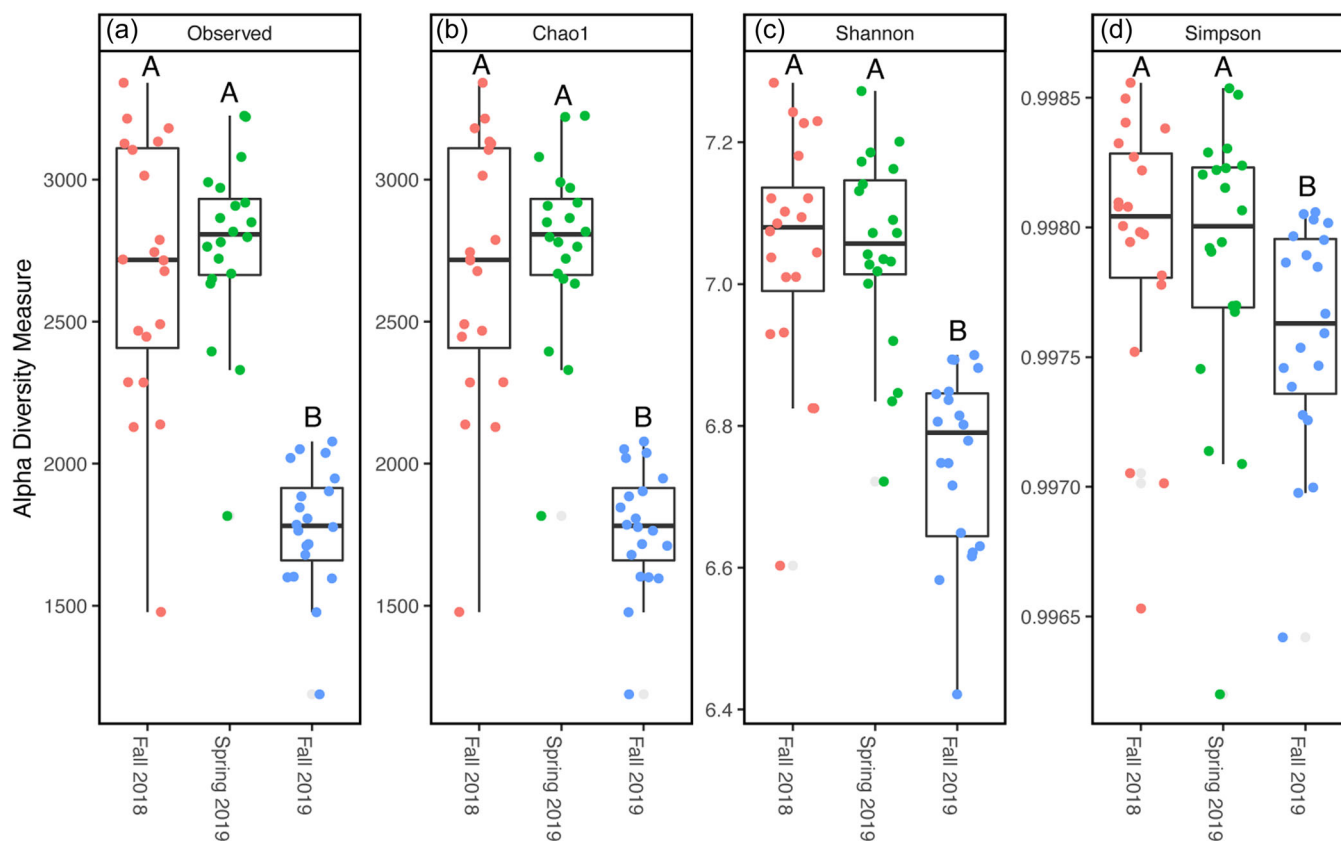
FIGURE 5 Bacterial richness of soil samples by collection time according to the (a) Observed richness, (b) Chao1 index, (c) Shannon diversity index and (d) Simpson's diversity index. Letters indicate significant differences among treatment groups.

The diversity of both bacteria and fungi in the plots remained consistent between treatments but varied significantly over time. While it has been widely reported that cover crops increase microbial diversity, many of these experiments capture changes in microbial communities after multiple growing seasons, and/or following the use of a diversity of cover crops (Adetunji et al., 2020; Vukicevich et al., 2016), which contrasts with the results found here. We do see changes in the microbial communities over time which supports the idea that expanding the length of this study to include multiple growing seasons would improve the chances of seeing an effect of the cover crop on microbial diversity. Interestingly, despite the fact there are no significant differences between the plots in terms of diversity, only 6.9% of fungal ASVs were shared between the two plots. This is in contrast with bacterial ASVs where 36.2% of bacterial ASVs were shared between the two plots. Taken together, these results suggest that while the use of a cover crop is not changing microbial diversity, species identity for both fungi and bacteria is changing due to the use of a cover crop.

Microbial traits can provide a useful means of capturing differences in microbial communities that may be masked by species-level analyses. The overwhelming majority of the fungi across both plots and through time were classified as saprotrophs (>50%), followed by those classified as plant pathogens (~25%). Within the saprotrophs, preferences for decay substrate and decay

type changed over time but did not differ by treatment. These results are inconsistent with previous studies which demonstrate an increase in fungi with saprobic functions over time due to the use of a cover crop (Adetunji et al., 2020). Instead, microorganisms associated with the decomposition of crop residues increased by the end of the experiment in both plots, suggesting accelerated nutrient cycling due to the release of inorganic nutrients as a result of crop growth (Coonan et al., 2020; Mary et al., 1996). This is particularly true for bacteria since most bacteria were classified as aerobic chemoautotrophs, which derive energy through the reduction of inorganic compounds (Grogan, 2012). Further, for those microbes where metabolic functions were identified, the overall community was dominated by microbes with metabolic functions related to carbohydrate metabolism and/or amino acid metabolism, suggesting they play a vital role in the C cycle (Falkowski et al., 2000). These functions did not vary through time or with treatment, suggesting they represent traits that are inherent to bacteria from dredged sediments, although future work with microorganisms from freshly dredged sediments is needed to confirm this. While cover crops have been shown to shift bacterial communities towards communities associated with decomposition in organic systems (Detheridge et al., 2016), conventional (nonorganic) cropping systems with corn have shown no effect (Chamberlain et al., 2020), which is consistent with the results presented here. On the whole, microorganisms associated
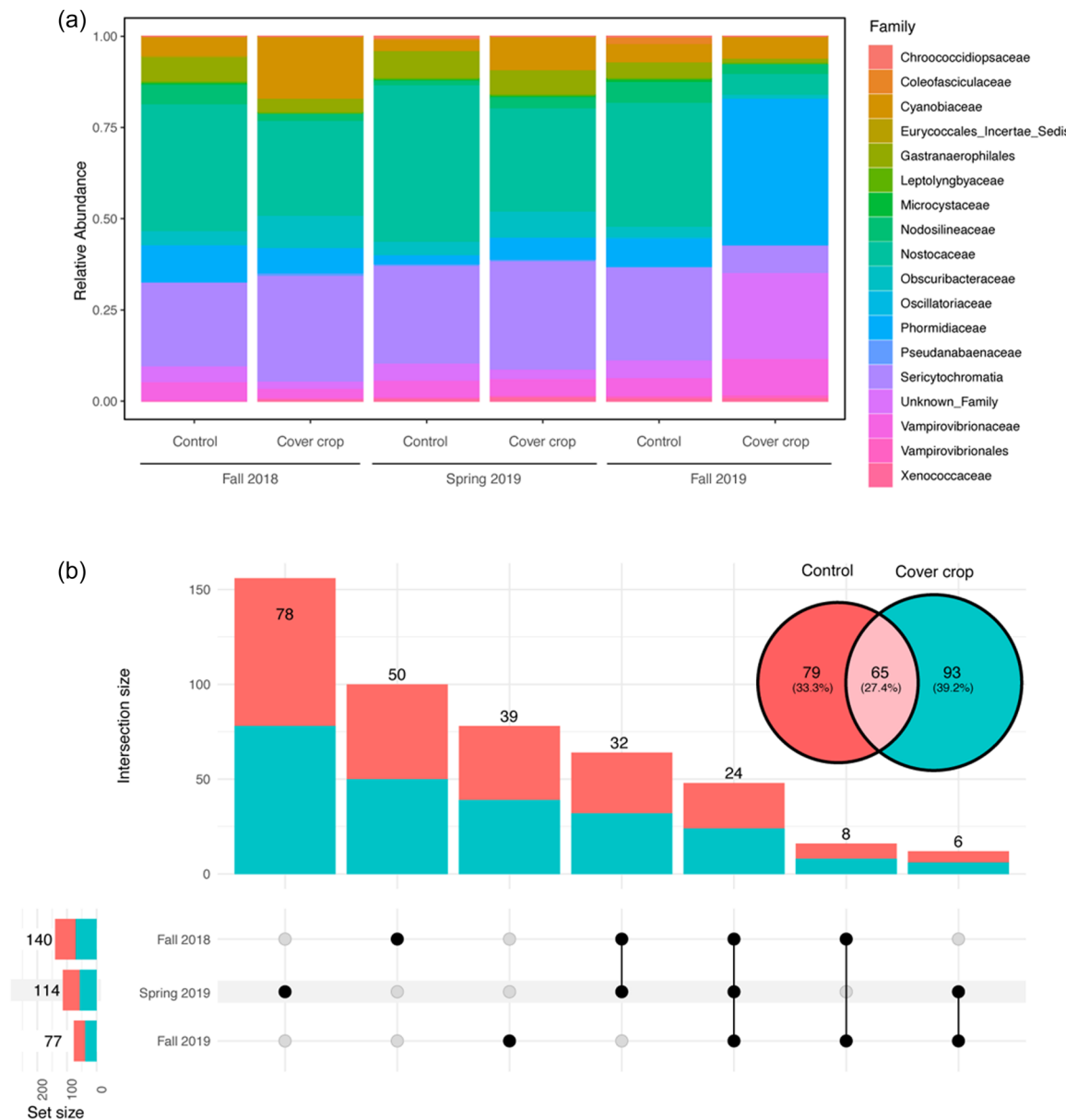
FIGURE 6 Relative abundance of different cyanobacterial families by sampling time and treatment (a) and upset plot of 16S ASVs that match bacteria in the phylum Cyanobacteria (b). The upset plot includes the total number of shared cyanobacterial ASVs (*X*-axis) at each time point (*Y*-axis) as a function of treatment. The inset Venn diagram reflects the shared 16S ASVs that match bacteria in the phylum Cyanobacteria by treatment, across time. ASV, amplicon sequence variant.

with the breakdown of plant material dominate dredged sediments and this can be enriched for fungi when cover crops are used. However, to fully elucidate the contribution of changes in the microbial community to nutrient cycling in dredged sediments following the use of a cover crop, future studies should not only take measurements of soil nutrients but also employ more in-depth metagenomic methodology to better evaluate microbial function.

We also examined corn roots directly for the presence of AM fungi and DSE, two fungi associated with nutrient cycling. While AM fungal colonization did not differ between the two plots, DSE colonization was greater for plants from the plot with the cover crop than the plot without the cover crop. These results are contrary to

previous research which has shown greater AM fungal colonization following the use of a cover crop (Bowles et al., 2017; Lehman et al., 2012) and lower DSE colonization following the use of a cover crop (Schulz et al., 2006). Taken together with the trait-based results, this suggests that fungal-driven nutrient cycling in dredged sediments changes over time, and, to a lesser extent, also changes following the use of a cover crop, but is not affected by their interaction. In contrast, bacterial-driven nutrient cycling is not affected by either time or the application of a cover crop.

Despite a full quarter of the overall microbial community consisting of plant pathogens, the only pathogen we observed infecting the plants in this experiment was corn smut. However, the

likelihood of infection did not vary between the cover crop plot and the plot left fallow. Interestingly, the causative agent behind corn smut, *U. maydis*, was not recovered in the soil microbiome, which suggests plants were infected through another pathway, probably airborne spore transfer (Kahmann et al., 2000). Corn smut is endemic throughout Ohio, including Lucas County where this experiment took place, further supporting the idea that the corn smut we observed in our experiment was transmitted locally rather than through dredged sediments. Furthermore, no guilds associated with animal biotrophic capacity or plant pathogenic capacity significantly varied by treatment, sampling time or their interaction. Thus, despite concern that dredged sediments would contain pathogenic microbes, our results with 100% dredged sediments suggest the pathogenic risk from dredged sediments is no different than the risk presented by agriculture in the region in general. Furthermore, since overall pathogen abundance was low, the use of a cover crop had little effect on pathogen abundance or composition.

Within this system, there was particular concern that the dredged sediments would contain microcystin-producing cyanobacteria (Rinta-Kanto et al., 2009), but we found very little evidence that this was the case. Instead, the most dominant member of the cyanobacteria phyla present in this system came from the family Nostocaceae, which are known for their abilities to fix nitrogen and form symbiotic relationships with plants (DeLuca et al., 2007; Rai et al., 2000; Tedersoo et al., 2018). Unfortunately, by the final collection period in the cover crop plot, the abundance of these cyanobacteria declined significantly, while the abundance of cyanobacteria from the family Phormidiaceae increased. The ecological function of the Phormidiaceae is largely undescribed but they are often found in aquatic (both fresh and saltwater) environments. Phormidiaceae can also be vital for preventing soil erosion and contributing to the C cycle through its involvement in the organic matrix of the soil as members of biological soil crusts in semiarid and arid environments (Belnap & Gardner, 1993; Pietrasiak et al., 2014; Warren & Eldridge, 2003). It is unclear why the relative abundance of these cyanobacteria increased in the cover crop plot compared to the control plot in the final time period, but it may reflect elevated levels of C cycling as alluded to by the dominance of saprobes in this plot.

5 | CONCLUSION

Soils worldwide are experiencing extensive levels of degradation. The use of dredged sediments as an amendment to restore soil function is gaining in popularity; however, concern remains about their potential to contain harmful microorganisms. This is particularly true for sediments dredged from Lake Erie, which regularly experience harmful algal blooms from microcystin-producing cyanobacteria. Our results demonstrate that these sediments contain both bacteria and fungi vital for C cycling and suggest their potential to drive increases in organic matter in degraded soils. They also suggest the use of a cover crop with dredged sediments is not necessary to acquire this benefit. This research represents an important first step

in understanding the use of dredged sediments as a soil amendment; however, to fully understand their use as a soil amendment in agricultural settings, future research linking the microbial community to changes in other soil properties and plant performance is vital.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available in the NCBI Sequence Read Archive with an accession number of PRJNA863636. All other data are available upon request.

ETHICS STATEMENT
The authors declare that they followed the ethics policies of the journal. This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the US Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this manuscript or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (https://energy.gov/downloads/doe-public-access-plan).

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