Agricultural land-use history and restoration impact soil microbial biodiversity

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Abstract

1. Human land uses, such as agriculture, can leave long-lasting legacies as ecosystems recover. As a consequence, active restoration may be necessary to overcome land-use legacies; however, few studies have evaluated the joint effects of agricultural history and restoration on ecological communities. Those that have studied this joint effect have largely focused on plants and ignored other communities, such as soil microbes.

2. We conducted a large-scale experiment to understand how agricultural history and restoration tree thinning affect soil bacterial and fungal communities within longleaf pine savannas of the southern United States. This experiment contained 64 pairs of remnant (no history of tillage agriculture) and post-agricultural (reforested following abandonment from tillage agriculture >60 years prior) longleaf pine savanna plots. Plots were each 1 ha and arranged into 27 blocks to minimize land-use decision-making biases. We experimentally restored half of the remnant and post-agricultural plots by thinning trees to reinstate open-canopy savanna conditions and collected soils from all plots five growing seasons after tree thinning. We then evaluated soil bacterial and fungal communities using metabarcoding.

3. Agricultural history increased bacterial diversity but decreased fungal diversity, while restoration increased both bacterial and fungal diversity. Both bacterial and fungal richness were correlated with a range of environmental variables including above-ground variables like leaf litter and plant diversity, and below-ground variables such as soil nutrients, pH and organic matter, many of which were also impacted by agricultural history and restoration.

4. Fungal and bacterial community compositions were shaped by restoration and agricultural history resulting in four distinct communities across the four treatment combinations.

5. Synthesis and applications. Past agricultural land use has left persistent legacies on soil microbial biodiversity, even over half a century after agricultural abandonment and after intensive restoration activities. The impacts of these changes on soil microbe biodiversity could influence native plant establishment, plant productivity and other aspects of ecosystem functioning following agricultural abandonment and during restoration.
INTRODUCTION

The conversion of natural ecosystems for human land uses is a leading threat to biodiversity (Foley et al., 2005; Newbold et al., 2015) and can leave long-lasting legacies on ecosystems (Flinn & Vellend, 2005; Foster et al., 2003). For example, former agricultural lands can support altered soils, plant communities and other properties for decades to millennia following farm abandonment, relative to ‘remnant’ ecosystems with no history of agricultural use (Bellemare, Motzkin, Foster, & Forest, 2002; Dupouey, Dambrine, Laffite, & Moares, 2002; Flinn & Marks, 2007). As a consequence, active restoration may be necessary to overcome land-use legacies in many ecosystems (Perring et al., 2015, 2016; Suding, 2011). Yet, our understanding of land-use legacies and the role of active restoration for mitigating legacy effects remains unresolved for several reasons.

First, incomplete understanding results from taxonomic biases in studies of land-use legacies and restoration. Plants have been a strong focus in both agricultural legacy and restoration research (Brudvig, 2011; Flinn & Vellend, 2005; Hermy & Verheyen, 2007). Yet, taxa respond differently to legacies and restoration activities (e.g. Jones et al., 2018) and many taxa remain poorly investigated. Here, we focus on responses of soil microbial communities, which can be strongly affected by land-use legacies and restoration practices (e.g. Barber, Chantos-Davidson, Amel Peralta, Sherwood, & Swingley, 2017; Freschet, Östlund, Kichenin, & Wardle, 2014; Hui et al., 2018; Jangind et al., 2011; Ma, De Frenne, Boon, et al., 2019; Xue, Carrillo, Pino, Minasny, & McBratney, 2018) but are poorly studied taxa in these fields (e.g. Brudvig, 2011).

Understanding soil microbial responses to agricultural legacies and restoration is especially important because these groups can have major impacts on plant diversity and productivity, as well as restoration success (van der Bij et al., 2018; van der Heijden, Bardgett, & Straalen, 2008; Wubs, Putten, Bosch, & Bezemer, 2016). For example, because of host preferences and their roles in mutualistic and antagonistic relationships, soil microbes can limit plant species distributions and alter plant community interactions (Kardol, Martijn Bezemer, & Putten, 2006; Wubs et al., 2016). In turn, inoculation of former agricultural fields with mycorrhizal fungi or whole soils from remnant ecosystems can affect plant establishment and community assembly dynamics during restoration (Koziol et al., 2018; Wubs et al., 2016). Thus, how agricultural legacies and restoration affect soil microbes may have broad-reaching implications for ecosystem recovery.

Second, studies of land-use legacies face study design challenges (De Palma et al., 2018), including biases introduced through decisions made in the past about where and how land was used by humans. For example, temperate forests on level ground, near roads and with higher pH soils are more likely to be converted to agricultural fields (Flinn, Vellend, & Marks, 2005). In turn, fields on steep slopes, located far from roads and with lower pH soils are more likely to be abandoned from agriculture (Flinn et al., 2005). As a consequence, underlying site properties might be mistakenly interpreted as agricultural legacy effects, when in fact they are simply consequences of the land-use decision-making process. Controlling for these land-use biases is particularly important for resolving how soil microbial communities respond to land-use legacies, given soil microbes’ responsiveness to soil conditions (Fierer & Jackson, 2006; Lauber, Strickland, Bradford, & Fierer, 2008; Ma, De Frenne, Vanhellemont, et al., 2019; Ma et al., 2018; Xue et al., 2018). Here, we control for land-use biases through a study design where post-agricultural plots and remnant plots with no known history of agriculture are paired in space, resulting in no bias in underlying soil types (Brudvig, Grman, Habeck, Orrock, & Ledvina, 2013).

Third, studies are needed to explicitly consider how restoration affects land-use legacies and, in turn, how land-use legacies affect restoration outcomes. Systems with a history of intensive human land use, such as agriculture, are a common focus of restoration efforts, and yet, whether and how restoration can overcome the legacies of past land uses remains unclear (Jones et al., 2018; Meli et al., 2017). Moreover, because land-use history can affect numerous system attributes, legacies alter the template onto which restoration acts. As a consequence, restoration outcomes may differ—perhaps substantially—for locations with differing land-use histories (Brudvig & Damschen, 2011; Turley & Brudvig, 2016). In other instances, however, restoration may have clear effects that are broadly similar to areas with and without a particular history (e.g. Brelend, Turley, Gibbs, Isaacs, & Brudvig, 2018). What is needed are controlled, replicated experiments to draw strong inferences about how restoration and land-use legacies interact. Yet experiments manipulating restoration treatments across areas differing in land-use history are rare. Ideally, such investigations would be coupled with measurements of key environmental variables hypothesized to affect the taxa of interest, including those affected by agricultural history and restoration. For example, within our focal system, bee responses to restoration may be mediated by the increase in flower cover resulting from restoration (Brelend et al., 2018). Therefore, determining mechanisms of legacy and restoration effects likely requires coupled measurements of key environmental variables along with the focal taxa.

We overcame these limitations through a replicated restoration experiment in longleaf pine savannas. Our experiment included a factorial manipulation of restoration (overstorey tree thinning) and agricultural history, whereby plots with and without a history of tillage agriculture received restoration or were left as unrestored controls. We arranged plots into blocks to control for land-use biases and considered how agricultural history, restoration thinning and their interaction affect soil bacteria and fungi.

KEYWORDS
agricultural history, bacteria, community ecology, fungi, land-use legacy, metabarcoding, restoration, soil microbe biodiversity
Previous work within our experiment has shown how agricultural history and restoration affect abiotic and biotic conditions in ways that we expect to influence soil microbial communities and diversity. In particular, compared to remnants, post-agricultural savannas support soils that are more compacted, support elevated phosphorus and reduced organic matter content and water holding capacity, as well as lower tree canopy cover and altered plant community composition (but comparable plant species richness; Brudvig et al., 2013). The restoration thinning treatment decreased tree canopy cover and litter accumulation; increased near-ground temperatures and sunlight reaching ground level; increased plant species richness; and altered plant community composition (Hahn & Orrock, 2015; Stuhler & Orrock, 2016; Turley & Brudvig, 2016). Based on these past findings, we suspected that soil microbial diversity and community composition will also be affected by both land-use history and restoration thinning.

We asked the following questions related to both soil bacteria and soil fungi from our field experiment:

1. What are the effects of agricultural land-use history and restoration on soil microbe diversity and composition?
2. Does the effect of restoration on diversity and composition depend on agricultural land-use history (i.e. do agricultural history and restoration interact)?
3. Do environmental variables correlate with microbial biodiversity?
4. Do correlations between microbe diversity metrics and environmental variables help explain the impacts of restoration and land-use history on soil microbe biodiversity?

2 | MATERIALS AND METHODS

2.1 | Study location and experimental design

Our research took place at the Savannah River Site (SRS), an ~80,000 ha National Environmental Research Park located on the upper coastal plain in South Carolina (33.20°N, 81.40°W). This area historically supported fire-maintained longleaf pine savanna in the sandy uplands (Kilgo & Blake, 2005)—an ecosystem characterized by sparse canopies dominated by longleaf pine trees *Pinus palustris* and a dense understorey plant layer of graminoids, forbs and shrubs (Noss et al., 2015). By the mid-20th century, most of the SRS uplands had been converted to tillage agriculture, primarily for cotton and corn (Kilgo & Blake, 2005). In 1951 the US government obtained SRS and began converting agricultural fields to longleaf, loblolly *Pinus taeda* and slash pine *Pinus elliottii* plantations (Kilgo & Blake, 2005). Following acquisition (and likely for decades prior to this), fire was excluded from ecosystems within SRS, until initiation of prescribed burning in the early 21st century (Kilgo & Blake, 2005).

At SRS, we conducted a factorial experimental manipulation of agricultural history and restoration tree thinning, across 126 1-ha plots arranged into 27 blocks (Figure 1). Each block was focused around a fragment of remnant longleaf pine savanna, with no known history of tillage agriculture, adjacent to a former agricultural field supporting closed-canopy pine (longleaf where possible) plantation at the initiation of the study (Brudvig et al., 2013). We determined land-use histories for each plot using historical aerial photos taken in 1951, at the time of SRS’s creation (Brudvig et al., 2013). Remnant and post-agricultural plots within blocks supported similar soil types and topographies (Brudvig et al., 2013), suggesting that the blocked experimental design adequately controlled for non-random land-use decision-making.

In 2011, prior to the start of the growing season, we applied a tree thinning treatment to restore open-canopy, savanna structure to half of the remnant and post-agricultural plots (Turley & Brudvig, 2016). This reduced tree densities from an average of 650 trees/ha to 10 trees/ha. All plots have subsequently been managed with one or more prescribed fires. The frequency of prescribed surface fire did vary among the 26 blocks since the initiation of the experiment; however, all plots, and thus all four treatment combinations, within a block were always burned together. Although fire could be an important factor shaping soil microbes within longleaf pine savannas (Semenova-Nelsen, Platt, Patterson, Huffman, & Sikes, 2019) looking at this is beyond the scope of this study.

![Figure 1](image-url)
2.2 | Soil sampling and processing

In Fall 2015 we collected ~1.2 L of soil from each of the 126 1-ha plots. Each soil sample was an aggregate of 30 ± 1.6 cm wide by 20 cm deep soil probes collected along two 50 m transects through the middle of each plot (Figure 1). The soil sampling transects ran on both sides of our already-present vegetation sampling transects (Turley & Brudvig, 2016; Figure 1). Before each probe the leaf litter, duff and sticks were brushed aside. To minimize contamination we used one soil probe for all remnant sites and another for all post-agricultural sites and between each plot we rinsed the inside and outside of the probe with a 10% bleach solution and then water. Aggregate soil samples were mixed thoroughly and split up for different purposes. About 50 ml was stored in a −20°C freezer for microbial analysis, and two other subsamples were used for environmental sampling.

For microbial analysis, we extracted soil DNA using MoBio PowerSoil Extraction Kit following the manufacturer’s instructions. We submitted DNA to the Michigan State University Core Genomics Facility for Illumina sequence library construction. Following their standard protocols, bacterial 16S V4 (S15f/806r) and ITS (ITS-F/ITS2) Illumina compatible libraries were prepared using primers containing both the target sequences and the dual indexed Illumina compatible adapters. The 16S and ITS1 amplicon pools were sequenced independently in a 2 × 250bp paired end format using independent v2 500 cycle MiSeq reagent cartridges.

The first of the soil subsamples was analysed by Brookside Laboratories Inc. for soil texture (percent sand, clay and silt), pH, organic matter, and nutrients and minerals. On the second subsample we measured soil water holding capacity (proportionate difference between saturated wet and oven dry weight) and gravimetric soil moisture using the same methods as Brudvig and Damschen (2011). Soil pH, water holding capacity, organic matter and several soil nutrients all decreased with agricultural history while soil phosphorus was strongly increased (see Table S1).

2.3 | Environmental data collection

We measured a set of environmental variables within each experimental plot at 10 m intervals along the 100 m vegetation transects (Figure 1) during the 2015 growing season. In 1 × 1 m plots we visually estimated the percent cover of leaf litter, down woody debris, bare ground and understory vegetation. At each of these plots we also measured the depth of leaf litter and canopy cover of overstory trees using a spherical densiometer. In 1 × 1 m and 10 × 10 m plots we recorded all plant species and calculated plant species richness. For all these environmental variables we averaged the 10 measurements across each transect to get one value per 1-ha plot. Restoration thinning resulted in strong declines in leaf litter and canopy cover and large increases in vegetation cover and understory plant richness (Table S1). Units and methods for measuring all of our environmental variables are available in Table S6.

2.4 | Bioinformatics

We processed and clustered bacterial and fungal reads into operational taxonomic units (OTUs). Reads from the bacterial community were chimera checked, quality filtered and merged using Trimomatic and Pandaseq (Bolger, Lohse, & Usadel, 2014; Masella, Bartram, Truszkowski, Brown, & Neufeld, 2012). Processed reads were clustered into OTUs at 97% identity level using UCLUST6.1 with the default settings (Edgar, 2010). Singletones were removed and contigs were screened using QIME 1.9.1 (Caporaso et al., 2010) with the default parameters. OTUs classified to chloroplast, mitochondria or with less than four reads across all samples were filtered out to avoid over splitting (Thiéry, Moora, Vasar, Zobel, & Ópik, 2012) and sequencing errors (Dickie, 2010). The resulting community was composed of 90,103 OTUs and 1,650,420 reads. Fungal reads were quality filtered and merged using the USEARCHv10 pipeline (http://drive5.com/usearch/; Edgar, 2010, 2013). Merged sequences were quality filtered to an expected error threshold of 1.0 fastq_filter (Edgar & Flyvbjerg, 2015) and primer sequences bases were removed. The combined reads were clustered into OTUs at 97% identity level then reference-based chimera checked (Edgar, 2016) and classified against the UNITE 7.1 ITS1 chimera and reference databases respectively (Kõljalg et al., 2013). All non-fungal OTUs and those with less than four reads were filtered from the community matrix. The resulting fungal community had 10,285 OTUs and 584,113 reads.

2.5 | Statistical analysis

We conducted all analyses in R version 3.5.1. We first removed two samples with extremely low reads: a bacteria sample with 471 reads and a fungal sample with 78 reads (compared to means of ~69,000 and 5,000 respectively). For measuring diversity we rarified the community datasets following Weiss et al. (2017) using the ‘rrarefy’ function (Oksanen et al., 2010). We set the minimum value in the rarefaction to the lowest observed read number in a sample. With those community datasets we calculated richness, evenness and inverse Simpson’s D. Our evenness metric was inverse Simpson’s diversity divided by species richness. We focus primarily on inverse Simpson’s D as our measure of biodiversity as this is recommended for microbial datasets (Haegeman et al., 2013). We evaluated correlations between average plot-level (1-ha) environmental variables and diversity metrics using Pearson’s correlations.

To test the effects of agricultural history and restoration thinning on biodiversity metrics we fit mixed effects models using the ‘lmer’ function (Bates, Mächler, Bolker, & Walker, 2015). We included restoration thinning, agricultural history (both two-level factors) and their interaction as fixed effects. Site (a 27-level categorical factor) and land-use history were included as random effects. Land-use history was nested within site to account for the pseudoreplication
inherent in the hierarchical experimental design. The model syntax was:

\[ Y \sim \text{thinning} \times \text{land use} + (1|\text{site/land use/thinning}). \]

We used the ‘ANOVA’ function (Fox & Weisberg, 2018) to calculate \( p \)-values using Type 2 sums of squares. We used Type 2 sums of squares because our models had non-significant interaction terms and this allowed us to interpret the main effects while keeping the interaction term in the model. We determined \( R^2 \) for the factors using the ‘r2beta’ function with the standardized generalized variance method (Jaeger, 2017). For community composition analyses we transformed the data using the ‘varianceStabilizingTransformation’ function with the ‘local’ fit type (Love, Huber, & Anders, 2014; Weiss et al., 2017). On the transformed datasets we created a distance matrix using Bray–Curtis dissimilarity which was abundance weighted by read number. We tested the effects of our factors on community composition by fitting PERMANOVA models with the ‘adonis’ function (Oksanen et al., 2010). We included the site factor as a ‘strata’ term. Because nesting is not possible with the ‘adonis’ function the degrees of freedom for these tests are inflated which could artificially reduce \( p \)-values. We visualized the effects of our treatments on community composition by performing a constrained analysis of principal coordinates using the ‘capscale’ function with default parameters then visualizing the ordination using the ‘ordiplot’ function (Oksanen et al., 2010). We used the ‘envfit’ function (Oksanen et al., 2010) to test for correlations between environmental variables (Bray–Curtis dissimilarity matrix) and the microbe community ordinations (non-metric multidimensional scaling with Bray–Curtis dissimilarity). To account for concerns of oversplitting due to open reference OTU clustering (Edgar, 2017), we ran the same PERMANOVA model on the bacterial Unifrac distance matrix. Accounting for phylogeny did not change the results, so we only present the Bray–Curtis-based results.

We explored the relationship among experimental treatments, environmental variables and microbial diversity variables using structural equation modelling. Because there were many, sometimes collinear, potential environmental variables to include in the analyses (Tables S3 and S4), we simplified the data into two composite variables using a principle components analysis (PCA). We standardized all variables to have a mean of 0 and standard deviation of 1, then fit SEM’s using the ‘sem’ function (Rosseel, 2012). We fit models with PC1 and PC2 as endogenous variables between the treatments and microbe biodiversity metrics. To test the importance of the environmental variables (PC1 and PC2) in the models, we fit SEM’s without them and compared the \( R^2 \) to the full models with them included.

### 3 | RESULTS

#### 3.1 | Question 1: Effects of agricultural history and restoration on soil microbial biodiversity

History of agricultural land use had opposite effects on bacterial and fungal diversity (inverse Simpson’s D) and also shaped community composition. For bacteria, agricultural history increased diversity by 53.7% (Figure 2a; Table 1) whereas for fungi, agricultural history reduced diversity by 18.5% (Figure 2b; Table 1). These results were driven primarily by changes in evenness for bacteria and richness in fungi (Table S2). Agricultural history also significantly affected microbial composition (Figure 3; Table 1) which explained 2.5% of bacterial and 3.9% of fungal community variation.

Restoration increased both bacterial and fungal diversity and impacted community composition. Restoration increased bacterial diversity by 13.8% (Figure 2a; Table 1) and fungal diversity by 60.1% (Figure 2b; Table 1). These changes in diversity were driven by increases in both richness and evenness (Table S2). Restoration thinning also shaped bacterial and fungal communities (Figure 3; Table 1) and this factor explained 1.2% and 2.6% of variation in communities respectively.

#### 3.2 | Question 2: Effects of agricultural history on restoration effects

Overall there was little evidence that the effects of restoration were dependent on agricultural history. There were no significant interactions between restoration and agricultural history for bacterial or fungal diversity (Table 1). There was a significant interaction between agricultural history and restoration on fungal community composition explaining 1% of variation.

![Figure 2](image-url) Effects of agricultural land-use history and restoration thinning on diversity (inverse Simpsons’s D) within a longleaf pine savanna experiment in South Carolina for (a) bacteria and (b) fungi. Remnant plots are savannas with no history of agriculture and post-agricultural sites had tillage agriculture that was abandoned over 60 years ago and then managed as pine plantation.
3.3 | Question 3: Correlations between environmental variables and soil microbial biodiversity

Many environmental variables were correlated with soil microbial diversity, richness and evenness (Tables S3 and S4). A PCA collapsed this variation into two composite variables. The first axis from this analysis (PC1) was associated mostly with below-ground variables. Negative values were associated with % sand, soil Fe and soil P, while positive values were associated with a wide range of soil micronutrients, soil organic matter, soil water holding capacity, % silt and soil pH (Figure 4; Table S5). PC2 was associated mostly with above-ground variables related to canopy density. Positive values of PC2 were associated with % canopy cover, leaf litter and bare ground, whereas negative values were associated with % cover bare ground (Figure 4; Table S5). Plant richness, % vegetation cover and % leaf litter were associated with both axes, with PC1 positively associated with plant richness and PC2 negatively associated with plant richness (Figure 4; Table S5).

The principle components of environmental variables predicted soil microbial richness and evenness, and diversity. The strongest correlations were between PC1 and richness (Table 2).
Locations with wetter, more nutrient rich and basic soils, and with greater plant species richness supported greater soil microbial richness, and this relationship was stronger in fungi than in bacteria (Figure 5). PC1 was somewhat negatively correlated with bacterial evenness and not significantly correlated with diversity (Table 2). PC1 had no relationship with fungal evenness and was positively correlated with fungal richness (Table 2). PC2 was negatively correlated with all measures of fungal and bacterial biodiversity (richness, evenness and Simpson’s diversity) with the exception of fungal richness (Table 2). Thus, plots with greater tree canopy cover and leaf litter had reduced soil microbial diversity, whereas plots with more bare ground, understorey vegetation and plant richness supported greater levels of microbial biodiversity.

Microbial community composition was also correlated with a wide range of environmental variables (Tables S3 and S4). Bacterial communities were correlated with most below-ground variables such as soil pH, nutrients, texture and water holding capacity (Table S3), but not with above-ground variables (with the exception of one measure of plant richness). Fungal communities were also correlated with below-ground variables, similar to bacteria, but were also correlated with above-ground variables such as plant richness, leaf litter and tree canopy cover (Table S4). Overall, environmental variables had significant correlations with community ordination for bacteria (Mantel test, $r = .21$, $p = .001$) and fungi (Mantel test, $r = .23$, $p = .001$).

### 3.4 Question 4: Do environmental variables help explain effects of treatments on microbe biodiversity

Our structural equation models (SEM’s) showed that agricultural land-use history and restoration treatments impacted microbial diversity (inverse Simpson’s D) and evenness mostly independently of the environmental variables we measured, while microbial richness was mostly predicted by environmental variables and not the treatments. The SEM’s showed that agricultural history and restoration thinning impacted both of the environmental PC axes (Figure 6) and the direct effects of the treatments on environmental variables are summarized in Table S1. Agricultural history was the strongest predictor of bacterial diversity, but the environmental variables were also significant (Figure 6a). The model overall explained 57% of the variation in bacterial diversity (Figure 6a). A SEM fit without the environmental variables as intermediates between the treatments and diversity still explained 53% of variation in bacterial diversity. The fungal diversity SEM had restoration thinning as a significant

![Figure 5](image-url) Relationship between the first principle component axis of environmental variables (see Figure 4) on (a) bacterial richness and (b) fungal richness. Richness was calculated from a rarefied community dataset. Negative values of PC1 are associated with % sand, Fe, P, leaf litter while positive values are associated with a wide range of soil micronutrients, soil organic matter, soil water holding capacity, % vegetation cover and plant richness.

![Figure 6](image-url) Structural equation model path diagrams showing the main treatment effects at the top, principle component axis of environmental variables in the middle and inverse Simpson’s diversity at the bottom for (a) bacteria and (b) fungi. The width of the arrows is proportional to the magnitude of the path coefficient. Black arrows are positive correlations, grey arrows are negative correlations and dashed arrows are non-significant paths.
predictor along with the environmental PC axes (Figure 6b) which explained a total of 30% of the variation in diversity. This model without the environmental variables explained 24% of variation in fungal diversity. The models for evenness (both for bacterial and fungal) showed similar patterns to those of diversity with the environmental variables explaining minimal variation (<2%) in evenness (Figure S4).

SEM explained little variation in microbial richness when environmental variables were excluded. The full model for bacterial richness explained 44% of variation in richness (Figure S3) but without environmental variables explained only 4%. Similarly, for fungal richness the full model explained 48% of variation in richness (Figure S3) while the model without environmental variables explained only 17%.

4 | DISCUSSION

Soil bacteria and fungi biodiversity were both affected by agricultural history, restoration thinning and environmental variables. Our results point to four major conclusions: (a) agricultural history increased bacterial diversity while reducing fungal diversity, (b) restoration thinning increased fungal and bacterial diversity, (c) agricultural history and restoration thinning resulted in four distinct bacterial and fungal communities across the four plot types and (d) environmental variables were important predictors of microbial diversity, mostly through their impacts on microbial richness.

4.1 | Possible explanations for changes in bacterial and fungal biodiversity

Agricultural land-use history increased bacterial diversity, similar to findings from other studies (Delgado-Baquerizo et al., 2017; Dong, Huai-Ying, De-Yong, & Huang, 2008; Hartman, Richardson, Vilgalys, & Bruoland, 2008; Jesus, Marsh, Tiedje, & Moreira, 2009; Rodrigues et al., 2013; Upchurch et al., 2008). Soil nutrients (Delgado-Baquerizo et al., 2017; Lauber et al., 2008) and soil pH (Jesus et al., 2009; Rodrigues et al., 2013) may be important factors mediating land-use history effects on microbial diversity. Similarly, we found a suite of variables that correlated with bacterial diversity (Figure 6) and richness (Figure 5) that were also impacted by agricultural history. In our system, post-agricultural sites had decreased soil organic matter, micronutrients (S, Ca, Mg, Al and K), moisture, and water holding capacity and increased soil P (Table S1). Given collinearities among these variables (Figure 4), it is difficult to say which of those that correlated with measures of bacterial metrics of biodiversity (Table S3) mechanistically influenced diversity. However, we did find a strong pattern that environmental variables, especially below-ground variables, were the most important predictors of bacterial richness, greatly increasing our predictive power of the effects of treatments on richness (Figure S3). However environmental variables explained much less variation in diversity (Figure 6), and almost none at all for evenness (Figure S4). This suggests that microbial evenness and richness are responding to fundamentally different environmental gradients in this system and illustrates the importance of considering multiple biodiversity measures when evaluating responses to disturbance and management.

In contrast to bacteria, fungal diversity was lower in post-agricultural plots, although the magnitude of this response was relatively small (Figure 2). Other studies have also found that agricultural land use lowers fungal diversity (Ding et al., 2013; Oehl et al., 2003; Wagg, Dudenhöffer, Widmer, & Heijden, 2018) and our analyses suggest that the above-mentioned environmental variables associated with bacteria could also be important factors shaping fungal diversity. It is also possible that post-agricultural recovery was limited by dispersal from remnant to post-agricultural plots for fungi, as we see for plants (Turley, Orrock, Ledvina, & Brudvig, 2017), or that fungi are relatively slower growing than bacterial and thus slower to recover following disturbance.

Restoration increased both bacterial and fungal diversity, although the effect was stronger for fungi (Figure 2). Decreases in canopy cover and leaf litter, along with increases in vegetation cover and plant richness, may help explain the increased bacterial richness and diversity in thinned plots as PC2 was a strong predictor of bacterial diversity (Figure 6a) and richness (Figure S3). However, this was less for fungi (Figure 6a; Figure S3). Restoration greatly increased plant species richness (Table S1; Turley & Brudvig, 2016), which may mediate the effects of restoration thinning on soil microbial communities by increasing the number of suitable plant hosts for host-specific microbes (Peay, Baraloto, & Fine, 2013; Prober et al., 2015), although it is also possible that microbial diversity enhanced plant richness. Finally, restoration thinning in savanna ecosystems can increase the variability in biota and environmental gradients (Brudvig & Asbjornsen, 2009), thereby increasing the number of potential niches within a site, for microbes of diverse life histories (Curd, Martiny, Li, & Smith, 2018). Such enhancement of heterogeneity may be particularly important when restoring post-agricultural ecosystems, like in our study, given reductions in heterogeneity that can persist for decades or longer following agricultural abandonment (Flinn & Marks, 2007).

4.2 | Community composition in response to agricultural history and restoration

Our results illustrate how agricultural legacies are long-lasting for soil microbial communities, persisting over half a century after agricultural abandonment despite post-agricultural and remnant plots being adjacent in our experiment. These findings add to a growing body of literature showing varying effects of land-use legacies on soil microbes (Fichtner, Oheimb, Härdtle, Wilken, & Gutknecht, 2014; Hartman et al., 2008; Hui et al., 2018; Jangind et al., 2011; Lauber et al., 2008; Upchurch et al., 2008), although some studies show no impacts of land-use history on soil bacteria (Ma, De Frenne, Boon, et al., 2019; Ma, De Frenne, Vanhellemont, et al., 2019). Our
community analyses show that both fungal and microbial communities cluster into four distinct community types (Figure 3; Table 1), which is very similar to how plant communities have responded to our treatments (Turley & Brudvig, 2016). This means that restoration did not result in post-agricultural communities being more similar to remnant communities. Similarly, Strickland et al. (2017) found that restored forests in Mississippi had soil microbial communities distinct from agricultural fields and from nearby remnant forests. They conclude that above-ground restoration focused on forest structure does little to drive microbial communities towards the remnant reference state, or perhaps that these changes will happen very slowly or be contingent on restoration of plant community composition. Alternatively, agricultural legacies could be due to priority effects where chance events early in community assembly results in different community outcomes that persist even with the recovery of environmental conditions (Keiser, Strickland, Fierer, & Bradford, 2011).

4.3 | Implications for management

We found little evidence that the effects of restoration thinning for soil microbes differed between remnant and post-agricultural plots. This finding suggests that agricultural history and restoration are independently operating on different groups of microbial species, with some species either dispersal limited or affected by altered environmental gradients following agricultural abandonment (e.g. elevated soil phosphorus) and a second group promoted by restoration thinning. This presents a mixed message for the prospects of soil microbial recovery during restoration. On the one hand, restoration can increase the diversity of soil fungi and bacteria in plots within either land-use history. On the other hand, restoration does not mitigate the legacies of historical agricultural land use. Thus, successful soil microbial restoration may require coupling of structural habitat manipulation to reinstate appropriate environmental conditions for a diverse suite of microbes with active reintroduction of soil microbes that do not recover passively following agricultural land use (e.g. Koziol et al., 2018; Wubs et al., 2016). In turn, active reintroduction of soil microbes may be important for re-establishing certain plant species during restoration (Harris, 2009; Kardol & Wardle, 2010). Evidence to date from our experiment does not support this, however, with a suite of understory herbs actually establishing better in post-agricultural plots and performing similarly when grown in soils inoculated with soil microbes from remnant and post-agricultural plots (Barker, Turley, Orrock, Ledvina, & Brudvig, 2019).

Whether and how soil microbial communities recover following human land use and active restoration efforts remains an open question (Harris, 2009) and our study adds to accumulating evidence that restoration actions manipulating ecosystem structure and plant diversity (directly or indirectly) also affect soil microbial communities (Banning et al., 2011; Barber et al., 2017; Dickens, Allen, Santiago, & Crowle, 2015; Potthoff et al., 2006). We further illustrate the potential for restoration to benefit soil microbes across sites supporting different land-use histories. Given the consequences of microbial communities for ecosystem dynamics during restoration (Kardol & Wardle, 2010), soil microbial differences resulting from land-use legacies and restoration actions may have broad-reaching implications for ecosystem recovery and restoration outcomes in degraded ecosystems.

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AUTHORS’ CONTRIBUTIONS

N.E.T. and L.A.B. conceived the research idea and wrote the paper; N.E.T. collected the field samples and analysed the data; L.B.-D. and S.E.E. conducted laboratory work and bioinformatics. All the authors edited the paper.

DATA AVAILABILITY STATEMENT

All raw sequence data from this study are available through the NCBI Sequence Read Archive under project PRJNA551504 and SRAs SRR9609456 - SRR9609568. Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.x3ffbg7fd (Turley, Brudvig, Bell-Dereske, & Evans, 2020).

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REFERENCES


SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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