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Soil microbial succession following surface mining is governed primarily by deterministic factors

Jennifer L. Kane

Thesis submitted
to the Davis College of Agriculture, Natural Resources, and Design
at West Virginia University

in partial fulfillment for the degree of
Master of Science in
Applied and Environmental Microbiology

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ABSTRACT

Soil microbial succession following surface mining is governed primarily by deterministic factors

Jennifer L. Kane

Understanding the successional dynamics governing soil microbial community assembly is a promising way to advance development of remediation strategies for lands disturbed by anthropogenic activities. The environmental and ecological influences shaping these communities following soil disturbance remain only partially understood. One example of a physical anthropogenic disturbance is extraction of minerals such as coal by surface mining. Surface mining removes natural soils and these soils may be replaced immediately on adjacent reclaimed areas or they may be stored in piles for later use. During reclamation, the soil is replaced on the landscape and the site is revegetated with grasses and trees. Throughout this process, the soil's physical and chemical properties are drastically changed and soil microbial communities are spatially displaced, causing changes in water relations and nutrient cycling, as well as microbial abundance and community composition. These changes have a large effect in eliciting selective pressure on microbial taxa (*i.e.*, deterministic processes). Dispersal and ecological drift are also important in shaping communities following disturbance (*i.e.*, stochastic processes). We investigated the influence of stochastic and deterministic factors in shaping the soil microbiome following reclamation using formerly surface mined and reclaimed areas ranging from 2 to 32 years since reclamation occurred. A suite of soil chemical and physical parameters were measured to quantify the influence of deterministic processes and time was considered a proxy for stochastic processes. Sequencing of bacterial and fungal rRNA gene amplicons coupled with a linear modeling approach revealed that the soil microbiome following mine reclamation is shaped by both deterministic and stochastic influences, but that deterministic factors influence microbial succession more than stochastic factors by a ~4-fold difference. Further, while microbial biomass and diversity did not consistently increase with time following reclamation, the abundance of ecologically important bacterial taxa (*e.g.*, Alpha- and Deltaproteobacteria) showed varying but significant responses, potentially due to the concomitant increases in soil nutrients such as carbon, nitrogen, and phosphorous. Our results suggest that management of deterministic soil characteristics over a sufficient time period could result in an accelerated recovery of the soil microbiome to pre-disturbance levels and composition, and therefore increased productivity of post-mining land uses.

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1. INTRODUCTION

1.1 Ecological Succession

Ecological succession theory predicts community compositional shifts following disturbance (Connell and Slatyer, 1977). Though ecological succession in plant communities has been widely studied (*e.g.*, Grime, 1979; Morris and Leger, 2016; Tilman, 1988), the mechanisms governing such patterns among microbial communities (*i.e.*, microbiota) remain unclear. Current theory in microbial ecology postulates that selection, diversification, dispersal, and drift mediate microbial community succession (Nemergut et al., 2013; Vellend, 2010). Still, questions remain regarding the relative influence of stochastic (*e.g.*, dispersal, ecological drift) and deterministic (*e.g.*, selection, diversification) processes on soil microbial communities across environmental and temporal gradients (Nemergut et al., 2013). To date, investigations of microbial community assembly mainly explore succession after natural disturbances like glacial retreat (Cline and Zak, 2014; Freedman and Zak, 2015; Jumpponen, 2003), fire (Ferrenberg et al., 2013; Gassibe et al., 2011; Hart et al., 2005), or volcanic eruption (Ibekwe et al., 2007; Zeglin et al., 2016). Far fewer studies focus on anthropogenic disturbances like land use change (Jesus et al., 2009; Lauber et al., 2008) or severe physical disturbances like tillage (Calderón et al., 2000; Lupwayi et al., 1998). Understanding microbial succession following disturbance is vital, since soil microbiota contribute to key ecosystem services (*e.g.*, nutrient cycling, mutualisms with plant hosts) and may contribute to future ecological success of a disturbed environment (Fierer et al., 2012; Harris, 2009).

Selection imposed by deterministic factors (*e.g.*, soil chemical and physical properties) affects the presence, absence, and relative abundance of microbial taxa within communities (Girvan et al., 2003; Lauber et al., 2008). For example, pH is a key driver of microbial

community composition at the global scale (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010), and other factors such as soil moisture, total carbon (C), total nitrogen (N), and C:N have also been noted as determinants of microbial community composition at smaller scales (Freedman and Zak, 2015; Högberg et al., 2007; Lauber et al., 2008; Romanowicz et al., 2016). Further, stochastic (*e.g.*, dispersal by wind, ecological drift) and deterministic processes can interact to influence microbial community composition in soil (Caruso et al., 2011; Cline and Zak, 2014; Ferrenberg et al., 2013; Freedman and Zak, 2015). While soil microbial community assembly in anthropogenically disturbed soils is not widely studied, deterministic factors such as bulk density, pH, and plant species can be influential in shaping communities following physical disturbance of the soil profile (Calderón et al., 2000; da C Jesus et al., 2009; Dimitriu and Grayston, 2010; Li et al., 2016).

1.2 Surface mining and microbial community assembly

One particularly severe anthropogenic disturbance to soil is surface mining for natural resources. In the United States, upwards of 2.5 million hectares of land have been surface mined over the past 100 years (Emerson et al., 2009). The goal of many post-mining land uses is aboveground productivity (*e.g.*, agriculture, forestry), which is dependent on adequate soil health (Figure 1; Macdonald et al., 2015; Skousen and Zipper, 2014). Despite the use of native topsoil (Skousen and Zipper, 2014; referred to as mine soils) these soils often exhibit adverse chemical and physical properties (*e.g.*, low nutrient content, high bulk density; Emerson et al., 2009). During the mining process, native soils are mixed together, stored, and replaced, and thereby the established communities become spatially displaced. Changes in soil chemical and physical properties accompanying storage and after reapplication potentially apply selective pressures on

microbial taxa. In this way, the nature of surface mining makes reclaimed mine soils a novel system to investigate soil microbial community succession.

To date, there has been some insight into how microbial communities respond to surface mining using chronosequence-based approaches (Banning et al., 2008; Dimitriu et al., 2010; Li et al., 2016; Sun et al., 2017). However, the relative influence of stochastic and deterministic factors on microbial communities during succession following surface mining remains unexplored. It has been established that physical disturbances such as mining, tillage, and land use change can cause distinct declines in ecologically important soil physical and chemical parameters (*e.g.*, bulk density, water-holding capacity, pH, C and N content) as well as microbial activity, biomass, and diversity, which is suggestive of deterministic selection (Banning et al., 2008; Calderón et al., 2000; da C Jesus et al., 2009; Dimitriu et al., 2010; Li et al., 2016; Sun et al., 2017). Stochastic processes also shape microbial succession following disturbance, for example, through genetic bottlenecks or barriers to dispersal (Schmidt et al., 2014; Sun et al., 2017; Verbruggen et al., 2012). If deterministic and stochastic processes are shaping microbial communities in concert, this must be considered during the reclamation process to strategically manage the soil environment for microbial communities and their function. This is important as it could potentially result in increased success of post-mining land uses, particularly those which are agriculturally-based.

1.3 Objective and Hypotheses

Here, we assessed the relative influence of deterministic and stochastic processes on soil microbial communities across a chronosequence of reclaimed mine sites in West Virginia (WV), USA. We hypothesized that, with time since reclamation, soil physical, chemical, and biological properties would trend towards that of undisturbed systems; namely, microbial biomass and

diversity would increase. Further, we hypothesized that while both stochastic and deterministic factors would explain distinct amounts of variation in community composition, the relatively stressful conditions imposed on soil microbiota following mining and reclamation would elicit strong deterministic influences on succession. To address our objectives, soil samples were taken at four reclaimed surface mine sites around Monongalia County, WV, USA that are similar besides time since mining and reclamation occurred. A nearby pasture, a common post-mining land use (Skousen and Zipper, 2014), served as a reference site. Microbiome composition was assessed by sequencing of bacterial and fungal rRNA gene amplicons. We utilized a modeling approach to determine the relative impacts of soil chemical and physical parameters (*i.e.*, deterministic factors) as well as time since reclamation (*i.e.*, a proxy for stochastic influences) on microbial community succession following surface mining.

2. METHODS

2.1 Site Description and Sample Collection

To investigate soil microbial community succession following surface mine land reclamation, four reclaimed mine sites in Monongalia County, WV were selected and considered as a chronosequence, as the time since reclamation varied from 2 to 32 years (Chaudhuri et al., 2013; Table 1). Due to their proximity, these sites experience similar climactic conditions and are of similar geology, which consists of sandstone, shale, siltstone, and limestone (Chaudhuri et al., 2013). Further, the four sites that comprised the chronosequence were reclaimed in approximately the same manner, which included backfilling followed by the application of approximately 20 cm of topsoil in accordance with the Surface Mining Control and Reclamation Act of 1977 (Chaudhuri et al., 2012). The sites were revegetated with mixed-grass leguminous

species such as orchard grass (*Dactylis glomerata)*, clover (*e.g.*, *Trifolium* spp.*)*, birdsfoot trefoil (*Lotus corniculatus),* and fescue grasses (*Festuca arundinacea;* Chaudhuri et al., 2013).

Sites were sampled in September of 2017, a time during which warm temperatures and high moisture favors high rates of microbial activity. According to the topographic relief of each site, a lowland and highland area was defined at each of the four chronosequence sites. From both the lowland and highland areas at each site, five random soil samples were taken to a 15-cm depth and the organic (O) and mineral (A) horizons were separated and homogenized by hand in the field. As a reference site, an undisturbed perennial pasture was sampled at the West Virginia University (WVU) Organic Farm in September of 2018 in a similar manner. Samples were kept on ice until return to the lab, where they were passed through a 2000 µm sieve to remove large roots and rocks. A subsample of sieved soil was kept at -80˚C for DNA analysis and the remainder was kept at 4˚C for biological and chemical analyses. Three replicates from each homogenized sample were included in each analysis (12 total samples per site).

2.2 Soil Physical and Chemical Analyses

Soil physical and chemical parameters were assessed that have been determined to influence soil microbial activity and diversity (Fierer et al., 2007; Freedman and Zak, 2015; Romanowicz et al., 2016). Gravimetric soil moisture was determined by drying 10 g of fresh soil at 105˚C for 24 hours. Soil pH was measured using a 1:5 soil:$CaCl_2$ suspension of air dried soil on an Accumet AE150 pH meter (Fisher Scientific, Hampton, NH, USA). Organic matter content was determined by loss-on-ignition, whereby dried soil from moisture analysis was combusted at 500˚C for 6 hours. Percent clay was estimated using the Texture-by-Feel method (Thien, 1979). Total C and total N were determined by combustion of ~250 mg of soil on a Vario MAX cube (Elementar, Landenselbold, Germany). The bioavailable carbon pool was estimated

according to the Permangenate Oxidizable Carbon Method (Weil et al., 2003). Soil N and P were extracted from bulk soil in 1 M KCl and Modified Morgan Extract respectively by shaking at 100 RPM for 90 minutes followed by filtration through a Whatman #42 filter (Whatman, Maidstone, UK). Extracts were stored at -20˚C until analysis using an AQ300 Discrete Chemical Analyzer (SEAL Analytical, Mequon, WI, USA) following U.S. Environmental Protection Agency (EPA) methods 353.2 for nitrate and nitrite, 350.1 for ammonium, and 365.1 for phosphate. Total organic N was estimated by subtracting the inorganic N (sum of nitrate, nitrite, and ammonium) concentration from total N content.

2.3 Soil Microbial Biomass and Extracellular Enzyme Activity

Microbial biomass was estimated by Substrate Induced Respiration (SIR; Anderson and Domsch, 1978) whereby 7 g (dry-weight equivalent) of soil were incubated with 7 mL of 1.2% yeast extract and CO_2 levels were measured using a LI-COR 6400XT fitted with a Trace Gas Sampler (LI-COR Biosciences, Lincoln, NE, USA) at 0, 2, and 4 hours. Carbon respired per hour per gram soil was calculated and used as a proxy for microbial biomass (*sensu* Fierer et al., 2003) . The capacity of the soil microbial community to mineralize C, N and P was estimated by determining the activities of β-glucosidase (βG), N-acetyl-glucosaminidase (NAG) and Acid phosphatase (AP), respectively. Enzyme assays were performed following the methods of Saiya-Cork et al. (2002). Briefly, 3 grams of fresh soil were suspended in acetate buffer and homogenized using a biohomogenizer (Bamix, Mettlen, Switzerland). In a 96-well plate, 4-methylumbelliferyl-labeled substrates specific to each enzyme were mixed with soil suspensions as well as standards and blanks and incubated for 2 hours (AP, NAG) or 5 hours (βG). The resulting fluorescence was measured at 365 nm excitation and 450 nm emission in a Synergy XR

plate reader (BioTek, Winooski, VT, USA) and were used to calculate enzyme activity as the amount of substrate hydrolyzed per hour per gram of soil.

2.4 DNA Extraction and Sequencing

DNA was extracted from 0.5 g of fresh soil using a DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA was quality checked and quantified using NanoDrop and Qubit, respectively (ThermoFisher Scientific, Waltham, MA). Bacterial 16S rRNA genes were amplified using primers 515fB (Parada et al., 2016) and 806rB (Apprill et al., 2015). Fungal ITS gene region amplicons were amplified using primers ITS1f (Gardes and Bruns, 1993) and ITS2 (White et al., 1990). All PCR amplifications were performed according to Earth Microbiome Project standard protocols (Thompson et al., 2017). Libraries were built from the resulting amplicons and sequenced at the University of Minnesota Genomics Center using the MiSeq platform (V3 chemistry; Illumina, San Diego, CA, USA) and a dual-indexed approach (Gohl et al., 2016). Amplicon sequences have been deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) repository under accession #PRJNA529237.

2.5 DNA Sequence Processing and Quality Control

Forward and reverse reads were merged by alignment to yield a single sequence using the –fastq_mergepairs command in USEARCH (Edgar, 2010). Prior to alignment, reads which had a quality score of less than 25 were truncated and sequences with more than 10 mismatches in the alignment were removed from the data set. Operational Taxonomic Units (OTUs) were picked using QIIME (Version 1.9.1; Caporaso et al., 2010) at the 97% similarity level using the open-reference method with default parameters. OTUs that occurred less than five times across the

dataset were removed. Bacterial taxonomy was assigned using the UCLUST algorithm (Edgar 2010) against the Green Genes database (Version 13.8, McDonald et al., 2012). Fungal taxonomy was assigned using the Ribosomal Database Project Naïve Bayesian Classifier against the UNITE database (Wang et al., 2007). Fungal OTUs with taxonomic assignments were then assigned to functional guilds using FUNGuild (Nguyen et al., 2016). Prior to analysis, bacterial and fungal sequences were rarefied to 5,000 and 1,300 sequences, respectively. For each site within the chronosequence, bacterial and fungal richness was calculated using the Chao1 metric (Chao, 1984) and β-diversity was estimated using Bray-Curtis dissimilarity (Bray and Curtis, 1957).

2.6 Statistical Analyses

All statistical analyses were performed in R (Version 3.4.1) using the Vegan package (Oksanen et al., 2019) or Primer (PrimerE, Version 7); an α level of 0.05 was considered significant while an α level of 0.10 was considered marginally significant. Residuals of the environmental variables were checked for normality using the Shapiro-Wilk Normality Test (Shapiro and Wilk, 1965). Non-normal data were normalized for linear regression by the transformation method indicated by superscript in Table 3.

To determine whether soil physical, chemical, and biological characteristics varied in a predictable manner over time after reclamation, linear regressions were used (Kenney and Keeping, 1954). To determine if taxonomic structure varied significantly across sites, soil horizon, and topography (*i.e.*, highland or lowland), three-way Permutational Multivariate Analysis of Variance (PerMANOVA; Anderson, 2001) was performed on Bray-Curtis dissimilarity matrices of bacterial and fungal OTU relative abundances with chronosequence site, soil horizon, and topography (*i.e.*, highland or lowland) as well as their interactions as factors. If

significant interactions were observed between factors, pairwise PerMANOVA was performed to determine differences between individual factor means across the chronosequence.

To determine the relative influence of stochastic (*e.g.*, time) and deterministic (*e.g.*, soil abiotic conditions) on variation in microbial community taxonomic structure across sites in the chronosequence, Distance Based Linear Modeling (DISTLM; Legendre and Legendre, 1998) was implemented using the adjusted-R^2 criterion. Environmental variables were first tested for collinearity using Draftsman Plots, with significant collinearity defined as $R^2>0.90$ (Hair Jr. et al., 2004). If variables emerged as collinear, one was selected for use in subsequent DISTLM model building procedures. Marginal DISTLM was initially performed to determine the relative influence of each factor on variation in microbial community taxonomic structure when considered alone, not accounting for the influence of other factors. Only significant ($P<0.05$) factors in the Marginal DISTLM were included in subsequent models. To determine if the variation in microbial community composition explained by time (*i.e.*, stochastic processes) was distinct or shared by other factors, a conditional DISTLM was performed with time added to the model last, after the variation attributable to all other significant factors had been accounted for. Lastly, '*best'* model selection was used to determine combination of factors that together accounted for the greatest variation in community composition.

3. RESULTS

3.1 Soil chemical properties

In the O horizon, lowland soils, phosphate content increased (+85%) and inorganic N content significantly decreased over time (-56%; Figure 2, Tables 2 and 3), with no significant change in soil pH, organic matter content, oxidizable C content, or organic N content. In O

horizon, highland soils, organic N content (+71%), phosphate content (+67%), and oxidizable C content (+30%), and pH (+6%) significantly increased whereas inorganic N content decreased (-54%) over time ($P<0.10$), with no significant change in organic matter content.

In the A horizon, lowland soils, there were increases in phosphate content (+92%), organic N content (+79%), inorganic N content (+69%), oxidizable C (+47%), organic matter content (+28%), and soil pH (+7%; $P<0.05$). In the A horizon, highland soils, organic N content (+83%), phosphate content (+80%), inorganic N content (+78%), organic matter content (+21%), and pH (+14%) all increased over time ($P<0.05$), with no significant change in oxidizable C content.

3.2 Microbial extracellular enzyme activity and biomass

In the O horizon, lowland soils, the activity of βG increased over time (+60%), whereas AP activity decreased over time (-81%) following reclamation ($P<0.05$), with no significant response of NAG activity or microbial biomass to time (Figures 3 and 4, Tables 3 and 4). In the O horizon, highland soils, there was a significant increase in NAG activity over time (+74%; $P<0.05$), but no change in βG activity, AP activity, or microbial biomass.

In the A horizon, lowland soils, AP activity decreased (-182%), whereas there was no change in βG activity, NAG activity, or microbial biomass over time ($P<0.05$). In the A horizon, highland soils, all enzyme activities assayed changed significantly over time, as there were significant increases in NAG activity (+52%) and significant decreases in βG (-418%; $P<0.05$), as well as a marginally significant decrease in AP activity over time (-148%; $P<0.10$). Further, microbial biomass significantly increased over time (+35%; $P<0.05$).

3.3 Microbial community composition

3.3.1 Microbial diversity and taxonomy

In the O horizon, lowland soils, fungal richness did not change significantly over time, but bacterial richness decreased (-31%; P<0.05; Figure 5, Table 3). Likewise, in O horizon, highland soils, there was no significant response of fungal richness to time, and bacterial richness significantly decreased (-31%; P<0.05). In the A horizon, lowland soils, fungal richness marginally increased over time (+21%; P=0.10) whereas bacterial richness marginally decreased (-14%; P<0.10). In the A horizon, highland soils, fungal richness did not significantly change over time, whereas bacterial richness decreased significantly (-30%; P<0.05).

Across the chronosequence, bacterial communities were dominated by members of the phyla Proteobacteria (23%-32%), Actinobacteria (7%-26%), and Acidobacteria (9%-22%). Within the Proteobacteria in the O horizon, lowland soils, the abundance of class Deltaproteobacteria marginally increased (+36%; P=0.10; Figures 6 and 7, Table 3) and the abundance of class Gammaproteobacteria significantly increased (+19%; P<0.05), while the abundance of class Alphaproteobacteria decreased (-70%; P<0.05). In the O horizon, highland soils, members of the Deltaproteobacteria increased (+24%) and members of the Alphaproteobacteria decreased (-50%) in abundance over time since reclamation (P<0.05), and the abundance of Actinobacteria marginally increased over time (+7%, P<0.10). In the A horizon, lowland soils, there were increases in abundance of members of the Delta- (+39%) and Gammaproteobacteria (+29%), and a decrease in the abundance of Alphaproteobacteria over time (-52%; <0.05; Figures 6 and 7, Table 3). In the A horizon, highland soils, there were

increases in abundance of members of the Delta- (+46%) and Gammaproteobacteria (+31%) and decreases in abundance of the Alphaproteobacteria (-50%; $P<0.05$). Further, the abundance of Acidobacteria significantly increased (+29%; $P<0.05$) and abundance of Actinobacteria marginally decreased (-30%; $P<0.10$).

Across the chronosequence, fungal communities were dominated by members of the phyla Ascomycota (26-69%) and Basidiomycota (5-66%). When fungal OTUs were assigned to functional guilds using FUNGuild, saprotrophic fungi were most dominant, ranging from 30%-80% relative abundance across the chronosequence. Other functional guilds considered were arbuscular mycorrhizae (0.1-14% abundance) and ectomycorrhizae (0-16% abundance).

In the O horizon, for both lowland and highland soils, there was no significant response of the abundance of Ascomycota, Basidiomycota, or abundance of assigned functional guilds (saprotrophs, arbuscular mycorrhizae, and ectomycorrhizae) to time (Figures 8 and 9, Table 3). In the A horizon, lowland soils, the abundance of Ascomycota significantly increased over time (+20%, $P<0.05$) and the abundance of Basidiomycota marginally decreased over time (-45%; $P<0.10$). Further, there was a marginally significant increase in the abundance of functionally saprotrophic fungi over time (+17%; $P<0.10$) but no significant response of functionally ectomycorrhizal or arbuscular mycorrhizal fungi. In the A horizon, highland soils, there was a marginally significant decrease in abundance of Basidiomycota (-30%; $P<0.10$), and a significant decrease in the abundance of functionally ectomycorrhizal fungi (-6%; $P<0.05$), but no change over time in abundance of Ascomycota, saprotrophic fungi, or arbuscular mycorrhizal fungi.

3.3.2 Microbial community dissimilarity and the influence of stochastic and deterministic processes on microbial community succession

Permutational multivariate analysis of variance (PerMANOVA) was implemented to assess changes in β-diversity between sites, topographic relief (*i.e.*, highland or lowland) and soil horizon from Bray-Curtis dissimilarity of OTU abundances. Bacterial and fungal communities were generally distinct across the chronosequence, and the degree of dissimilarity between communities varied in a manner coherent with time (Figure 10). However, this difference varied by site, horizon and topography, as a significant three-way interaction of site × topography × horizon was observed ($P<0.05$; Tables 5 and 6). Though all were statistically significant, the magnitude of the effect of site exceeded that of topography and horizon as well as the site × topography, site × horizon, topography × horizon, and site × topography × horizon interactions for both bacterial and fungal communities as determined by Pseudo-F values (Table 5).

In the O horizon, lowland soils, there were significant differences observed between the taxonomic structure of bacterial communities for the comparisons of the 2-year to 10-year, 2-year to 32-year, 10-year to 32-year, 2-year to reference, 15-year to reference, and 32-year to reference sites ($P<0.05$), and a marginal differences between the 2-year and 15-year, 10-year and 15-year, 15-year and 32-year, and 10-year and reference sites $P<0.10$). Fungal communities were significantly different between each site combination in the O horizon lowland soils ($P<0.05$). In the O horizon, highland soils, there were significant differences between the bacterial communities of all site comparisons besides the 2- to 15-year and 15- to 32-year comparisons, which were marginally significantly different ($P<0.10$). In the O horizon, highland soils, fungal communities varied significantly ($P<0.05$) between sites in all comparisons besides the 10-year

to 15-year and the 15-year to 32-year site comparisons, which were marginally different ($P<0.10$), and the 2-year to 15-year site comparison, which did not show a significant difference.

In the A horizon, lowland soils, bacterial communities were significantly different ($P<0.05$) between every site comparison besides that of the 2-year to 10-year sites, which was marginally significantly different ($P<0.10$), and fungal communities emerged as significantly different between every site comparison ($P<0.05$). In the A horizon, highland soils, bacterial communities were significantly different ($P<0.05$) in every site comparison besides that of the 2- to 20-year sites, which was marginally significant ($P<0.10$). Fungal communities of the A horizon, highland soils were significantly different ($P<0.05$) in all comparisons besides that of the 2-to 10-year sites and the 10-to 15-year sites, which were both marginally significantly different ($P<0.10$).

Marginal DISTLM determined that all chemical and physical soil characteristics included in the model accounted for a significant proportion of bacterial and fungal β-diversity across the chronosequence ($P<0.05$; Tables 7 and 8). For bacterial communities, soil clay content and pH accounted for the greatest proportion of taxonomic variation (19% and 21% respectively; $P<0.05$). The '*best'* model procedure determined that all measured factors together account for the greatest proportion of bacterial dissimilarity across the chronosequence, with a total of 39% of variation explained by the model (Table 9). For fungal communities, soil clay content and time accounted for the greatest proportion of taxonomic variation (15% each; $P<0.05$; Table 10). The 'best' model determined that all factors besides oxidizable C content accounted for the greatest proportion of fungal dissimilarity across the chronosequence, with a total of 38% of variation explained by the model (Table 10). Lastly, conditional DISTLM was performed to determine whether stochastic processes alone influenced bacterial and fungal succession. Indeed,

time since reclamation accounted for a distinct proportion of taxonomic variation in both bacterial and fungal communities once all variation attributable to environmental factors was accounted for (6% and 4% additional variance explained, respectively; P<0.01; Tables 9 and 10).

4. DISCUSSION

In this study, we investigated whether soil microbial communities exhibit successional patterns across a ~30-year mine land reclamation chronosequence by surveying soil chemical and physical characteristics, determining microbial activity, diversity, and community composition, and then testing relationships between measured factors using linear modeling. Microbial communities varied in a manner coherent with time since reclamation, and moreover, deterministic (*e.g.*, pH, texture, soil C, N) and stochastic (*e.g.*, dispersal and drift) factors emerged as influences on the post-disturbance succession of these communities. Deterministic factors together accounted for ~4 times more variation in communities than stochastic factors.

4.1 Abundance of microbial taxa, but not total microbial biomass or richness, show trends following reclamation

It was expected that microbial biomass would increase over time in the mine reclamation chronosequence, as has been shown in other investigations of microbial community succession following a disturbance (Banning et al., 2008; Brown and Jumpponen, 2014; Harris, 2003). Counter to these predictions, microbial biomass exhibited a variable response to time since reclamation, with biomass only significantly increasing with time since reclamation in the A horizon in highland soils (+35%). One plausible explanation for this result is that the reclamation

strategy used for these sites involved long-term storage and reapplication of native topsoil, thus soils were not sterile (or nearly sterile) at time zero, as is common other studies of microbial community succession following disturbance (*e.g.*, after a volcanic eruption). However, soils in this study were subjected to a distinct physical disturbance during mining and subsequent spreading of topsoil on the sites, and therefore may mirror successional dynamics more closely related to a disturbance such as tillage. Indeed, the response of microbial biomass to physical disturbance (*e.g.*, tillage) is variable and is dependent on factors such as soil moisture, soil nutrient content, and management practice (Anderson et al., 2017; Calderón et al., 2000). This highlights the influence of disturbance type and the physical and chemical state of soil in shaping microbial responses. Additionally, the method used here (*i.e.*, Substrate Induced Respiration) is limited in that it cannot distinguish between biomass attributable to bacteria and that of fungi. It also does not capture and represent the portion of the microbial community that do not readily degrade labile C substrates.

The relative abundance of some members of the phylum Proteobacteria showed statistically significant responses to time since reclamation. The abundance of Alphaproteobacteria decreased and Deltaproteobacteria increased significantly over time in every soil horizon and topographic location (i.e., highland or lowland). In a similar study, the abundance of Alphaproteobacteria increased with time since reclamation, the opposite response of that observed here. However, the initially high abundance of Alphaproteobacteria followed by a decline over time is consistent with a ruderal life strategy, which is characterized by rapid growth initially following disturbance, but not necessarily a sustained dominance over time depending on factors such as competition and stress (Grime, 1977; Ho et al., 2017; Ho et al., 2013). Deltaproteobacteria showed an increase in relative abundance over time, aligning with a competitor-stress tolerator

life strategy, whereby a group is relatively inactive initially following the disturbance, but becomes more prevalent with time (Ho et al. 2013). It is possible for organisms to exhibit characteristics of more than one life strategy and to adapt their life strategy over time (Ho et al. 2013), and this could account for the complex and highly variable responses of the abundance of microbial taxa seen in this study. Further, the groups discussed here harbor incredible amounts of taxonomic and functional diversity within. For this reason, is not likely that generalizations about function can be made with complete validity at broad taxonomic levels; finer levels of taxonomic resolution have been shown to reveal a much more robust picture of microbial community function (Ho et al., 2013; Morrissey et al., 2016). The trends seen in this study are also potentially dependent on factors that are difficult to quantify, such as historical land-use. For example, Nacke et al. (2011) found that forest soils harbor a higher abundance of Alphaproteobacteria than grassland soils, and these sites were forested prior to surface mining and subsequently management as pastureland. This, coupled with observed trends in the compositional response of microbial communities to nutrient quality and quantity, could explain the shift in abundance of Alphaproteobacteria and Deltaproteobacteria with time following reclamation. This is further supported by the trend of abundance of these taxa over time since reclamation to approximately that of the undisturbed grassland reference site (Figure 6).

We expected that the abundance of fungal taxa would change over time following reclamation to mirror that of the undisturbed reference grassland. When considered as predicted functional guilds, 53% of fungal OTUs were assigned to the saprotroph guild on average across the chronosequence. Though there were not clear responses of the abundance of saprotrophs over time, their clear dominance highlights their functional importance in this disturbed system. This functional guild represents an extremely diverse group of fungal taxa, including some members

of the Ascomycota and Basidiomycota, which were also highly abundant (53% and 32% of fungal communities on average, respectively) but showed variable responses to time following reclamation. Other investigations of fungal succession after mine reclamation have shown that Ascomycota and Basidiomycota did not change in abundance over time since (Banning et al., 2008; Sun et al., 2017), supporting our variable results. Basidiomycota are capable of degrading recalcitrant C compounds, with Ascomycota degrading less chemically complex C compounds (Floudas et al., 2012; Riley et al., 2014; Treseder and Lennon, 2015). This further supports the seemingly important role of the Basidiomycota and Ascomycota's ability to degrade C of varying levels of recalcitrance in this system as time progresses following reclamation. Further, these taxa are often seen in high abundance together as they are here, potentially due to their differential substrate preference and the subsequent lack of competition between the two taxa (Ma et al., 2013).

It was expected that microbial richness would increase over time in the mine reclamation chronosequence as has been observed in other studies (Brown and Jumpponen, 2014; Freedman and Zak, 2015; Li et al., 2016), though in this study the opposite response was observed. However, responses of some ecologically important microbial taxa to time since reclamation indicate that the microbial community, while not more taxonomically diverse, could still be adapted to the desired post-mining land use conditions. Further, the link between microbial diversity and community function following disturbance is highly dependent on many factors, such as disturbance type, land use history, and soil properties (Griffiths and Philippot, 2013; Tobor-Kapłon et al., 2006). In this study, the observed trends in relative abundance of taxa coupled with changes in soil nutrient content (*e.g.*, increase in oxidizable C and organic N) supports that successional patterns may be observable using more resolved metrics of microbial

community composition and are dependent on the chemical and physical properties of the soil. Further, though trends in extracellular enzyme capacity (EEC) were not totally consistent, they align somewhat with concomitant changes in soil chemical properties (Figures 2 and 3). For example, activity of acid phosphatase, associated with the mineralization of organic P, decreased as phosphate levels increased. This is suggestive of functional adjustments by the microbial community in response to changing chemical (*i.e.*, deterministic) factors. Changes in EEC have been shown to be attributable to shifts in community composition (Chaer et al., 2009), which could also explain the trends in EEC following reclamation. In order to fully understand these dynamics, a more robust functional and phylogenetic analysis is needed to accurately quantify the association between microbial community composition, function, and above-ground productivity following surface mining disturbance.

4.2 Microbial community succession after mine reclamation is driven by both stochastic and deterministic factors.

As expected, bacterial and fungal communities were compositionally distinct across the chronosequence (Figure 10) and these differences were explained by both stochastic (*e.g.*, time) and deterministic (*e.g.*, soil chemical and physical factors) factors. In Marginal DISTLM model building, all soil chemical and physical factors considered explained significant proportions of taxonomic variation in both bacterial and fungal communities and most measured soil factors were included in the *'best'* model (Tables 7-10). Further, after accounting for the variance attributable to all measured environmental factors, time since reclamation accounted for 4% and 6% of additional variation explained in bacterial and fungal communities, respectively, suggesting the modest, but significant influence of stochastic factors in shaping microbial community succession across this chronosequence (Tables 9 and 10). This aligns with other

findings that both stochastic and deterministic factors shape microbial communities following disturbance (Caruso et al., 2011; Cline and Zak, 2015; Dumbrell et al., 2010; Ferrenberg et al., 2013; Freedman et al., 2015).

As in our results, other studies have shown that successional patterns following disturbance vary between bacteria and fungi (Brown and Jumpponen, 2014; Li et al., 2016; Sun et al., 2017). While bacterial and fungal communities were both influenced by stochastic and deterministic factors, the amount of variance in communities explained by the individual factors varied between the two. Fungi are larger in size, and therefore may be more susceptible to stochastic influences such as dispersal limitation (Brown and Jumpponen, 2014; Cline and Zak, 2014; Schmidt et al., 2014). In our study, slightly more distinct variance was explained by time (*i.e.*, stochastic factors) in fungal communities (6%) than bacterial communities (4%) in the conditional DISTLM. However, pH (*i.e.*, a deterministic factor) explained more variance in bacterial communities (21%) than fungal communities (14%) in the Marginal DISTLM, again supporting the distinct successional processes occurring in bacterial and fungal communities. Further, oxidizable C content emerged as a factor in the *'best'* model for bacterial communities, but not fungal communities. This could be due to the generally more diverse C metabolism of fungi (Treseder and Lennon, 2015).

It is possible that this study did not account for all environmental variables contributing to taxonomic dissimilarity between sites in the chronosequence. Though it is not feasible to measure all potentially deterministic soil factors, to minimize the effects of this we strategically selected environmental factors that have been well documented as deterministic drivers of soil microbial community composition (Fierer et al., 2007; Freedman et al., 2015; Högberg et al., 2007; Lauber et al., 2008; Romanowicz et al., 2016) in order to account for as much

deterministic influence on succession as possible. Still, the factors measured here could underestimate the influence of soil chemical factors on microbial communities. Additionally, these sites were chosen because they harbor minimal heterogeneity between them in plant communities, climate, and management regime, further minimizing confounding variation which could contribute to community dissimilarity. Still, ~60% of variation for both bacterial and fungal communities remained unexplained in the model output. This could be accounted for by microbe-microbe interactions, such as competition, predation, mutualism, and order-of-colonization (Fukami, 2015; Jiang and Patel, 2008; Vannette and Fukami, 2013), which were not quantified in this study.

4.3 Conclusions

Taken together, our results support the hypothesis that the successional trajectory of microbial communities following mine reclamation is influenced by both deterministic and stochastic processes. Though stochastic processes did emerge as distinct, the influence of deterministic factors was ~4 times greater. This suggests that remediation of lands from a physical anthropogenic disturbance such as surface mining is attainable through adequate soil management over the appropriate amount of time. Specifically, management of factors such as pH and soil texture, which both emerged as highly explanatory of variance among communities in this study, may promote favorable succession. These findings provide an enhanced understanding of the factors influencing soil microbial communities, and therefore overall ecosystem function, following mine land reclamation.

5. FIGURES

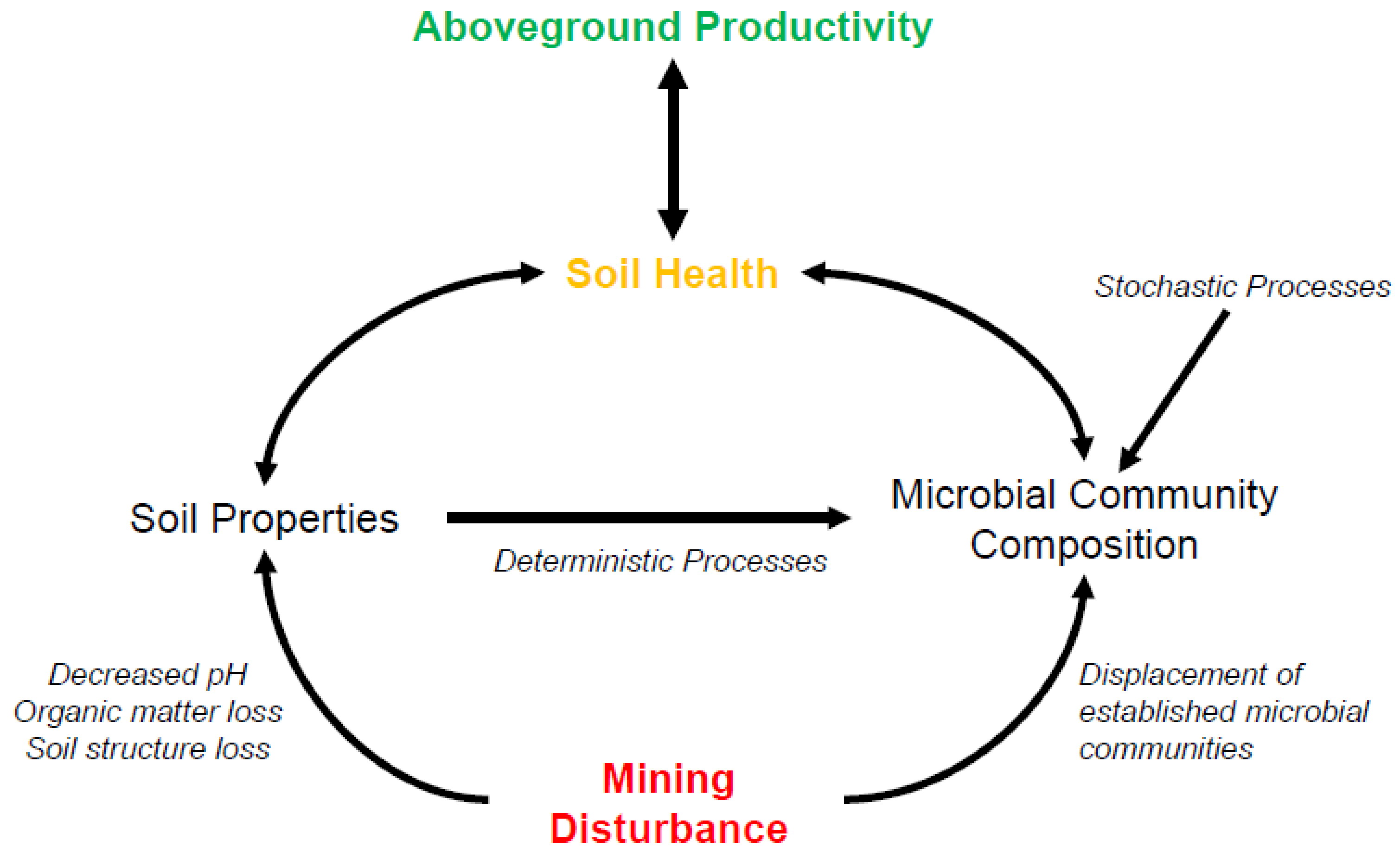

Figure 1. Conceptual model of the effects of mining disturbance on soil microbial communities, including the interaction between deterministic (*e.g.*, soil chemical and physical properties) and stochastic (*e.g.*, time) processes during succession and how it influences post-mining aboveground productivity.

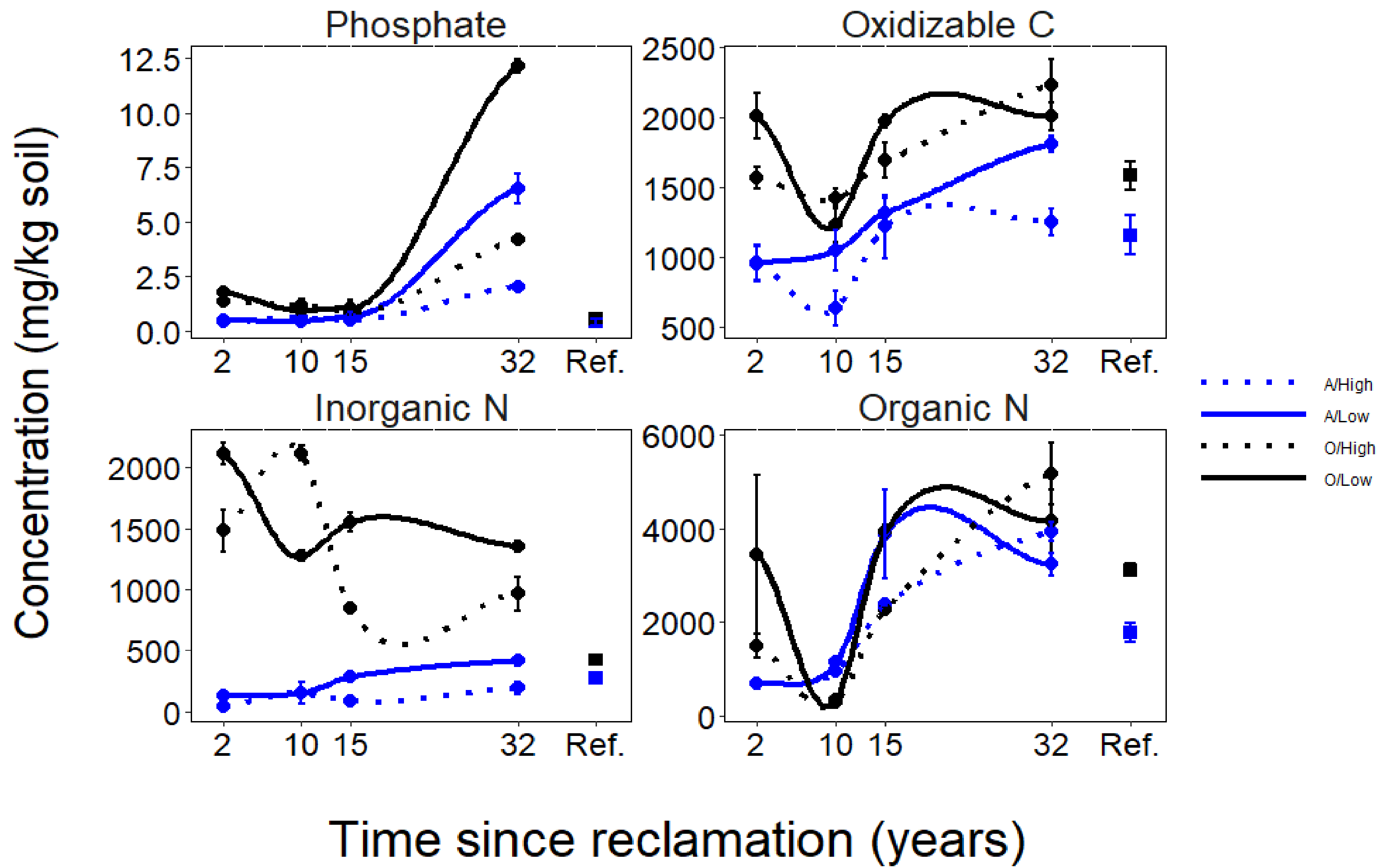

Figure 2. Soil phosphate, oxidizable C, inorganic N, and organic N concentrations over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils. Error bars represent +/- SE, n=3.

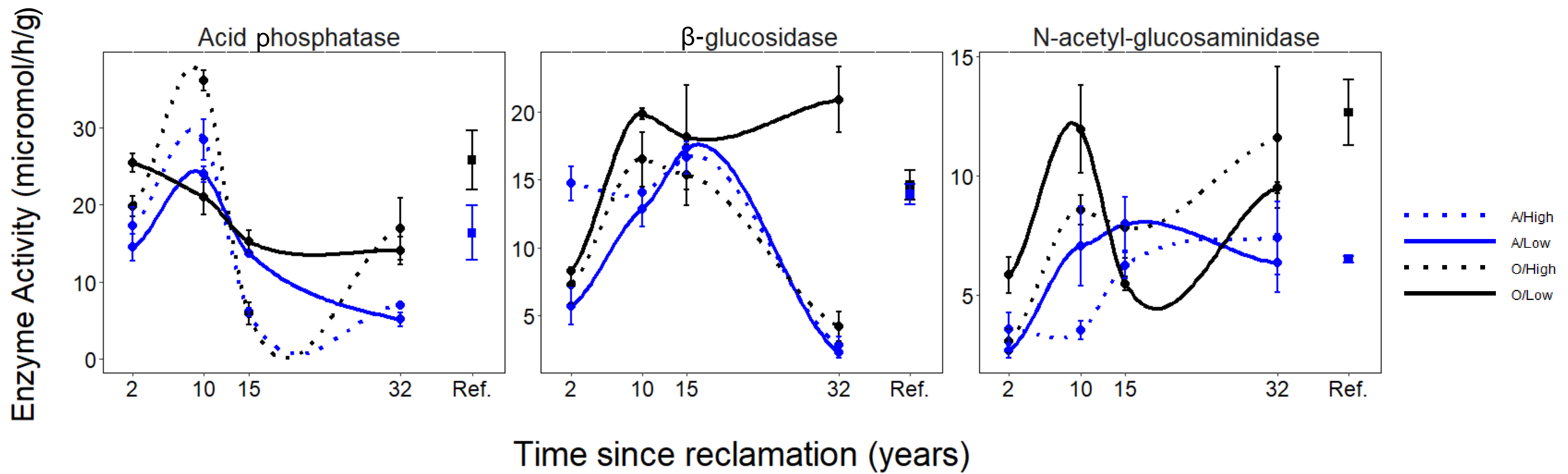

Figure 3. Extracellular enzyme capacity (EEC) over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils.. Error bars represent +/- SE, n=3.

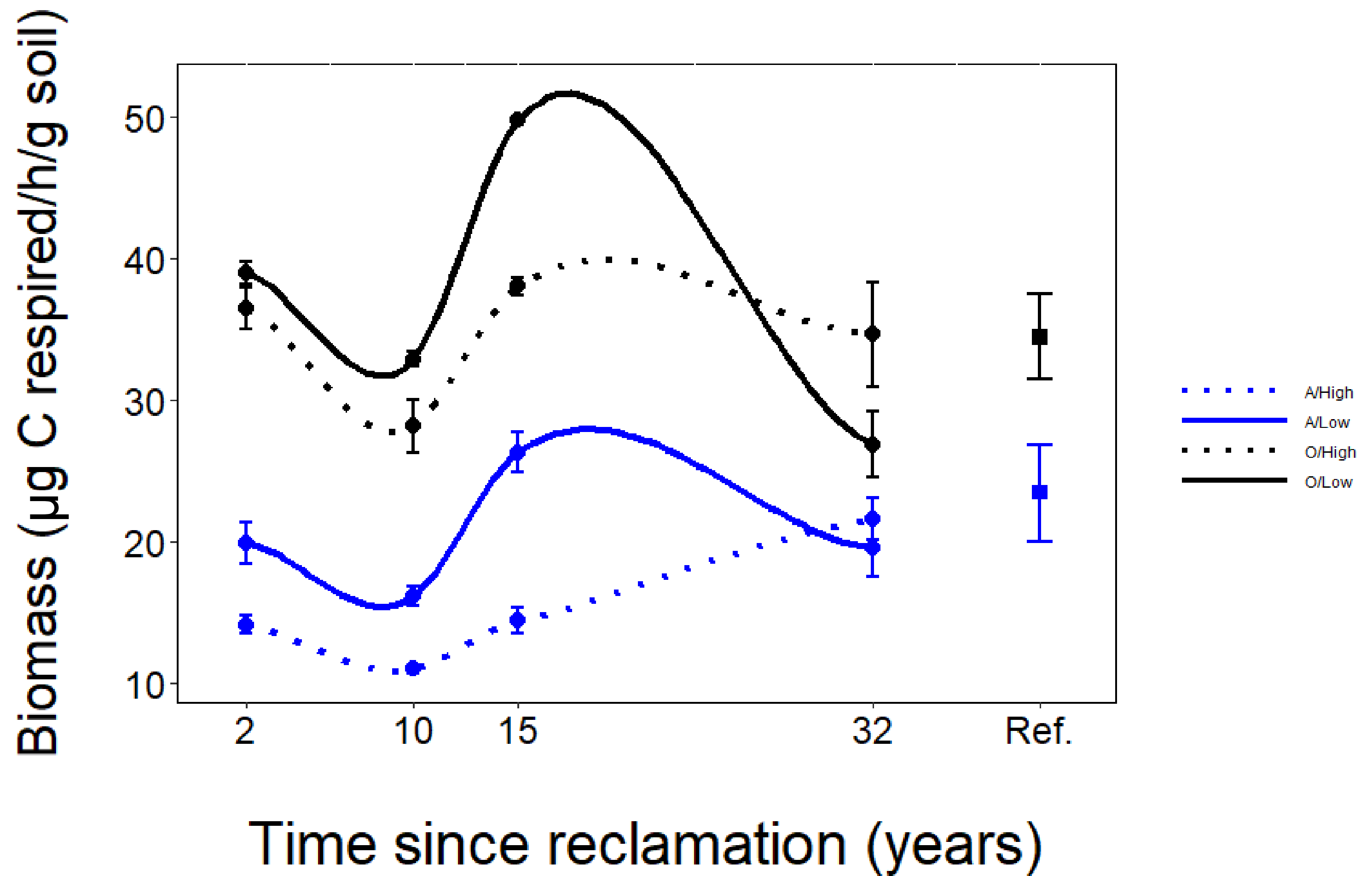

Figure 4. Estimated microbial biomass over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils. Error bars represent +/- SE, n=3.

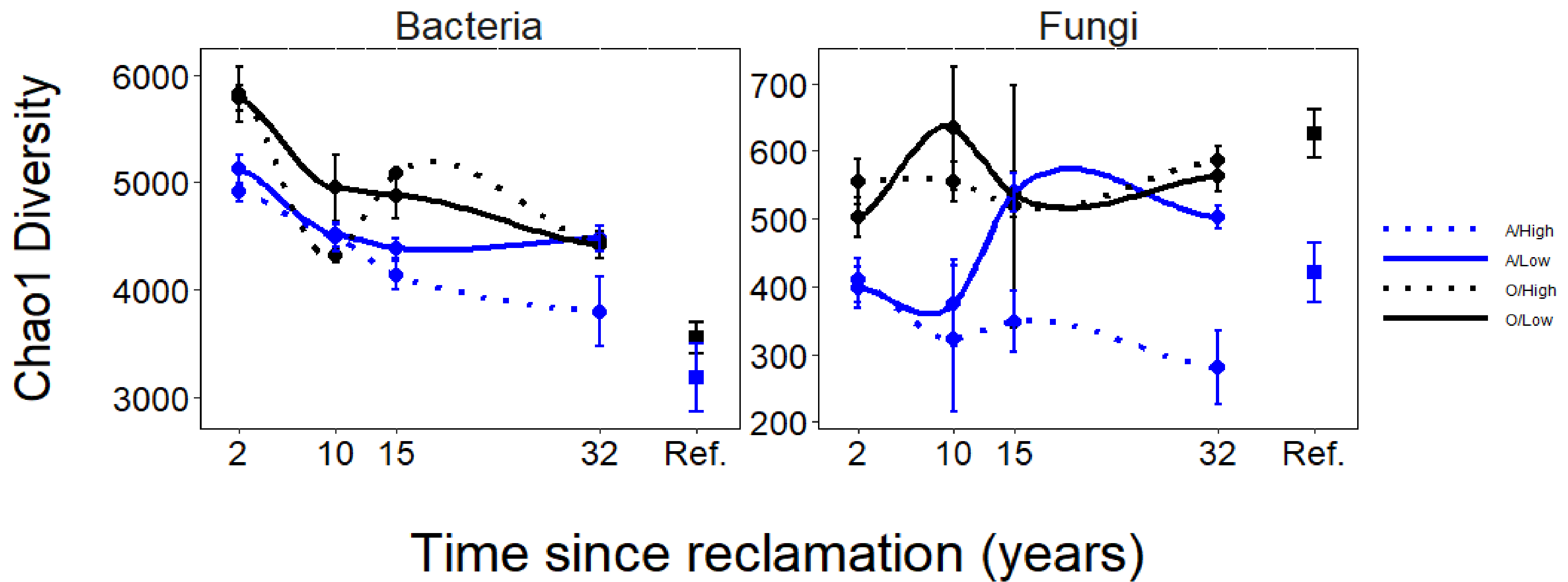

Figure 5. Bacterial and fungal richness over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils. Error bars represent +/- SE, n=3.

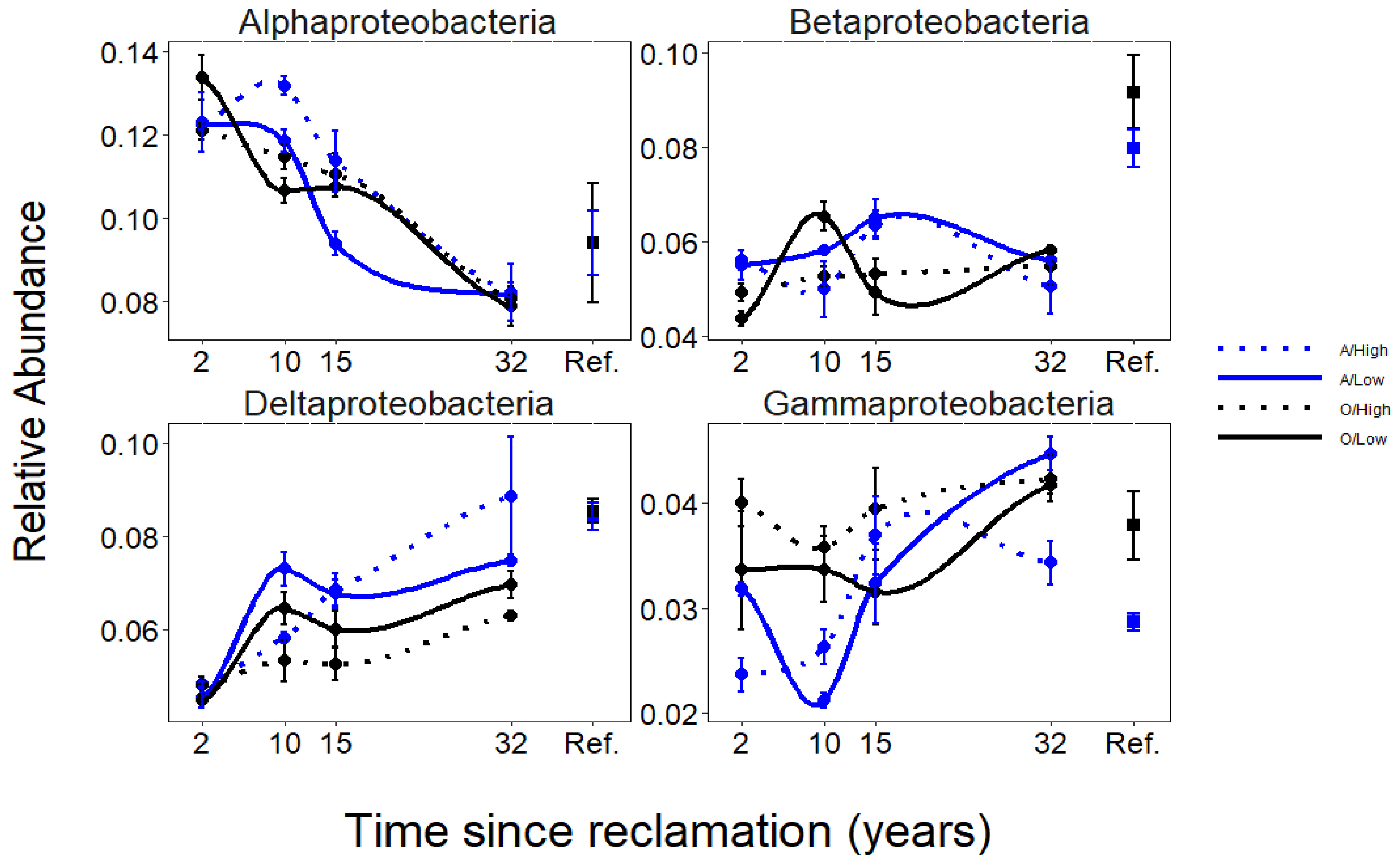

Figure 6. Relative abundance of Alpha-, Beta-, Delta-, and Gammaproteobacteria over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils. Error bars represent ±SE, n=3.

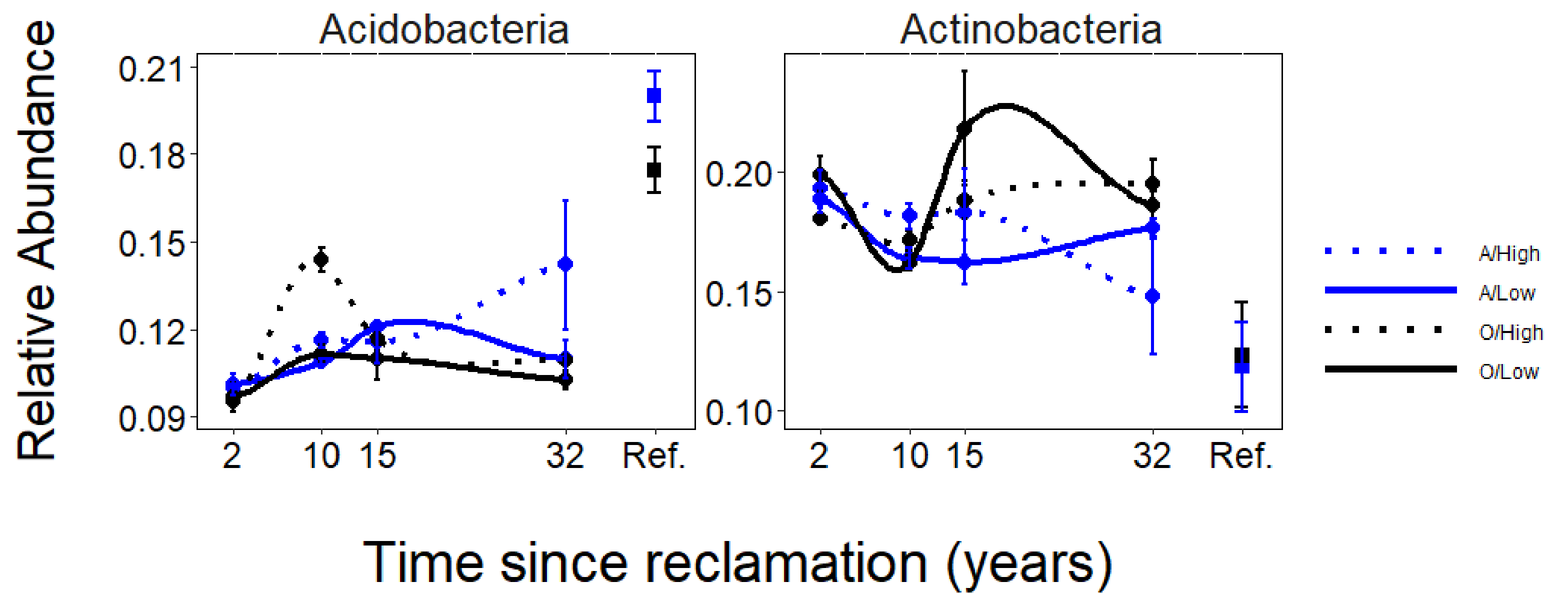

Figure 7. Relative abundance of class Acidobacteria and Actinobacteria over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils. Error bars represent ±SE, n=3.

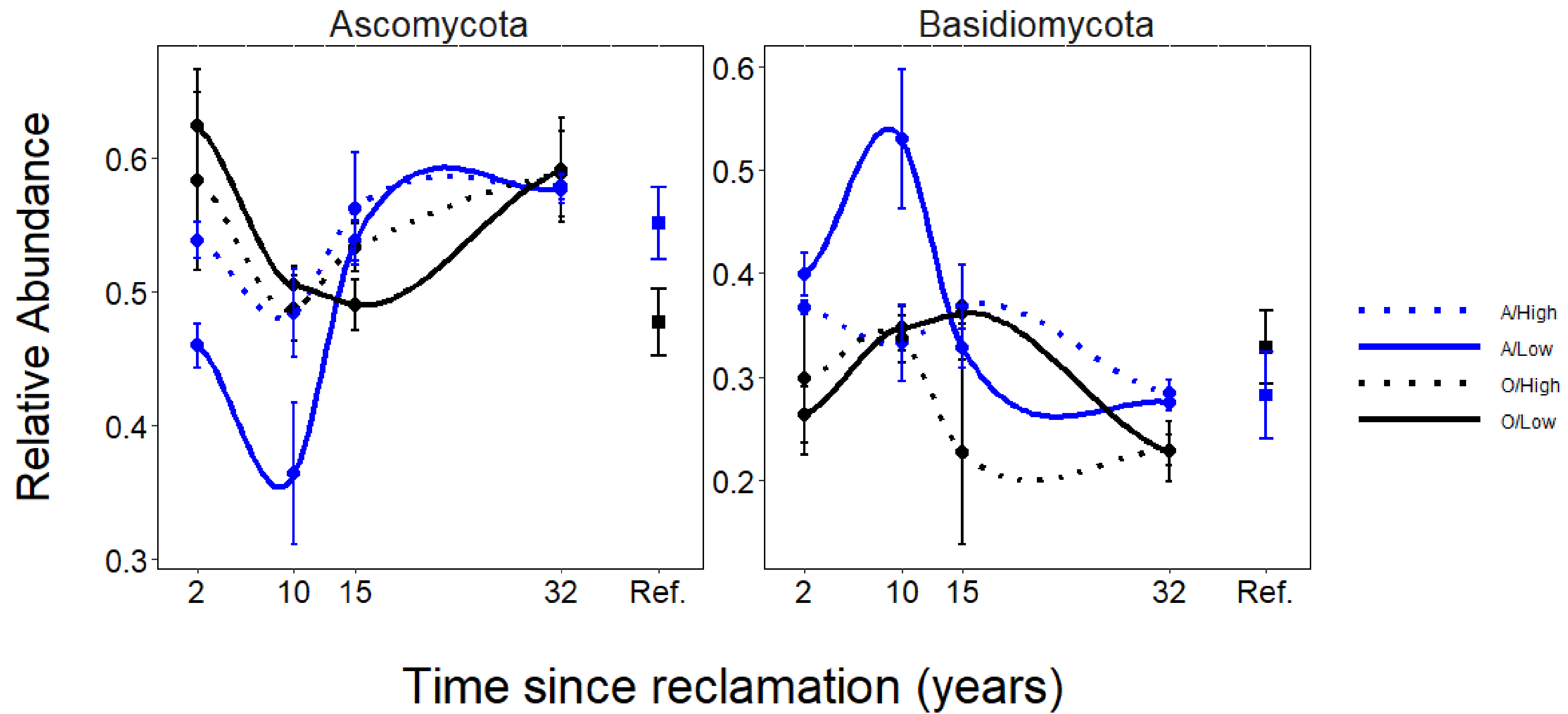

Figure 8. Relative abundance of Ascomycota and Basidiomycota over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils. Error bars represent ±SE, n=3.

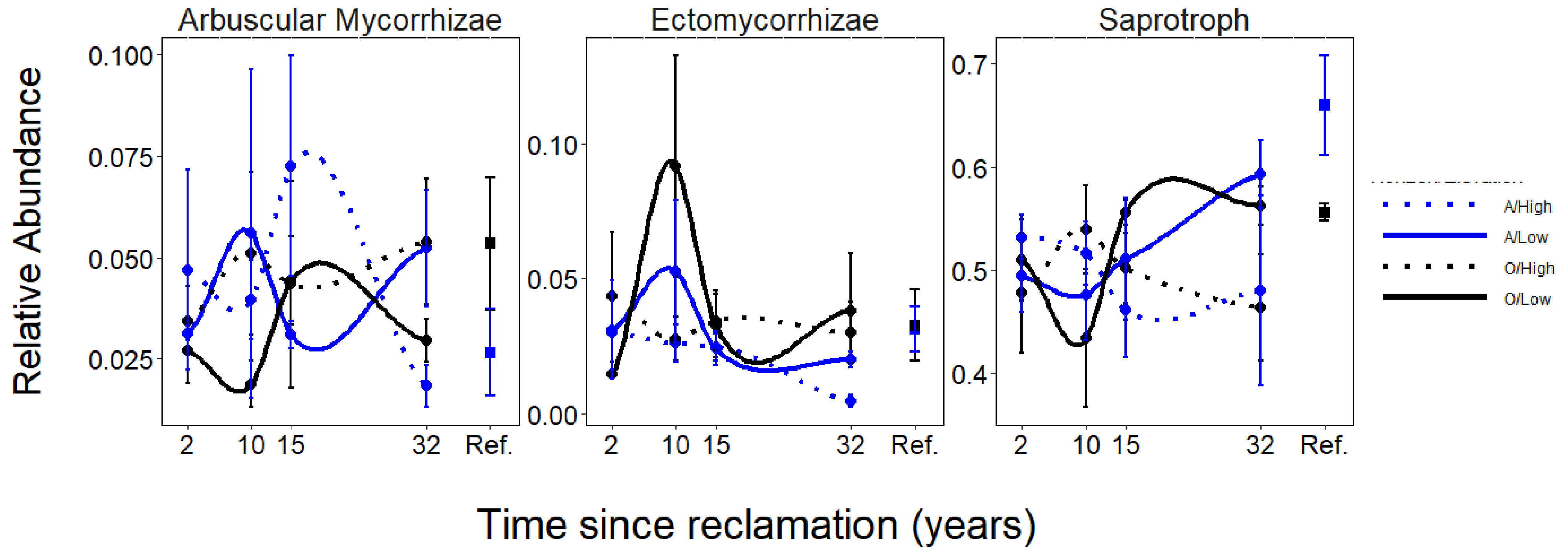

Figure 9. Relative abundance of putative functional guilds as determined by FUNGuild over time since reclamation and at the reference grassland site in both highland/lowland and organic/mineral soils. Error bars represent ±SE, n=3.

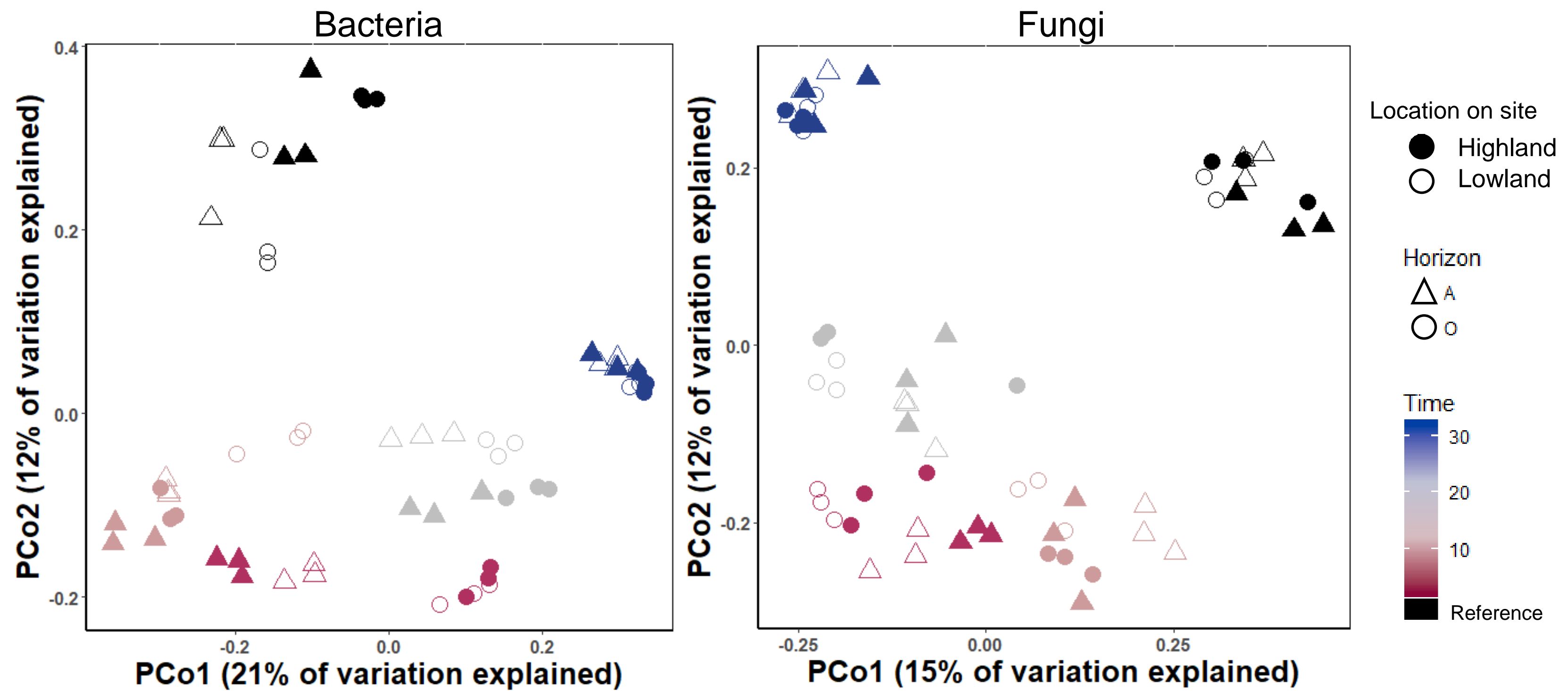

Figure 10. Principle coordinate analysis of Bray-Curtis dissimilarity in bacterial and fungal community composition between chronosequence sites and the reference site in both highland/lowland and organic/mineral soils. Pairwise significance is shown in in Table 5.

Table 1. Description of chronosequence sites. Sites were all reclaimed in the approximately same manner following surface mining, only differing in time since the mining and reclamation process. A more detailed site description can be found in Chaudhuri et al. (2013).

Site	Years since reclamation	Soil Texture Class	Land Type
New Hill	2	Clay Loam	Grass-Legume Pasture
Shafer	10	Silty Clay Loam	
Metz	15	Silty Clay Loam	
Mylan	32	Silty Clay Loam	

Table 2. Average soil physical and chemical properties +/- SE in each site of the chronosequence as well as in the reference site (n=3).

Time (Years Since Reclamation)	Horizon/Elevation	Soil Chemical/Physical Factor						
		Soil pH	carbon:nitrogen	Organic Matter (%)	Oxidizable C (mg/kg soil)	Inorganic N (mg/kg soil)	Organic N (mg/kg soil)	Phosphate (mg/kg soil)
2	A/High	5.93±0.01	11.06±0.29	5.18±0.10	953.06±125.96	41.14±14.44	685.53±89.65	0.41±0.01
	A/Low	6.14±0.03	9.73±0.48	5.59±0.07	958.31±127.73	124.97±26.44	691.70±39.93	0.52±0.03
	O/High	6.39±0.02	10.91±0.47	10.50±0.21	1567.54±76.32	1481.11±171.90	1492.23±259.83	1.37±0.24
	O/Low	6.7±0.05	12.47±0.25	10.74±0.59	2005.98±159.97	2113.20±90.73	3446.81±1682.09	1.81±0.06
10	A/High	4.76±0.03	8.97±0.72	4.28±0.06	632.42±120.83	147.12±11.42	949.55±69.12	0.6±0.07
	A/Low	5.14±0.003	8.39±0.16	4.59±0.03	1042.46±144.67	148.93±86.48	1147.74±121.90	0.45±0.07
	O/High	4.78±0.10	11.10±0.23	9.27±0.21	1416.05±72.29	2116.51±62.11	290.16±112.33	1.17±0.31
	O/Low	5.21±0.06	11.62±0.57	9.02±1.47	1233.17±125.74	1271.81±46.17	358.19±114.34	0.93±0.06
15	A/High	6.16±0.01	6.38±0.24	6.02±0.08	1217.28±225.11	81.84±13.05	2388.16±27.95	0.5±0.05
	A/Low	5.68±0.05	6.80±1.44	7.30±0.06	1315.50±100.72	280.87±31.49	3872.47±940.03	0.67±0.19
	O/High	6.58±0.01	10.11±0.11	9.43±1.51	1688.31±121.94	840.56±24.01	2282.77±54.99	0.89±0.11
	O/Low	5.86±0.05	8.94±0.24	12.18±0.1	1972.78±48.61	1550.15±73.614	3924.19±143.68	1.07±0.33
32	A/High	6.87±0.05	5.32±0.18	6.60±0.29	1247.60±94.83	189.14±45.18	3924.20±199.15	2.07±0.05
	A/Low	6.61±0.01	6.09±0.41	7.71±0.16	1806.78±59.33	409.49±39.53	3230.52±252.18	6.55±0.67
	O/High	6.78±0.02	7.30±0.68	11.15±0.12	2226.58±181.41	963.57±140.39	5169.78±654.82	4.19±0.12
	O/Low	6.52±0.02	7.57±0.80	10.50±0.08	2002.65±97.61	1354.65±47.12	4145.35±671.95	12.15±0.29
Reference	A/High	5.67±0.02	14.79±0.81	9.92±0.11	1445.38±104.75	277.76±5.72	2222.24±32.04	0.51±0.24
	A/Low	5.24±0.01	10.22±0.08	5.26±0.07	863.15±15.62	260.16±23.77	1343.17±23.63	0.22±0.03
	O/High	5.59±0.03	12.95±0.06	7.51±0.09	1707.17±160.84	430.73±3.67	3382.60±62.82	0.48±0.03
	O/Low	5.29±0.05	10.11±0.13	8.70±0.09	1454.10±80.98	407.37±12.12	2832.63±35.10	0.62±0.20

Table 3. Linear regressions of microbial activity, soil properties, bacterial and fungal diversity, and abundance of dominant bacterial and fungal taxa with time since reclamation.

	O/Low			O/High			A/Low			A/High		
Microbial Activity	R^2	slope	P	R^2	slope	P	R^2	slope	P	R^2	slope	P
Biomass per gram soil	0.14	-0.3675	0.13	<0.01	0.0161	0.91	<0.01	0.0271	0.83	0.60	0.2979	<0.01
N-acetyl-glucosaminidase[4]	<0.01	0.0140	0.34	0.46	0.0462	0.01	0.09	0.0219	0.17	0.47	0.0296	0.01
Beta-glucosidase	0.34	0.3415	0.03	0.05	-0.1951	0.23	0.02	-0.1848	0.29	0.64	-0.4164	<0.01
Acid phosphatase	0.57	-0.3662	<0.01	<0.01	-0.2720	0.41	0.42	-0.0427	0.01	0.25	-0.4832	0.06
Soil properties												
pH[1]	<0.01	28.7700	0.80	0.20	211.7000	0.08	0.31	189.3700	0.03	0.60	336.0600	<0.01
% Organic Matter[2]	<0.01	0.0001	0.85	<0.01	0.0003	0.37	0.51	0.0008	<0.01	0.48	0.0006	<0.01
Bioavailable C	<0.01	7.4680	0.49	0.58	25.1020	<0.01	0.77	29.6750	<0.01	0.14	13.8160	0.13
Phosphate	0.68	0.0801	<0.01	0.57	0.0326	<0.01	0.80	0.0660	<0.01	0.84	0.0272	<0.01
Organic N	<0.01	55.6000	0.33	0.69	143.6500	<0.01	0.35	87.8800	0.03	0.92	114.0100	<0.01
Inorganic N[3]	0.29	-0.00479	0.04	0.23	-0.0085	0.07	0.44	0.0196	0.01	0.36	0.0193	0.02
Bacteria												
Richness	0.58	-41.0500	<0.01	0.60	-34.6600	0.04	0.26	-17.3240	0.05	0.61	-36.2900	<0.01
Alphaproteobacteria	0.85	-0.0020	<0.01	0.87	-0.0013	<0.01	0.81	-0.0014	<0.01	0.64	-0.0015	<0.01
Betaproteobacteria	0.04	0.0030	0.25	0.08	0.0002	0.19	<0.01	<0.0001	0.95	<0.01	-0.0001	0.57
Deltaproteobacteria[3]	0.51	0.0050	<0.01	0.54	0.0040	<0.01	0.44	0.0060	0.01	0.74	0.0080	<0.01
Gammaproteobacteria	0.17	0.0003	0.10	<0.01	0.0001	0.35	0.44	0.0006	0.01	0.62	0.0004	0.03
Actinobacteria	<0.01	-0.0001	0.92	0.19	0.0006	0.09	<0.01	-0.0002	0.60	0.27	-0.0010	0.05
Acidobacteria[3]	<0.01	0.0003	0.76	<0.01	0.0004	0.82	<0.01	0.0010	0.33	0.39	0.0040	0.02
Fungi												
Richness	<0.01	0.8757	0.74	<0.01	1.1170	0.78	0.17	4.0180	0.10	0.08	-3.7930	0.20
Ascomycota	0.10	-0.0001	0.97	0.07	0.0011	0.59	0.32	0.0051	0.03	0.09	0.0020	0.19
Basidiomycota	0.03	-0.0020	0.27	0.02	0.0029	0.29	0.27	-0.0057	0.05	0.23	-0.0026	0.07
Saprotrophs	0.06	0.0030	0.22	<0.01	-0.0011	0.62	0.21	0.0037	0.07	<0.01	-0.0017	0.47
Ectomycorrhizae[3]	<0.01	0.0042	0.70	<0.01	-0.0030	0.69	<0.01	-0.0050	0.58	0.39	-0.0200	0.02
Arbuscular mycorrhizae	<0.01	0.0002	0.67	<0.01	0.0005	0.50	<0.01	0.0005	0.60	0.01	-0.0009	0.33

[1]Power transformed
[2]Arcsine transformed
[3]Natural logarithm transformed
[4]Square-root transformed

Table 4. Average extracellular enzyme capacity and microbial biomass +/- SE (n=3).

Time (Years Since Reclamation)	Horizon/Topographic Location	N-acetyl-glucosaminidase (μmol/hr/g soil)	Acid phosphatase (μmol/hr/g soil)	β-glucosidase (μmol/hr/g soil)	Microbial Biomass (g C respired/h/g soil)
2	A/Low	2.64±0.31	14.46±1.73	5.71±1.35	19.87±1.47
	A/High	3.55±0.68	17.26±2.35	14.72±1.28	14.09±0.62
	O/Low	5.83±0.76	26.36±1.20	8.28±0.22	38.95±0.78
	O/High	3.03±0.49	19.78±1.27	7.25±1.31	36.49±1.44
10	A/Low	7.05±1.68	23.91±0.97	12.86±1.32	16.08±0.67
	A/High	3.51±0.39	28.4±2.58	14.03±0.20	10.93±0.28
	O/Low	11.95±1.84	20.95±2.28	19.85±0.38	32.89±0.53
	O/High	8.65±0.62	36.05±1.36	16.50±2.00	28.15±1.86
15	A/Low	7.96±1.13	13.69±0.31	17.31±0.12	26.30±1.38
	A/High	6.22±0.54	6.11±0.53	16.63±1.17	14.36±0.92
	O/Low	5.47±2.27	15.23±1.40	18.13±3.83	49.76±0.25
	O/High	7.81±1.28	5.87±1.45	15.35±2.25	38.04±0.65
32	A/Low	6.33±1.23	5.13±0.90	2.34±0.45	19.49±1.98
	A/High	7.37±1.55	6.97±0.38	2.84±0.64	21.55±1.46
	O/Low	9.48±0.24	14.05±1.78	20.88±2.42	26.84±2.35
	O/High	11.6±2.97	16.85±4.06	4.22±1.08	34.62±3.69
Reference	A/Low	6.57±0.29	9.26±1.04	13.01±1.3	15.81±0.16
	A/High	6.42±0.04	23.44±3.45	14.90±0.38	31.08±0.24
	O/Low	14.47±2.03	33.32±3.80	14.25±1.83	29.10±2.74
	O/High	10.84±1.41	18.21±0.28	15.00±1.61	39.84±2.88

Table 5. Main result of PerMANOVA performed on Bray-Curtis dissimilarity matrices for bacterial and fungal communities.

	Bacteria	Fungi
Source	Pseudo-F	Pseudo-F
Site	11.47*	13.72*
Topography	1.91*	4.52*
Horizon	5.86*	5.54*
Site × Topography	2.18*	3.65*
Site × Horizon	2.13*	2.59*
Topography × Horizon	1.51*	1.52*
Site × Topography × Horizon	1.50*	1.88*

*$P<0.05$

Table 6. Pairwise comparisons of each Horizon × Topography (*i.e.*, highland or lowland) × Site age combination as determined by a pairwise PerMANOVA.

	Comparison (Site ages)		Bacteria t	Fungi t
A/Low	2	10	1.61^	2.59*
	2	15	1.79*	3.40*
	2	32	2.15*	2.91*
	10	15	1.91*	2.42*
	10	32	2.56*	2.92*
	15	32	1.83*	2.62*
	2	Ref.	2.01*	2.53*
	10	Ref.	1.94*	1.91*
	15	Ref.	2.12*	2.34*
	32	Ref.	2.45*	2.59*
A/High	2	10	1.57^	1.82^
	2	15	1.73*	2.26*
	2	32	2.23*	2.58*
	10	15	2.14*	1.71^
	10	32	2.54*	1.94*
	15	32	1.79*	2.15*
	2	Ref.	2.07*	2.89*
	10	Ref.	2.28*	2.00*
	15	Ref.	2.11*	3.34*
	32	Ref.	2.10*	2.62*
O/Low	2	10	1.91*	2.59*
	2	15	1.61^	2.38*
	2	32	1.95*	2.60*
	10	15	1.74^	2.40*
	10	32	2.31*	2.43*
	15	32	1.70^	2.24*
	2	Ref.	2.12*	3.01*
	10	Ref.	1.65^	2.32*
	15	Ref.	1.91*	2.76*
	32	Ref.	2.38*	2.60*
O/High	2	10	2.39*	2.25*
	2	15	1.51^	1.41
	2	32	1.96*	2.17*
	10	15	2.37*	1.88^
	10	32	2.85*	3.22*
	15	32	1.59^	1.69^
	2	Ref.	2.4*	2.44*
	10	Ref.	2.64*	3.50*
	15	Ref.	2.23*	2.01*
	32	Ref.	2.35*	3.54*

*P<0.05
^P<0.10

Table 7. Proportion of bacterial taxonomic variation explained by soil physical and chemical factors as determined by a marginal distance based linear model.

Factor	Pseudo-F	Proportion of Variance Explained
Soil C:N	2.13*	0.04
Nitrate	2.65*	0.05
% Organic Matter	5.43*	0.11
Phosphate	5.82*	0.11
Nitrite	6.25*	0.12
Bioavailable C	7.84*	0.15
Time	9.62*	0.17
% Clay	10.78*	0.19
Soil pH	11.96*	0.21

*P<0.05

Table 8. Proportion of fungal taxonomic variation explained by soil physical and chemical factors as determined by a marginal distance based linear model.

Factor	Pseudo-F	Proportion of Variance Explained
Nitrate	2.56*	0.05
% Organic Matter	3.07*	0.06
Soil C:N	2.93*	0.06
Bioavailable C	4.21*	0.08
Nitrite	4.86*	0.10
Phosphate	5.03*	0.10
Soil pH	7.51*	0.14
% Clay	8.10*	0.15
Time	8.06*	0.15

*P<0.05

Table 9. Proportion of and cumulative bacterial taxonomic variation accounted for by each soil chemical/physical property as well as time since reclamation as determined by a conditional distance based linear model (DISTLM) with time added last. Overall best solution was determined using 'best' DISTLM.

Variable	Adjusted R^2	Pseudo-F	Variance Explained	
			Proportion	Cumulative
+% Organic Matter[a]	0.09	5.43*	0.11	0.11
+carbon:nitrogen[a]	0.13	3.21*	0.06	0.17
+Oxidizible C[a]	0.16	2.89*	0.05	0.22
+Nitrite[a]	0.22	3.98*	0.07	0.28
+Nitrate[a]	0.24	2.49*	0.04	0.32
+Phosphate[a]	0.26	2.07*	0.03	0.36
+% Clay[a]	0.31	3.58*	0.05	0.41
+Soil pH[a]	0.35	3.96*	0.05	0.46
+Time since reclamation[a]	0.39	3.43*	0.04	0.51
[a]Overall Best Solution	0.39			

*P<0.05

Table 10. Proportion of and cumulative fungal taxonomic variation accounted for by each soil chemical/physical property as well as time since reclamation as determined by a conditional distance based linear model (DISTLM) with time added last. Overall best solution was determined using 'best' DISTLM.

Variable	Adjusted R^2	Pseudo-F	Variance Explained	
			Proportion	Cumulative
+% Organic Matter[a]	0.04	3.07*	0.06	0.05
+carbon:nitrogen[a]	0.09	3.40*	0.07	0.13
+Oxidizable C	0.11	1.82*	0.04	0.16
+Nitrite[a]	0.15	3.40*	0.06	0.23
+Nitrate[a]	0.18	2.44*	0.04	0.27
+Phosphate[a]	0.22	2.87*	0.05	0.32
+% Clay[a]	0.27	4.26*	0.07	0.38
+Soil pH[a]	0.33	4.13*	0.06	0.44
+Time since reclamation[a]	0.38	4.52*	0.06	0.50
[a]Overall best solution	0.38			

*P<0.05

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