MONITORING THE EFFECTS OF SURFACE COAL-MINE-RECLAMATION ON SOIL BIOLOGICAL PROPERTIES

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Pamela Rose Block

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Range Science

November 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Monitoring the effects of surface coal-mine-reclamation on soil biological properties

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MASTER OF SCIENCE

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ABSTRACT

Surface coal mining is a large-scale disturbance that disrupts soil properties that requires reclamation. Reclamation monitoring and success typically focuses on vegetation communities, but soil monitoring may improve our understanding of barriers to reclamation success. In this study, we assessed biological presence in stockpiled soils using seed viability screening and phospholipid fatty acid analysis. We found microbial communities had distinct shifts in structure, declines in overall abundance of organisms with increased stockpile depth, and that total seedling emergence was lower in stockpiled soils than the reference site. In addition, we measured carbon pools, vegetative cover, and other edaphic properties in a chronosequence of reclaimed mine land (chapter two) in order to quantify how reclamation affects soil properties. We found disturbance affected all soil properties at every treatment (years since reclamation) and that all soil carbon pools measured were significantly ($p < 0.005$) lower than that of the reference site.

ACKNOWLEDGEMENTS

I would like to thank the members of my committee, Dr. Caley Gasch, Dr. Aaron Daigh, and Dr. Ryan Limb. I am especially grateful and honored to have worked with Dr. Caley Gasch, who provided not only endless scientific guidance during my masters but who is an extremely hard working scientist and served as an absolutely inspiring role model. Without your support and encouragement I would have never made it through this process, I thank you for showing me what a good advisor looks like.

I am eternally grateful to my friends that I made at NDSU during my time in Fargo. You guys were pivotal in my mental sanity, and I'm so grateful for your unyielding support and that you always had open arms. Also shout out to all the other friends in my life for keeping me laughing during some very rough patches (Craig Whale Pass). Lastly, I would like to thank Owen. You truly have been through the thick and thin and seen and supported me during such lows— I genuinely could never ever have done this without you. You have inspired me to push myself and to stay strong and kept my soul ignited.

This work was funded by the North Dakota Industrial Commission Lignite Energy Counsel. I would like to thank BNI for providing the sites to perform this research on, along with all their friendly staff.

DEDICATION

I would like to dedicate this work to my family, who instilled inspiration and curiosity in me at a young age and always encouraged me to be true to myself. Dad, I wish you were here to read this and know you would be proud—I miss you every day but am grateful that I can feel you here with me, supporting me even from beyond.

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INTRODUCTION

Anthropogenic soil disturbances, ranging from agricultural practices to mineral extraction, result in varying levels of soil degradation—depending on the intensity and duration of the disturbance (Berga et al., 2012). Large scale, high intensity disturbances (i.e., surface mining) are expected to increase, in order to meet increased energy demands. Disturbances such as these often completely disrupt plant-soil communities, altering both the composition and structure of vegetation and the soil microbial community (Allison et al., 2005; Holl, 2002). Soil microorganisms are responsible for and are key players of the cycling of organic compounds, and influence the composition of the vegetative community (Van der Heijden et al., 2008; Sayer et al., 2017). Currently, reclamation success in reclaimed mine sites is monitored largely via above-ground visual parameters (native/exotic vegetative species presence, slope lengths, elevation) however lacks any soil biological parameters (Schumann, 1999). If the goal of reclamation is to facilitate the reestablishment of a stable and productive ecosystem, it is imperative to quantify the relationship between common edaphic disturbances and their effect on the biological components of the soil in order to improve reclamation success (Moreno-de las Heras, 2009).

The soil microbial community facilitates many ecosystem functions, namely enabling nutrient uptake for plants through nutrient transformation (i.e. mineralization) (Horner-Devine et al., 2004; Wubs et al., 2016). Disturbance has been shown to negatively affect abundance, and composition of the soil microbial community—potentially leading to affects at the ecosystem scale, i.e. reduced plant diversity and production, and changes in nutrient cycling rates (Muller et al., 2002, Philippot et al., 2013). Due to their small size and ability to rapidly reproduce, microbes are important organisms to measure in terms of change to environmental conditions as they respond quickly (Anderson, 2003). The establishment of a productive and stable plant community will rely on the quality of the soil— and in particular the soil microbial community, as soil microbes provide the plant community with the essential link between inaccessible organic nutrients and usable inorganic nutrients (Jacoby et al., 2017).

The Surface Mining Control and Reclamation Act (OSM, 1977) requires mining companies to achieve reclamation success via a verified bond release process. This process mandates backfilling, stream channel reconstruction, top and subsoil separation and application, vegetation establishment, and monitoring of the composition of vegetation over a ten year period. This process involves field

inspections, annual reports and reviews, and accounts for roughly 30% of a mine company's total budget. Surface mining inherently disrupts soil and vegetation, and the reclamation process in itself is a form of disturbance. The goal is to have reclamation facilitate an improved ecosystem, and to maximize ecosystem recovery by implementing and monitoring reclamation via the most efficient processes (Hobbs and Harris, 2001). Meaning, in order to maximize ecosystem recovery—we need to understand how surface mining and reclamation processes are affecting every portion of the ecosystem, including belowground biology.

One particular disturbance frequently used during the mining process, along with many civil engineering projects, is the storage of topsoils (Miller et al. 1985). In order to expose the desired minerals, which are often found 50-150 feet below the surface, soils are stripped and stored using heavy machinery. These stockpiles are commonly over eight meters tall and may remain piled for 5-30 years (Rosenzweig et al., 2012; Wick et al., 2008). Effective stockpiling means that stockpiled soils that are dismantled and then re-spread (during reclamation process) are still viable physically, chemically, and biologically. Without any amendments, prolonged stockpiles may drastically disrupt soil biological activity; in this study, we wish to determine whether topsoil experiences depth-related changes in chemical, physical, and microbial characteristics after long-term storage.

The physical, chemical, and biological components of stockpiles are largely unknown, and when these stockpiles are dismantled and used during the re-spread process, they are treated under the assumption that stockpiling does not affect topsoil properties. Soil microbial abundance and activity is likely altered by disturbance and stockpiling (Birnbaum et al., 2017). Seeds in the stockpile act as a propagation source of indigenous, native, vegetation – however seed viability in stockpiles is unknown. In this study, we wish to determine how long-term topsoil stockpiling changes the biological characteristics and auxiliary soil physical and chemical properties. In theory, stockpiled soils that are dismantled and then re-spread (during reclamation process) should have physical, chemical, and biological characteristics that facilitate successful reclamation.

Disturbed soil and vegetative properties are expected to improve with time, meaning they will become increasingly similar to their native reference site. However, studies previously conducted on the same area of this study found that soil parameters such as soil compaction have shown little

improvement and that plant communities are becoming increasingly non-native (Bohrer et al., 2017). These results indicate a missing link when assessing reclamation success, and without any belowground monitoring and intervention, reclaimed land may never recover to its full potential. In this study, we aim to assess how the surface mine and reclamation process affects soil parameters. We hypothesize that vegetation will be limited in diversity in reclaimed years and that microbial biomass carbon will increase with reclamation age. In order to test this, we sampled soil and vegetation on a 42-year-chronosequence of reclaimed strip mines and measured soil physical, chemical, and biological properties.

Objectives

The objectives of this study are to: 1. Assess soil microbial community structure and seed viability from multiple depths in a stockpile to understand effects of long-term topsoil storage on belowground biological integrity, 2. Assess belowground changes in labile carbon pools (as measured by MBC and POXC) along with other edaphic and vegetation properties, to evaluate and monitor the recovery of soil quality in reclaimed mine soils over time in comparison to a reference site. We hypothesize that biological activity will be limited via stockpile biogeochemical conditions, and that the upper most portion of the stockpile will be the most biologically active. We also hypothesize that older reclaimed sites will more closely resemble the reference site in terms of microbial activity, carbon content, and edaphic properties than more recently reclaimed sites.

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CHAPTER 1: BIOLOGICAL INTEGRITY OF MIXED-GRASS PRAIRIE TOPSOILS SUBJECTED TO LONG-TERM STOCKPILING

Abstract

Surface mining often requires storage of topsoil in large piles for long periods of time (1-30 years). Such soil handling and storage results in physical and biogeochemical changes and may alter the soil microbial community and seedbank. Microbial activity regulates nutrient cycling and soil quality, and revegetation of most native forb species relies on a viable seedbank. Soil condition and biological viability influence the establishment and success of the aboveground plant community, and therefore should be considered in quantifying successful reclamation. In this study, we characterized the seed bank and microbial community in order to quantify their viability in a stockpile (depths of 15cm-750cm). Microbial community structure was measured using phospholipid fatty acid analysis, which provides abundance estimates of soil microbial groups at broad taxonomic levels. The seed bank was assessed using the seedling emergence method under greenhouse conditions. We hypothesized that with increasing depth, soil biota would decrease in abundance and perhaps exhibit a shift in community structure, and that stockpiled soils would display different communities than an undisturbed reference site. Using principal component analysis, we found microbial communities had distinct shifts in terms of community structure and declines in overall abundance of organisms with increased depth. Furthermore, overall abundance of microbes within the top 15 cm of stockpiled soil was nearly 1/3 less than our native reference site, and soil at deeper depths were similarly depleted. Total seedling emergence from soil collected at all depths of the stockpile was lower (20 viable seeds) compared to the reference soil emergence (36 viable seeds). Our results demonstrate that stockpiling greatly affects soil microbial communities and that stockpiled topsoil is not a dependable source for forb seeds. Reclamation may require forb seeding and soil amendments to facilitate whole-system restoration.

Introduction

Soil disturbance results in widespread biogeochemical changes and alters biotic and abiotic soil properties (Griffiths et al., 2004; Tischer et al., 2015). Anthropogenic disturbances negatively affect soil biodiversity (Beare et al., 1995). Reduced diversity has been linked to decreases in soil microbial

functions, which then influences the available nutrients needed for a productive plant community (Wagg et al., 2014). Soils subject to surface mining and reclamation are degraded, often characterized by high levels of compaction, limited water infiltration, and poor soil structure (Helingerová et al., 2010; Mummey et al., 2002b; Yuan et al., 2017). These factors are reflected through sub-optimal establishment and diversity of the vegetative community following reclamation.

Anthropogenic soil disturbances require reclamation, with the end goal often targeted at the reestablishment of a productive and healthy vegetative community. While vegetative (aboveground) recovery is the most commonly used metric to monitor and determine reclamation success, it does not necessarily indicate full recovery of ecosystem functionality (Harris et al., 1989; Ingram et al., 2005; Mummey et al., 2002a). Microbial communities influence and shape plant community structure (Reynolds et al., 2003), with microbes mediating most soil processes that affect nutrient availability, plant health, and primary production (Brussard et al. 1997, van der Heijden et al. 2008). Although reclaimed land often meets aboveground expectations, this does not mean the ecosystem is performing at full potential, particularly for belowground ecosystem components (Bohrer et al., 2017). Therefore, it is important to monitor and understand soil recovery in addition to vegetation communities to provide a more robust assessment of reclamation success and overall ecosystem recovery.

Surface mining is a destructive process that destroys existing vegetation and disturbs belowground biological activities (Boyer et al., 2011; Harris et al., 1993). Pre- and post-surface mining activities follow strict soil handling requirements to ensure soil conservation and facilitate reclamation success (OSM, 1977). Top- and sub-soil are separated during the mining process to retain topsoil quality, and each type of soil is either directly re-spread on previously mined areas or stockpiled for future reclamation. Perennial native vegetation is established through a combination of manual seeding, natural recolonization, and by viable seeds remaining in the soil after soil handling and storage (Baskin and Baskin, 1998; D'Antonio and Meyerson, 2002). In the Northern Great Plains, much of the native vegetation (especially warm season grasses) are dependent on symbiotic and beneficial microorganism associations; therefore, retaining topsoil biological integrity is important for long term reestablishment of a diverse native plant community.

Topsoil handling during the mining and reclamation process may influence the trajectory of plant community establishment and overall reclamation success. When soils are re-spread immediately following excavation, changes to the physical, chemical and biological properties are minimized (Koch, 2007). However, when soils are stockpiled for prolonged periods of time edaphic properties are likely to change, causing shifts or losses in the soil microbial community (Abdul-Kareem & McRae, 1984; Wick et al., 2009). An immediate effect of soil disturbance is the destruction of aggregates (Abdul-Kareem, 1984; Wick et al., 2008) which control the pore distribution and therefore soil water and air distribution throughout the soil—both which are critical to the growth and survival of soil microorganisms (Kuzyakov et al., 2015). Destruction of soil aggregates, combined with topsoil mixing, results in an increase of carbon (C) and nitrogen (N) mineralization through the exposure of physically protected nutrients to microbes and oxygen (Ingram, 2005; Rustad et al., 2001; Williamson et al., 1990). Mineralization of previously unavailable nutrients leads to net loss and dilution of nutrients in the disturbed soil, and stockpiling for long periods of time may prevent soil properties from recovering to pre-disturbance levels. These soil changes may ultimately affect ecosystem processes, such as primary productivity, decomposition rates, carbon storage, and nitrogen fixation (Ussiri et al., 2005; Visser et al., 1984). Edaphic properties, such as pH and moisture content, are the most influential properties on the soil microbial composition when compared across land-use types (Lauber, et al., 2008), and they may also be altered by soil disturbance and stockpiling. Thus, with these foreseen changes in the physical and chemical environment caused by soil handling, and their potential to be exacerbated by long-term stockpiling, we expect soil disturbance and stockpiling to be associated with shifts in microbial abundance and the microbial community structure.

Vegetation plays a major role in improving disturbed soils over time, providing structural stability, with roots providing the main source of carbon needed to jumpstart soil recovery (Zhang et al., 2016). Due to the height and size of stockpiles, ranging from 5-18 m in height and 1-22 total hectares, the rooting depth of vegetation is limited to the shallow surface soil of the stockpile—meaning deep incorporation and cycling of soil organic matter may be limited with depth. This combined with the initial flush of mineralization of previously protected organic matter, via the breakup of aggregates, may contribute to an overall decline in organic matter and persistent low organic matter content in stockpiled

soils (Schwenke, et.al, 2000). Depleted organic matter will decrease the soil's cation exchange capacity and potentially result in nutrient leaching. In addition, compaction caused during the creation of the stockpile, combined with the lack of vegetative roots—which would normally aid in breaking up heavily compacted soil—will result in reduced air and water flow throughout the stockpile. Years of limited air and water exchange will likely hinder biological activity and may cause topsoil to appear more characteristically like subsoil.

In addition to the changes expected in soil biological communities, habitats, and substrates, as a result of stockpiling, the viability of the soil seed bank is also important to consider. The reestablishment of vegetation, particularly in the Great Plains, largely depends on the germination of seeds found in the seedbank to propagate indigenous, native, species (Faist et al., 2013). For many of these plant species, longevity of seeds found in the soil benefit recruitment (Stocklin and Markus, 1999) meaning that a high seed longevity in the stockpiled soil will increase chances of successful recruitment once the soil is re-spread during reclamation. Mines rely entirely on the seedbank for forb species reestablishment; however, the viability and abundance of seeds exposed to long-term stockpiling is largely unknown. Therefore, understanding the relationship between common edaphic disturbances and native plant persistence in the seedbank may facilitate reclamation success and in understanding the susceptibility of reclaimed soils to invasion by non-natives.

Soil handling during surface mining has effects on soil physico-chemical characteristics, which are most likely exacerbated via long-term stockpiling (Wick et al., 2009), and which may ultimately delay reclamation success. This is contrary to mine reclamation practices that treat stockpiled topsoil as though it has the same properties as "fresh" topsoil throughout the pile. Inclusion of belowground properties in assessing effects of long-term stockpiling will aid in identifying practices that promote ecosystem rehabilitation after disturbance. In this study, we aim to determine the effect of long-term topsoil stockpiling on the soil microbial community and the seedbank. In practice, stockpiled soils that are dismantled and then re-spread (during the reclamation process) are assumed to have physical, chemical, and biological characteristics that facilitate successful reclamation. Our study will assess this according to the following objective: Assess soil microbial community structure and seed viability from multiple depths in a stockpile to understand effects of long-term topsoil storage on belowground biological integrity. We

hypothesize that biological activity will be limited via stockpile biogeochemical conditions, and that the upper most portion of the stockpile will be the most biologically active.

Methods

Study site description

Soils were collected from one topsoil stockpile at BNI Coal mine in central North Dakota (47°3′13″N 101°19′45″W). This stockpile was 230 x 60 x 9.5 m, 1 hectare in size, and a similar dimension to other stockpiles in this area. BNI is an active lignite strip mine that has been in production for 48 years, located in Oliver County, North Dakota. The pre-mined soil developed on siltstone or sandstone, with loamy to silty-clay loam textures, dominated by the Williams (Typic Argiustolls) and Cabba *(*Typic Ustorthents) soil series (NRCS, 2017). In general, the soils in this area are deep, well-drained to moderately well-drained, with a friable loam subsoil and carbonate accumulation at depth (Weiser, 1975). The area has a continental climate regime, with an annual mean temperature of 6.7° C and precipitation of 406.4 mm. Minimum temperatures occur in January averaging -16.7° C and maximum temperatures in July average 22.8° C from 1991-2017 (NDAWN, 2017). Vegetation on the stockpile was well established, dominated mostly by invasive grasses—with no flowering forbs at the time of sampling.

Field sampling methods

To address soil microbial integrity of stockpiled soil, we collected soil samples in August of 2017 from a stockpile originally formed in 1992-1993. Topsoil removed during the creation of this stockpile was originally found in the A horizon (Scott, 2010). Following formation, the stockpile was planted with non-native perennial vegetation for stabilization. Soil samples were collected using a mechanical core extraction probe (Diedrich D-50, Diedrich Drill, LaPorte, IN) (diameter= 3.5 cm) at ten locations randomly selected along a 100 m transect. Cores were segmented into seven depth increments (0-15 cm, 30-45 cm, 60-75 cm, 90-120 cm, 300-350 cm, 450-490 cm, 750-790 cm) at each of the ten locations (7 depths x 10 locations = 70 samples total). We also extracted soil with a hammer-driven corer at three depths (0-15 cm, 90-120 cm, and 300-350 cm) for bulk density estimation. Samples were kept on ice while in the field and frozen (-20° C) within two hours of sampling. Additionally, we collected ten soil samples (0-15 cm) from an undisturbed native reference site at 10 m increments along a 100 m transect. The reference site has a similar textural class, is approximately 7.2 km away and was assumed to be representative of the

pre-disturbance state of the stockpiled soil. The reference areas serve as a vegetation benchmark for BNI, and is not used specifically as a target for soil reclamation.

Seedbank sampling and screening

To survey the residual soil seed bank, we collected soil samples at five locations from the same seven depths during dismantle of the stockpile in September of 2017. Each sample was extracted, placed in a sterile plastic bag, and transported to the greenhouse in a tub under dark conditions. Fresh soil samples were weighed to 100 g and spread over 100 g of vermiculite to 1 cm thickness in germination trays (25cm x 20 cm in surface area and 10 cm depth). Greenhouse conditions were set to 15 hr days (26 °C) and 9 hr nights (18 °C) and were watered twice daily until seedling emergence. Following emergence, seedlings were transplanted to individual pots (5 cm x 5 cm) containing potting mix and watered once per day. Individual plants were grown until species identification was possible.

Soil biological analysis

Phospholipid fatty acid (PLFA) analysis was used to assess soil microbiological communities and microbial group abundance of the stockpiled soil. This method provides abundance estimates of soil microbial groups at a broad taxonomic level (bacteria, saprophytic fungi, arbuscular mycorrhizal fungi, actinomycetes, and eukaryotic microbes), and it is a robust method for detecting microbial community differences across treatments (Ramsey et al., 2006). Frozen soil samples were lyophilized and ground to pass through a 2 mm sieve, then analyzed for PLFA identity and abundance by Microbial Identification (MIDI) Labs, Inc. (Newark, DE). The MIDI lab follows lipid extraction procedures described by Buyer and Sasser (2012) and quantitative analysis with gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) and peak identification using Sherlock software version 6.2 (MIDI, Inc., Newark, DE) and PLFAD2 version 2.0. The abundance of each microbial group was treated in terms of absolute abundance (nmol fatty acid / g soil) as well as in relative terms (each group divided by the total abundance).

Soil chemical and physical analysis

All soil samples used for soil physical and chemical analyses were air-dried and sieved to 2 mm. Soil pH and electrical conductivity (EC) were determined in a 1:1 soil:water slurry with an Oakton pH meter and an Orion Star A112 conductivity meter (Rhoades, 1996; Thomas, 1996). Bulk density was estimated from intact volumetric cores, oven-dried at 105 °C according to Blake and Hartge (1986). We

used the pressure plate method (Richards et al., 1943) at -1/3 Bar to determine gravimetric water content at field capacity of ground samples. Total carbon and inorganic carbon were analyzed using a Skalar Formacs total organic carbon high temperature catalytic combustion analyzer. Soil organic carbon was calculated as the difference between the two pools. Permanganate oxidizable carbon represents a labile, active fraction of non-living SOC and was assessed following Weil et al., 2003. We calculated particle size analysis on a subset of samples (n=3 per depth) following hydrometer method in order to determine soil texture (Gee and Bauder, 1986).

Statistical analysis

Each depth increment was considered as an independent set of observations in the analysis. Descriptive statistics were calculated for soil properties and seed viability within each depth. Differences in means across depths were determined using an analysis of variance (ANOVA) with Tukey's Honest Significant Difference post-hoc test. Bray-Curtis dissimilarity indices were calculated between the microbial community at each depth compared to the reference in absolute terms, representing differences in abundance and composition.

To understand how the microbial communities and soil properties varied across depth, and in relation to one another, we conducted two multivariate analyses. To identify changes in microbial community composition across depth, we performed a principal component analysis (PCA) on PLFA data alone. To explore how general soil properties varied and co-varied across depths, we used a linear discriminant analysis (LDA) with the following input properties: pH, EC, field capacity, inorganic carbon (IC), active carbon (POXC), organic carbon (SOC), total nitrogen, total microbial abundance, and the fungal-to-bacterial ratio.

All data analysis was completed in R (R Core Team, 2018) using the 'vegan' (Oksanen et al., 2015), 'pastecs' (Grosjean et al., 2014), 'plyr' (Wickham, 2011), 'MASS' (Venables and Ripley, 2002), 'RColorBrewer' (Neuwirth, 2014), 'fields' (Nychka et al., 2015), 'scales' (Wickham, 2014) 'ggord' (Beck and Mikryukov, 2018), 'zoo' (Zeileis and Grothendieck, 2005), 'ggplot2' (Wickham, 2016), and 'multcompView' (Graves et al., 2015) packages for data manipulation, analysis, and visualizations.

Results

Total seedling emergence from soil collected at all depths of the stockpile was lower (20 viable seeds) compared to the reference soil emergence (36 viable seeds) (Fig 1.1). The total number of species emerging from all stockpiled soils was four species. The shallowest depth (15 cm) had the highest number of seedlings emerge, with eight total seedlings, compared to that of the reference. As depth increased, seedling emergence decreased steadily—with the second depth (45 cm) having half the seedling emergence of the top depth (15 cm). Grass species dominated the emergent vegetation in the reference soil, accounting for 50% of total emerged species.

As stockpile depth increased, total microbial abundance significantly decreased (Fig. 1.2 A, Table 1). The mean total microbial abundance concentration was 47 nmol g^{-1} soil in the shallowest depth (15 cm), compared to 6 nmol g^{-1} soil in the deepest depth and 134 nmol g^{-1} in the reference soil (Table 1.1). Total microbial abundance was significantly ($p < 0.05$) different between all depths and the reference soil. Dissimilarity indices of absolute abundance revealed that, community structure became less similar to the reference as depth increased (Fig 1.2 A). Moreover, we found that relative abundance of actinomycetes and Gram-positive bacteria increased proportionally with depth—becoming dominant at the deepest depths (Figure 1.1 B). The fungal to bacterial (F:B) ratio serves as an indicator of community shifts, and decreased with depth throughout the stockpile until the deepest depth. The F:B was significantly different beyond the 45 cm depth compared to the reference site (Fig 1.3). Surprisingly we found that relative abundance of saprophytic fungi was the highest at the deepest portion of the stockpile (Fig 1.2, B).

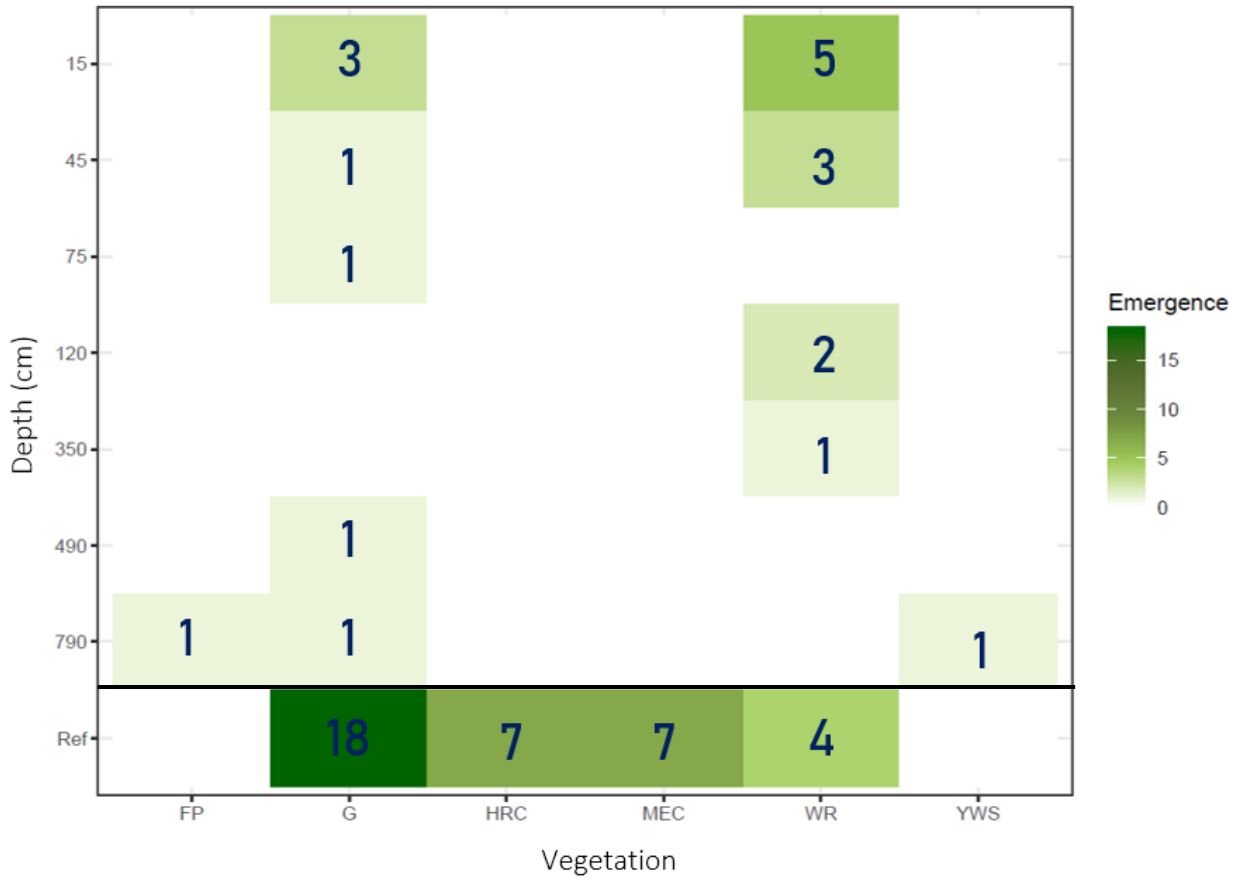

Figure 1.1. Heatmap representing emergence rate of vegetation from the seedbank from soils collected from multiple stockpile depths. Values represent emergence of vegetation at each depth. Total seedling emergence in the reference site n=36, showing that stockpiling negatively affects seedling emergence. FP – Field pennycress (*Thlaspi arvense*), G—grass, HRC – Hairy rock cress (*Arabis pycnocarpa*) , MEC – Mouse-ear chickweed (*Cerastium vulgatum)*, WR—Western ragweed (*Ambrosia psilostachya*), YWS – Yellow wood sorrel (*Oxalis stricta)*. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

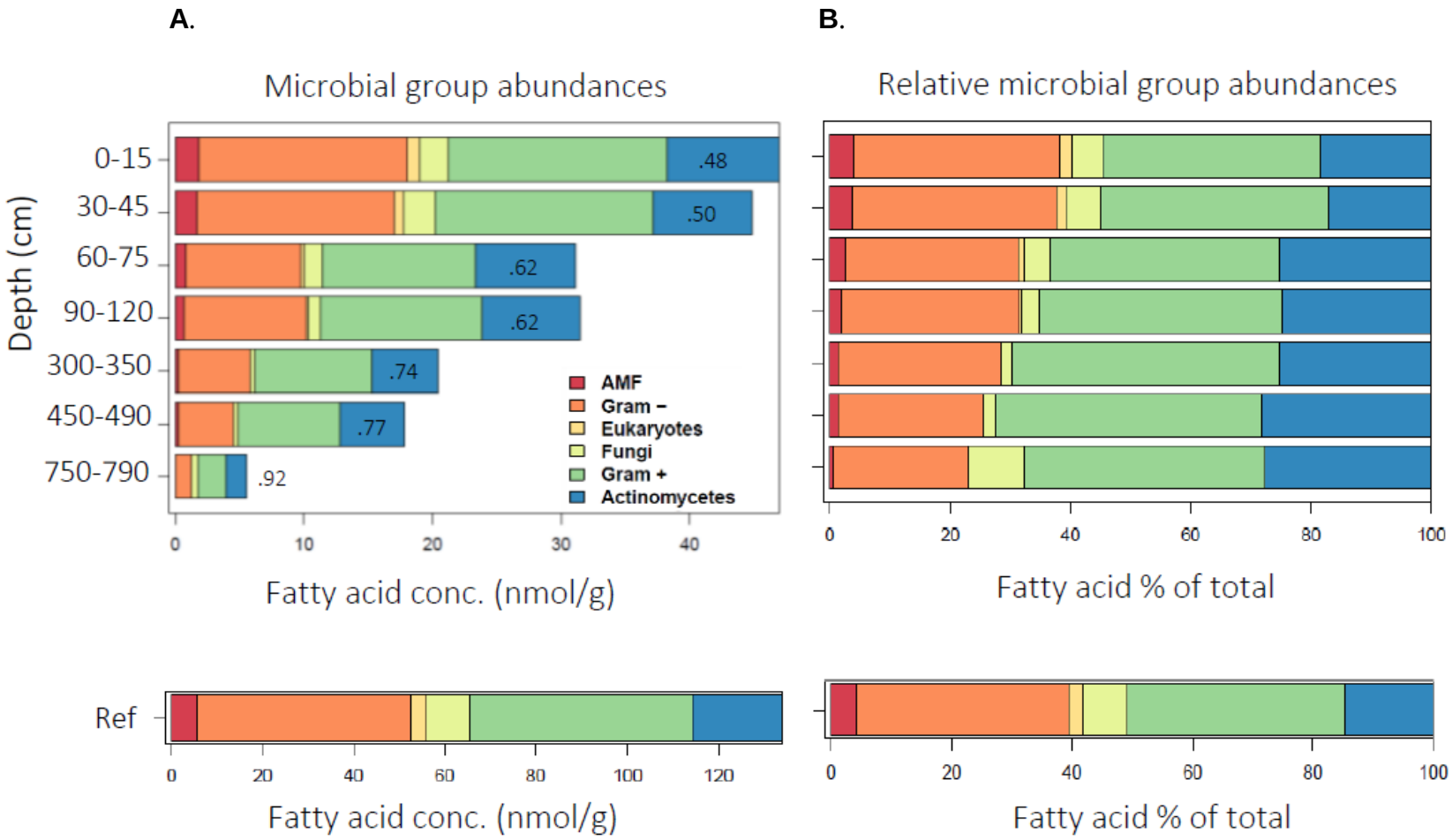

Figure 1.2. A. Concentration (nmol g^{-1} soil) of microbial groups (phospholipid fatty acid analysis) in soil collected from increasing depths of a stockpile (n=10), values are dissimilarity indices (Bray-Curtis) as compared to the reference site, based on the mean values of each group x depth, showing that as depth increases, the microbial community becomes increasingly dissimilar to the reference community. B. Relative abundance of microbial groups along with a nearby reference soil, calculated as the percent of each group in relation to the total microbial abundance showing that Gram - and AMF are most negatively affected by stockpiling. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

Table 1.1. Means with standard deviation in parenthesis of relative (%) and absolute (nmol g^{-1} soil) abundances of microbial groups from phospholipid fatty acid analysis in soils collected from multiple depths in a stockpile. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

	----------Relative Abundance (%)----------						----------Absolute Abundance (nmol FA g^{-1} soil)----------							
Depth (cm)	AMF	Gram -	Eukaryotes	Fungi	Gram +	Actinomycetes	Total Abundance	AMF	Gram -	Eukaryotes	Fungi	Gram +	Actinomycetes	F:B
0-15	3.87	34.35	1.97	5.13	36.2	18.48	46.94	1.82	16.23	0.93	2.25	17.04	8.67	0.13
	(0.48)	*(1.5)*	*(0.9)*	*(2.65)*	*(1.44)*	*(0.82)*	*(11.74)*	*(0.5)*	*(4.49)*	*(0.43)*	*(0.72)*	*(4.45)*	*(2.27)*	*(0.05)*
30-45	3.78	33.94	1.6	5.76	37.77	17.15	44.81	1.7	15.3	0.73	2.52	16.9	7.67	0.14
	(0.54)	*(1.73)*	*(0.34)*	*(1.47)*	*(4.87)*	*(4.39)*	*(11.68)*	*(0.51)*	*(4.37)*	*(0.27)*	*(0.67)*	*(4.6)*	*(2.87)*	*(0.03)*
60-75	2.63	28.67	0.97	4.35	38.23	25.16	31.15	0.81	8.94	0.32	1.38	11.93	7.77	0.11
	(0.22)	*(0.99)*	*(0.7)*	*(1.36)*	*(0.92)*	*(1.63)*	*(5.08)*	*(0.13)*	*(1.55)*	*(0.26)*	*(0.58)*	*(2.08)*	*(0.91)*	*(0.02)*
90-120	2.04	29.49	0.34	3.04	40.27	24.83	31.47	0.69	9.5	0.14	0.95	12.59	7.61	0.08
	(0.64)	*(3.07)*	*(0.48)*	*(1.04)*	*(1.63)*	*(2.53)*	*(10.6)*	*(0.47)*	*(4.33)*	*(0.25)*	*(0.38)*	*(3.82)*	*(1.63)*	*(0.02)*
300-350	1.3	27.32	0	1.68	44.45	25.26	20.42	0.27	5.59	0	0.35	9.05	5.17	0.04
	(0.42)	*(2.74)*	*(0)*	*(0.76)*	*(5.11)*	*(5.69)*	*(3.31)*	*(0.1)*	*(1.2)*	*(0)*	*(0.2)*	*(1.54)*	*(1.41)*	*(0.02)*
450-490	1.28	24.22	0	2.09	44.28	28.13	17.82	0.23	4.31	0	0.35	7.88	5.05	0.05
	(0.27)	*(2.26)*	*(0)*	*(1.5)*	*(1.61)*	*(2.94)*	*(3.26)*	*(0.06)*	*(0.83)*	*(0)*	*(0.2)*	*(1.42)*	*(1.31)*	*(0.02)*
750-790	0.62	22.44	0	9.19	40.17	27.59	5.5	0.05	1.21	0	0.5	2.21	1.54	0.17
	(1.02)	*(4.64)*	*(0)*	*(3.19)*	*(6.48)*	*(6.92)*	*(2.93)*	*(0.08)*	*(0.66)*	*(0)*	*(0.3)*	*(1.27)*	*(0.82)*	*(0.07)*
Ref	4.16	35.24	2.53	7.28	36.08	14.73	134.01	5.53	47.05	3.36	9.69	48.53	19.85	0.17
	(0.36)	*(1.36)*	*(0.58)*	*(0.71)*	*(1.45)*	*(1.06)*	*(16.9)*	*(0.41)*	*(4.4)*	*(0.8)*	*(0.99)*	*(7.83)*	*(3.66)*	*(0.01)*

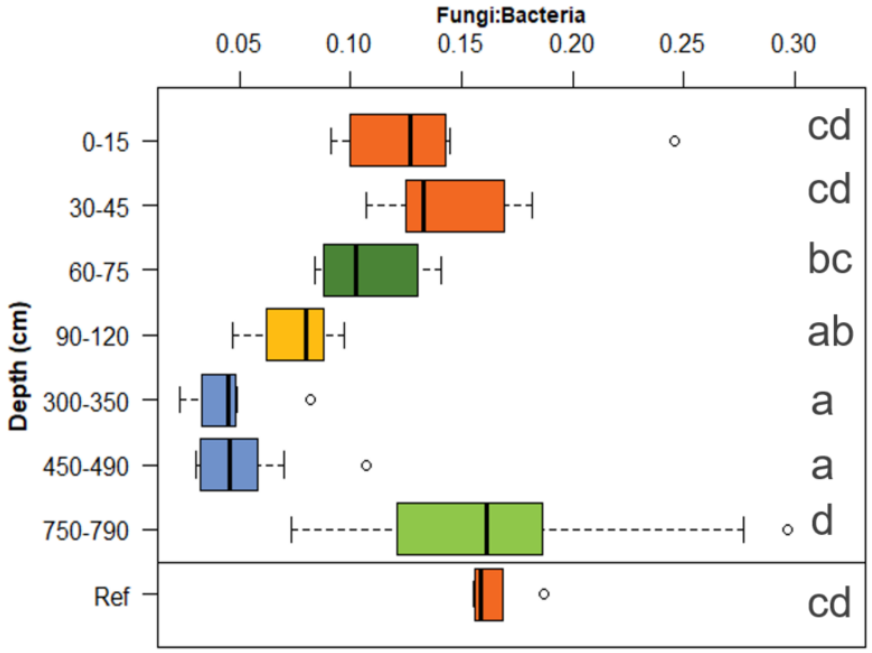

Figure 1.3. Box-and-whisker plots of fungi:bacteria (F:B) ratio in soils collected from depths in a stockpile, showing that the fungi:bacteria ratio decreased with depth throughout the stockpile until the deepest depth. Letters indicate significant differences between all depths and the reference site ($p < 0.05$) according to ANOVA with Tukey's posthoc HSD test. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

Principal component analysis of PLFAs revealed that the microbial community composition differed across stockpile depth (Fig 1.4). The combined PCA explained 78% of the variance, with the first principal component accounting for 59.4% and the second principal component representing 18.6% of the variance across all observations. Samples tended to separate based on microbial group and depth. The top portion of the stockpile was most similar to the reference soil, with arbuscular mycorrhizal fungi, Gram-negative, and eukaryotic groups underlying those similarities along the first component. Abundance of Gram-positive bacteria and actinomycetes were associated with the deeper depths of the stockpile, while fungi were associated with the second component and the deepest sampled depth.

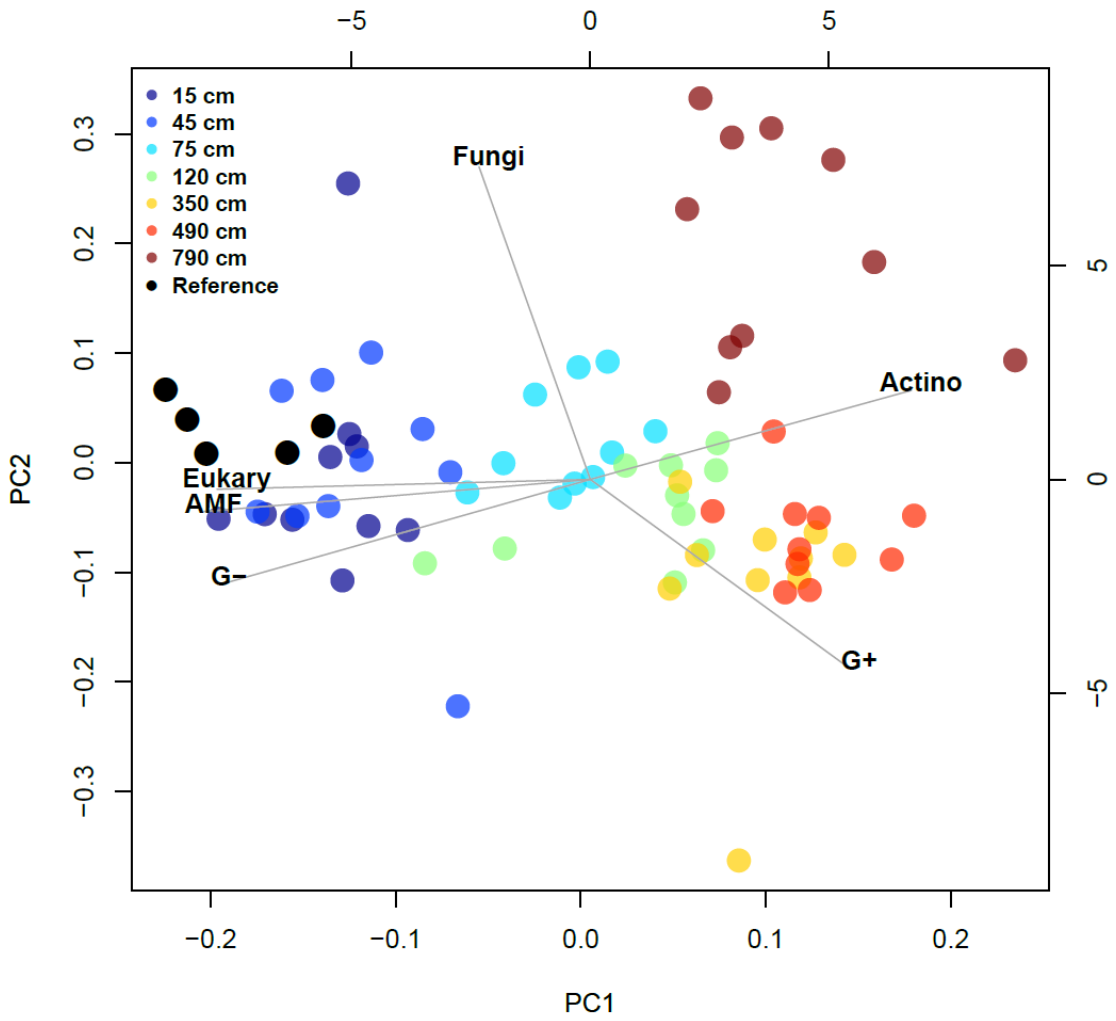

Figure 1.4. Principle component analysis for microbial communities across stockpile depth, proportion of variance of PC1 (59.4%) and PC2 (18.6%) of microbial groups. The principal component analysis was created using relative abundance of microbial groupings in order to explore multivariate relationships between groups and depth in relative terms. Data shows that microbial composition varied across depth and shallow stockpiled soil is the most similar to the reference soil. Eukary – Eukaryotes, AMF – Arbuscular mycorrhizal fungi, G- – Gram-negative bacteria, G+ – Gram-positive bacteria, Actino – Actinomycetes. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

Stockpiling did not greatly affect texture; particle size analysis revealed texture throughout the stockpile was largely silty clay loam (Table 1.2). Bulk density (Table 1.2) was consistent throughout the stockpile (mean = 1.68, 1.66 & 1.71 g/cm^3) however was significantly ($p < 0.05$) higher compared to the reference soil (mean = 1.09 g/cm^3). In general, edaphic properties were different throughout the stockpile (Fig 1.5). Soil pH and electrical conductivity were variable at depths deeper than 90 cm. Total nitrogen was not significantly different throughout the stockpile, however was significantly lower than the reference

soil. Active carbon and soil organic carbon were low throughout the stockpile, and lowest in the deepest depth. Inorganic carbon was significantly different ($p < 0.05$) only in the deepest depth. Field capacity (Appendix A, Figure 1) was significantly different at each depth compared to the reference.

Table 1.2. Particle size analysis used to determine soil texture, averaged across subsamples (n=3) for each depth and bulk density (n = 10). Table shows that texture and bulk density remain consistent throughout the stockpile. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

Depth (cm)	% Sand	% Silt	% Clay	Texture	Bulk Density (g/cm^3)
0-15	20	53	27	Silt loam	1.68
30-45	19	48	34	Silty clay loam	NA
60-75	19	53	28	Silty clay loam	NA
90-120	18	47	35	Silty clay loam	1.66
300-350	20	48	33	Clay loam/Silty clay loam	1.71
450-490	22	53	25	Silt loam	NA
750-790	21	49	29	Clay loam	NA

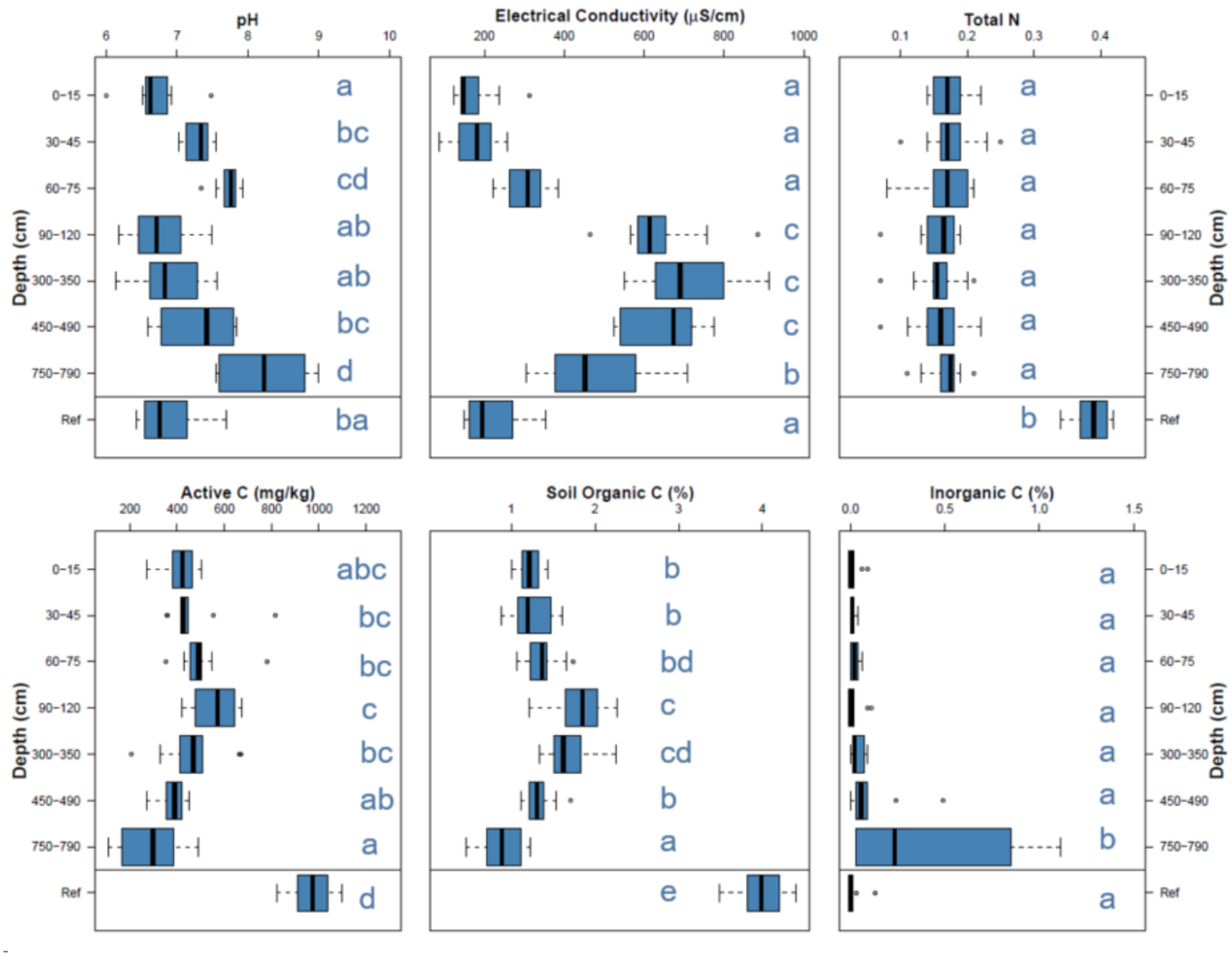

Figure 1.5. Box-and-whisker plots of soil properties across stockpile depths. Letters indicate significant differences between depths and the reference site ($p < 0.05$) according to ANOVA with Tukey's posthoc HSD test. Physico-chemical properties were highly affected by stockpiling, with carbon content significantly reduced throughout all stockpile depths. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

The combined linear discriminant analysis explained 92.09% of the variability in multivariate properties across depths with 80 and 12% of the variability explained via LD1 and LD2 functions respectively. LD1 accounts for the most variance between stockpiled soils and the reference soil; we found that soil properties at all depths were significantly different than the reference (Fig 1.6). Total microbial abundance, total nitrogen, soil organic carbon, and POXC had the strongest positive correlation out of all properties, (0.98 and 0.90, respectively) with LD1 (Table 1.3). Electrical conductivity had the strongest negative correlation with LD1 (-0.55). Soil properties in shallower depths (0-15 cm, 30-45 cm, 60-75 cm) were grouped together and were distinctly separated from deeper depths (90-120 cm, 300-350 cm, & 450-490 cm), with electrical conductivity and the F:B ratio driving that separation. Soil properties in the deepest depth (750-790 cm) overlapped all but the shallowest depths.

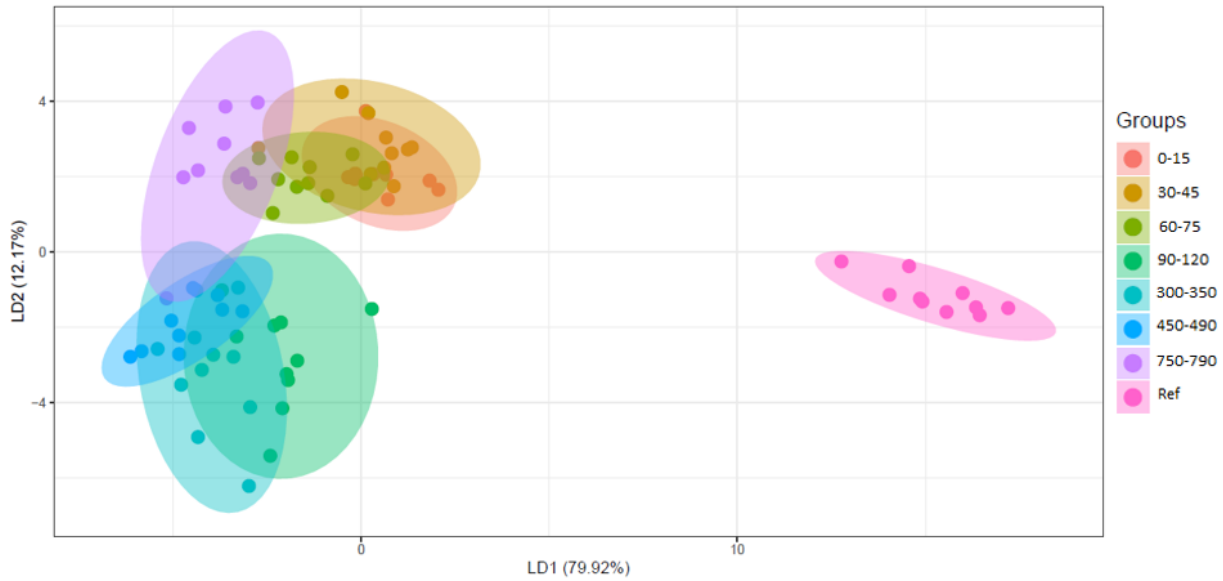

Figure 1.6. Linear discriminant biplots using edaphic properties (plus total microbial abundance and F:B ratio), including the first two discriminant functions (LD1 and LD2). Ellipses represent confidence intervals around the mean of each group (alpha=0.05). Groupings are stockpile depths (cm). Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

Table 1.3. Correlations between properties and linear discriminant coefficients (structure loadings) of linear discriminant analysis, (POXC – permanganate oxidizable carbon, F:B fungal to bacterial ratio). Correlations greater than ± 0.50 are bold. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

Explanatory variable	LD1 – 79.92%	LD2 – 12.17%
pH	-0.28	0.45
Electrical conductivity	**-0.55**	**-0.75**
Field capacity	**0.58**	-0.11
Inorganic carbon	-0.22	0.20
POXC	**0.88**	-0.43
Soil organic carbon	**0.88**	-0.43
Total N	**0.90**	-0.09
Total microbial abundance	**0.98**	-0.07
F:B	0.48	**0.62**

Discussion

Stockpiling is a common strategy for temporarily storing soil materials. Currently, mining companies are mandated to keep topsoil separate from subsoil, often resulting in stockpiles. The purpose of stockpiling is to ensure that there is viable topsoil to re-spread when mined areas are ready to be reclaimed. The objective of this study was to determine the effects of stockpiling on the soil microbial community, edaphic properties, and the soil seed bank in order to assess whether stockpiling topsoil for long periods of time causes changes in important soil properties. We found that soil physical, chemical, and biological properties were altered through stockpiling. These changes indicate that soil subject to long-term stockpiling should not be considered to have the same characteristics as fresh topsoil.

Stockpiling drastically hindered the seedling emergence rate of the seedbank compared to the reference seedbank. Not only was seedling emergence in the entire stockpile low, depths beyond the shallowest depth (15 cm), total seedling emergence did not exceed four seedlings. Time and duration of stockpiling likely reduced seed viability, along with high levels of compaction and limited water availability (Baskin and Baskin, 2001). Mining companies assume that stockpiling does not affect the seedbank, and do not seed forb species during reclamation for this reason. Understanding seedbank characteristics, prolificity, and viability is essential for reclamation planning (Roovers et al. 2006; Vécrin et al. 2007). This study found that stockpiled topsoil is not a dependable source for forb seeds, and management should reflect this finding by seeding for forbs at an appropriate time after reclamation.

In this study, we found that even the highest microbial abundance (found in the shallowest depth) was less than a third of the reference site. Decreased biological activity in stockpiled soils will likely have a lasting impact on land reclaimed with stockpiled topsoil. Decreased microbial activity may be due to the reduction of available water combined with anoxic conditions, which has been found in other studies (Abdul-Kareem and McRae, 1984). Contrary to our initial hypothesis and results from previous studies (Harris, 1993) we found that depth did not influence bulk density. This could be due to repeated traffic with heavy machinery during stockpile creation, and a lack of root or water penetration to allow for any break up of compaction. However, despite the uniform bulk density throughout the stockpile, bulk density was significantly higher throughout the stockpile compared to the reference soil. Highly compacted soil in the stockpile will have limited nutrient and gas exchange due to decreased porosity and may be a cause of decreased microbial activity (Breland and Hansen, 1996).

Gram-negative bacteria decreased in relative abundance with depth throughout the stockpile, while Gram-positive bacteria and actinomycetes (a type of Gram-positive bacteria), were relatively less impacted by depth. Limited contact with roots that feed the microbial community likely resulted in differences in abundance and composition of the microbial community, which could disrupt nutrient cycling and rates of decomposition (Cleveland et al., 2014). Limited access to water may explain the shift in community structure, as Gram-negative bacteria are more sensitive to water stress compared to Gram-positive bacteria (Manzoni et al., 2012). The lesser impact on the Gram-positive bacteria may be due to both being more tolerant of water stress, along with the capability of spore formation, allowing them to be able to withstand the harsh condition of a deep stockpile (Hueso et al., 2012). We also found decreased abundance of eukaryotes, with a complete lack of eukaryotes past 120 cm. This is likely due to complete lack of sunlight access (algae) and very limited water availability (protozoa). The highest relative abundance of saprophytic fungi at the deepest depth could be due to hyphae from soil underneath the stockpile growing into the stockpile from below.

We found the F:B ratio to be similar ($p < 0.05$) to the reference soil in the first two depths (0-15, 30-45 cm) and that F:B ratio steadily decreased beyond 45 cm, eventually bouncing back up at the deepest depth. The F:B ratio is an important parameter, showing the ratio of major decomposer groups (fungi and bacteria) and has been linked to soil carbon cycling and storage potential (Malik et al., 2016).

Nutrient availability (i.e. quantity and quality of root exudates) has been shown to be one of the biggest drivers of F:B dominance (Bardgett and McAlister, 1999). We found homogenous, compacted soils (bulk density 1.7 g/cm^3) throughout the stockpile indicating that compaction and reduced gas exchange is most likely not the reason for a change in the F:B ratio. We found that total nitrogen and carbon throughout the stockpile was minimal compared to the reference soil— indicating a lack of available nutrients, likely due to a lack of root contact. We expect that this is the main driver of decreased fungal presence because the decreased F:B ratio occurs below 45 cm, where nutrient inputs from plant roots is very limited. We also found however that the F:B ratio was the highest and had an identical mean to the reference in the deepest depth (Fig1.2 B). This may be due to hyphae growing upwards into the stockpile from below, having found deposits of organic matter from roots and vegetation mixed up into the pile during the initial stockpile creation. Owing to the fact that hyphae under the stockpile prior to disturbance was never physically ripped.

We found that arbuscular mycorrhizal fungi were the most important driver in the PCA linking the top most portion of the stockpile with the reference soil (Fig 1.4). Arbuscular mycorrhizal fungi are an important symbiont for mixed-grass prairies, providing increased root surface area and therefore helping the plant capture nutrients— most importantly phosphorous (Barea, 1991; Lin et al., 2015). However, their filamentous growth structure (hyphae), are extremely sensitive to physical damage, and cannot survive long periods of time without a root host and are therefore likely damaged by stockpiling. Many invasive grass species do not require arbuscular mycorrhizal fungi to establish (Wilson et al., 2012). With stockpiled soils negatively affecting the abundance of arbuscular mycorrhizal fungi, when stockpiled topsoils are re-spread during reclamation, invasive plant species may hold a competitive advantage compared to native grasses which in the great plains region usually require arbuscular mycorrhizal fungi to live (Jordan et al., 2011).

Management strategies that aim to increase soil function, stability, native vegetation stands and overall ecosystem health should be the main focus in post-reclamation. We can conclude from this study that stockpiling negatively affects soil microbial abundance, and causes distinct shifts in the microbial community composition. In addition, the vegetative seed bank is negatively affected by stockpiling. If the purpose of stockpiling is to retain viable topsoil in order to have successful reclamation when this soil is

re-spread, this study demonstrates that stockpiled topsoil more closely resembles subsoil and may not support a successful, fully restored ecosystem. We recommend only mandatory use of stockpiling, and to limit the height of these piles in order to retain maximum root-to-soil contact. Stockpiled soils used during reclamation should be amended with organic matter in order to facilitate recovery of the decreased biological activity, chemical variability, and physical destruction caused by stockpiling. Future research is needed to determine the recovery time of the soil microbial community on reclaimed areas using stockpiled soil.

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CHAPTER 2: MICROBIAL BIOMASS AND LABILE CARBON IN RECLAIMED COAL SURFACE MINES IN A MIXED-GRASS PRAIRIE OVER A 42-YEAR-CHRONOSEQUENCE

Abstract

Disturbed soils are those that have experienced alterations to the soil's physical, chemical, and biological components. Soils found on reclaimed mine sites are degraded, often characterized with high levels of compaction, limited water infiltration, and reduced aggregation— factors that affect the establishment and often the diversity of the vegetative community during reclamation. The rate of transformations, pathways, and fate of plant litter inputs is primarily controlled by the composition and activity of the microbial community. In this study, we assessed soil microbial abundance measured via microbial biomass carbon, labile carbon pools, edaphic properties and vegetation in order to quantify the recovery of reclaimed surface mine soils along a chronosequence spanning 42 years (1975-2017). We found that plant composition was different in reclaimed sites compared to the reference with vegetation in older reclaimed sites dominated by exotic grasses with limited native grasses or forb species. We also found that disturbance affected all soil properties at every treatment (years since reclamation) and that all soil carbon pools measured were significantly ($p < 0.005$) lower than that of the reference site, with microbial biomass carbon of the oldest reclaimed year (329 mg C/ kg soil) containing almost half the microbial biomass carbon of the reference soil (647 mg C/ kg soil). Due to the fact that all soil properties measured were not similar to the reference site, it appears that without a change in management or soil handling practices during the mining and reclamation process, full ecosystem recovery is hindered. We recommend the incorporation of belowground monitoring during the post reclamation, pre-bond release phase in order to better facilitate recovery of reclaimed disturbed land.

Introduction

Soil disturbance results in widespread biogeochemical changes, altering the soil's physical, chemical, and biological components (Griffiths et al., 2004; Tischer et al., 2015). Large-scale disturbances (i.e., surface mining) result in rapid change in soil properties, including a decrease in soil organic matter (SOM) (Mummey et al., 2002) and destruction of aggregates (Wick et al. 2008)—potentially reducing a soil's ability to store and cycle nutrients (Ingram et al. 2005). Recovery of the soil is imperative for full

ecosystem recovery as it provides the foundation for the ecosystem and affects successful plant community recovery. Therefore, it is important to monitor and understand the linkages between plant-soil biota in reclaimed soils and provide a more robust assessment of reclamation success and ecosystem recovery.

Surface mining is a common anthropogenic disturbance that results in severe disruption of ecosystem properties. After the desired resources have been removed, mine companies are required to reclaim disturbed land (OSM, 1977). One of the primary goals of reclamation is to return land to pre-mining conditions (OSM, 1977). When pre-mining conditions are not documented, or post-mining land use differs from pre-mining land use, characteristics of an appropriate reference site are used as a basis for reclamation success. Although reclaimed land often meets aboveground expectations (i.e. established plant community and reconstructed topography), this does not mean the ecosystem has recovered, particularly for belowground components (Bohrer et al., 2017). The lack of belowground monitoring, in particular soil chemical and biological variables, limits our ability to detect ecosystem recovery, due to the role these characteristics play in establishing and maintaining a productive and diverse biological community (Jacoby et al., 2017).

Recovery of a severely disturbed ecosystem depends on the recovery of belowground properties, i.e. the physical, chemical, and biological aspects of the soil (Rastetter et al., 2013). Recovery of soil microbial communities are of particular importance because they influence and shape plant community structure (Reynolds et al., 2003), and mediate most soil processes that affect nutrient availability, plant health, and primary production (Brussard et al. 1997, Van Der Heijden et al. 2008). Measuring and management of SOM is a critical component of monitoring terrestrial ecosystem recovery after disturbance (Akala et al., 2001; Liu et al., 2017). However, accumulation of SOM in soil is slow, on the order of decades to centuries, and measurable shifts in SOM in response to management are difficult to detect (Awale et al., 2017; Ussiri et al., 2005). In contrast, active, labile carbon pools—such as microbial biomass carbon (MBC) and permanganate oxidizable carbon (POXC) fractions—are sensitive to changes in soil condition and act as indicators of overall soil health (Anderson et al., 2008; Awale, 2017; Hurisso et al., 2016; Yang et al., 2012).

Soil MBC is a measurement used to estimate the carbon content of microorganisms in the soil, and is often considered a component of the total SOM pool on account of the fast turnover time of microbial cells. Microbial biomass carbon is a common metric when evaluating soil health—particularly in disturbed systems such as tilled agriculture fields (Morrow et al., 2016), and offers insight in understanding soil biotic activity and soil fertility. Permanganate oxidizable carbon is an easy and cheap method used to quantify the labile fraction of the SOM pool and represents a fraction of carbon that is most readily degradable by microorganisms (within days to years) (Culman et al., 2012; Wang et al., 2017). Moreover, POXC has been shown to have a significant positive relationship with MBC, and is sensitive to changes in management or environmental variation (Hurisso et al., 2016). Meaning, in the context of reclamation, POXC may have the ability to give insight into soil biochemical recovery.

Reclaimed mine sites offer a unique opportunity to study belowground responses to drastic disturbances, resulting in a surge of carbon-related mine reclamation studies in the last two decades (Das and Maiti, 2016; Frouz, 2013; Yuan et al., 2017, 2018). A majority of these studies measure organic carbon via the active pool (microbial biomass carbon, dissolved organic carbon, respiration, etc.) due to the difficulty of measuring and interpreting the conglomerate of various carbon compounds that make up the passive, stable carbon pool (i.e. humic materials). Reports of MBC recovery to pre-disturbance or reference levels after disturbance and reclamation vary greatly, ranging from five (Clayton et al., 2009) to 50 years. Surprisingly, some reclaimed mine soils never reach MBC quantities found in undisturbed reference soils (Insam and Domsch, 1988; Mummey, 2002).

To quantify the recovery of reclaimed surface mine soils, we propose inclusion of more belowground characteristics than are currently considered in reclamation practice. In particular, we expect that indicators associated with active carbon pools will contribute to our ability to estimate ecosystem recovery. It is important to provide a more robust assessment of reclamation success and overall ecosystem recovery. The goal of this study is to assess belowground changes in labile carbon pools (as measured by MBC and POXC) along with other edaphic and vegetation properties, to evaluate and monitor the recovery of soil quality in reclaimed mine soils over time in comparison to a reference site. We hypothesize that older reclaimed sites will more closely resemble the reference site in terms of microbial activity, carbon content, and edaphic properties than more recently reclaimed sites.

Methods

Study site description

We sampled soil and vegetation on fifteen reclaimed sites at BNI Coal in central North Dakota (47°6′54″N 101°18′1″W) across a 6400-hectare area. Mined strips differed in time-since-reclamation, collectively providing a reclamation chronosequence spanning 42 years (1975-2017). We also sampled an intact native prairie as a reference site. The reference site is classified as a loamy ecological site (USDA NRCS, 2004) and situated on the same ecological sites as reclaimed locations (054-Rolling Soft Shale Plain). The soils at all sites developed on siltstone or sandstone, with loamy to silty-clay loam textures dominated by the Williams (Typic Argiustolls) and Cabba (Typic Ustorthents) soil series (NRCS, 2017). In general, the soils in this area are deep, well-drained to moderately well-drained, with a friable loam subsoil and carbonate accumulation at depth (NRCS, 1975). Seeding takes place after topsoil has been re-spread, sourced from local suppliers and seeded at 45 kg/ha and included two C3 grass species: green needlegrass (*Nassella viridula*) at 25% western wheatgrass (*Pascopyrum smithii*) at 10%, and five C4 species: switchgrass (*Panicum virgatum*) at 20%, bluegrama grass (*Bouteloua gracilis*) at 15% and sideoats grama (*Bouteloua curtipendula*) at 15%, little bluestem (*Schizachyrium scoparium*) at 10%, and big bluestem (*Andropogon gerardii*) at 5%. Forbs were not used in the seed mix. Following plant establishment, post reclamation management strategies were a combination of season-long cattle grazing and haying. The sites are located in a northern mixed-grass prairie under management for livestock grazing. The area has a continental climate regime, with an annual mean temperature of 6.7° C and precipitation of 406.4 mm. Minimum temperatures occur in January averaging -16.7° C and maximum temperatures in July average 22.8° C from 1991-2017 (NDAWN, 2017). The 2017 growing season received below-average precipitation (235.3 mm) with a record-setting highest rainfall event accounting for 33% of the entire growing season's rain in one day (2017-08-12) (NDAWN, 2017).

Vegetation survey methods

We established one 120-meter transect for the basis of vegetation and soil sampling within each reclaimed strip. On each reclaimed strip, we surveyed vegetative cover and soil surface characteristics using a modified Daubenmire classification system (Daubenmire 1959). Vegetation was surveyed using a ¼ m x ¼ m frame at 10 m increments along the 120 m transect. We surveyed vegetation during the

growing season, however it should be noted the summer of 2017 had extreme drought conditions (NDAWN, 2017). In addition to assessing living vegetation, we documented annual litter and bare ground cover.

Soil sampling methods

We collected soil samples at the same time as vegetation surveys at 10 m increments along the length of the 120 m transect and combined six soil samples taken within a ¼ m x ¼ m area using a bucket auger to 0-15 cm depth — providing a total of 12 samples for every transect (reclaimed strip). We removed vegetation prior to soil sampling in order to exclude litter from samples. We also collected one volumetric sample at each quadrat using a slide-hammer corer (AMS, Inc., 5 cm diameter) to 15 cm depth. We immediately placed a portion of the composite sample intended for MBC on ice and transported to a -20C° freezer. Soils were air-dried, ground and sieved to 2-mm prior to processing for chemical and physical analysis.

Soil analysis

We estimated microbial biomass carbon using the chloroform-fumigation extraction method (Beck et al., 1997; Joergensen, 1996; Vance et al., 1987) using 30 g thawed samples. We assessed permanganate oxidizable carbon following Weil et al. (2003). We determined soil pH and electrical conductivity in a 1:1 soil:water slurry with an Oakton pH meter and an Orion Star A112 conductivity meter (Rhoades, 1996; Thomas, 1996). We used intact volumetric cores to determine bulk density of volumetric samples dried at 105°C (Blake and Hartge, 1986). We used the pressure plate method (Richards and Fireman, 1943) at 1/3 bar to determine field capacity. We analyzed total carbon and inorganic carbon using a Skalar Formacs total organic carbon high temperature catalytic combustion analyzer. We calculated soil organic carbon as the difference between the two pools.

Statistical analysis

We treated each reclaimed strip and the reference site as independent treatments in the analysis. We calculated descriptive statistics for soil and vegetation properties within treatments. Non-parametric means comparison between each treatment mean and the reference mean were determined for each property using a Mann-Whitney U test. Linear discriminant analysis (LDA) is a classification method for multivariate data sets and was used in order to assess how all soil and vegetation properties collectively

varied across treatments. For the LDA, vegetation was grouped into “bare ground,” “leaf litter,” and “combined cover” categories in order to maximize explanatory vegetative variables. We employed LDA to see how reclamation treatments separate from the reference site, and each other, based on our explanatory variables. We executed all data analysis in R (R Core Team, 2018) using the ‘vegan’ (Oksanen et al., 2017), ‘pastecs’ (Grosjean and Ibanez, 2014), ‘plyr’ (Wickham, 2011), ‘MASS’ (Venables and Ripley, 2002), ‘RColorBrewer’ (Neuwirth, 2014), ‘fields’ (Nychka et al., 2015), ‘scales’ (Wickham, 2014), and ‘ggord’ (Beck and Mikryukov, 2018) packages for data manipulation, analysis, and visualizations.

Results

Plant composition was different in reclaimed sites compared to the reference (Fig 2.1 & Appendix Table 2). Older reclaimed sites showed an increase in relative cover of exotic species compared to younger reclaimed sites (Fig 2.1). There was a decrease in native grasses about seven years after reclamation, with 2014 having the highest relative cover of native grass followed by a sharp decline of native grasses in 2010. The reference site had more than seven times more native-forb cover compared to the average of all reclamation years. Yellow sweet clover (*Melilotus officinalis*) and alfalfa (*Medicago sativa*) were the dominant exotic forbs found in reclaimed sites. Bare ground dominated the most recently reclaimed sites (2015, 2016, 2017), with 78, 95, and 97% bare ground respectively. All sites including the reference were dominated by leaf litter four years after reclamation, ranging from 23-76% across all sites.

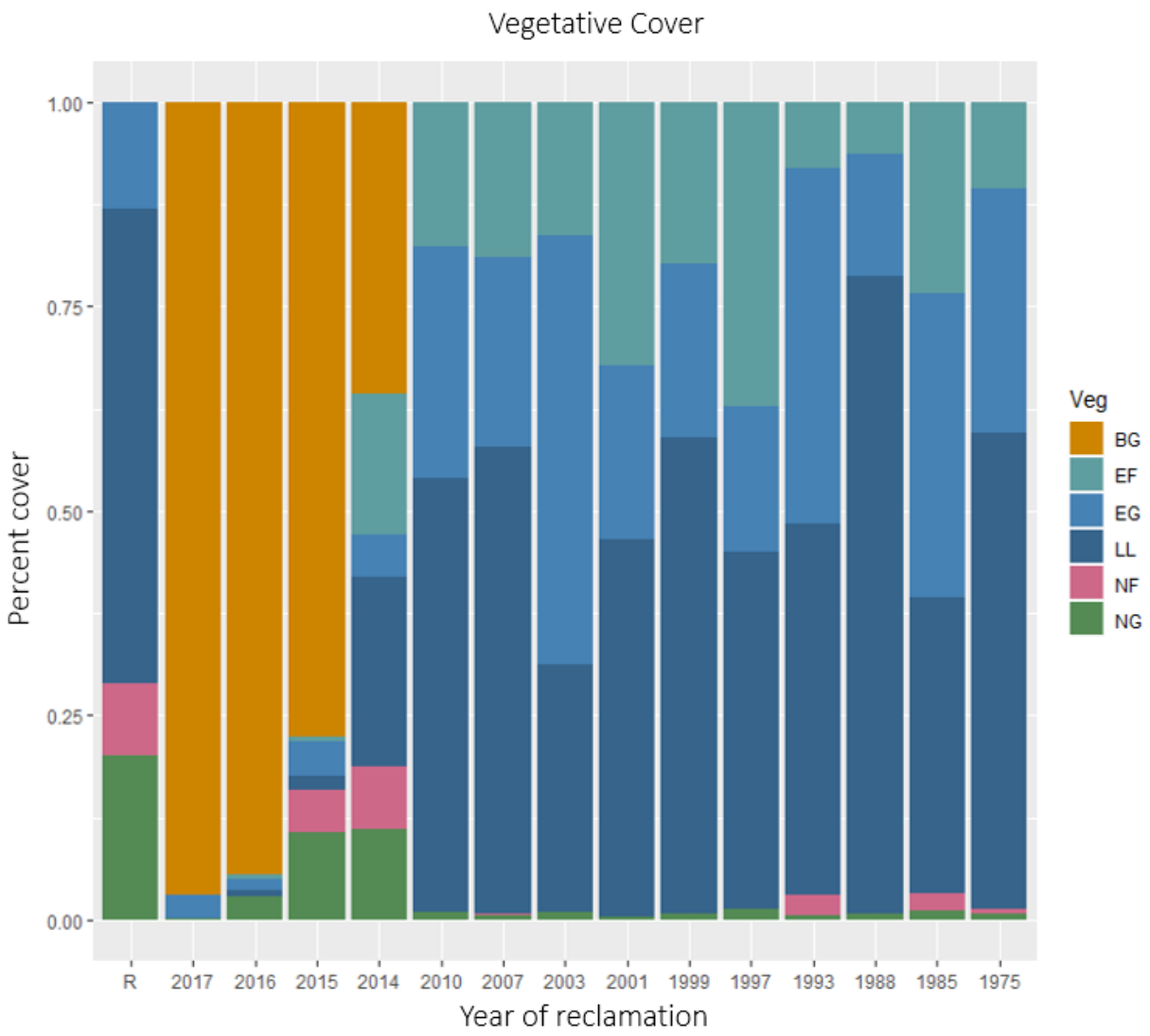

Figure 2.1. Mean proportion of vegetation and surface cover across reclamation treatments. Vegetation was surveyed using a ¼ m x ¼ m frame at 10 m increments along the 120 m transect across each reclamation year along with the reference site. Bare ground dominates reclaimed sites during initial years, and exotic grass dominates the older reclaimed years. BG- bare ground, EF- exotic forb, EG- exotic grass, LL- leaf litter, NF- native forb, NG- native grass. Vegetation cover surveyed during summer of 2017 from BNI Coal mine near Center, ND, USA.

Compared to the reference site, all reclaimed soils had higher ($p<0.005$) pH and BD, and most reclaimed soils had higher EC and inorganic carbon (Fig 2.2). The mean pH of the reference site was 6.6, compared to 1975 which was 7.9 indicating a clear change in soil chemistry. Recently reclaimed soils also displayed higher pH values. Similarly, reclaimed soils consistently contained more conductive ions (as measured by EC), including carbonates (IC), which likely contributed to the observed pH values. While variable, the BD was also consistently higher in reclaimed soils compared to the reference, ranging

from 21 – 53% higher than the mean reference BD (Table 2.1). Soil moisture was generally very low in all soils. Field capacity was not different between sites or between each treatment and the reference site.

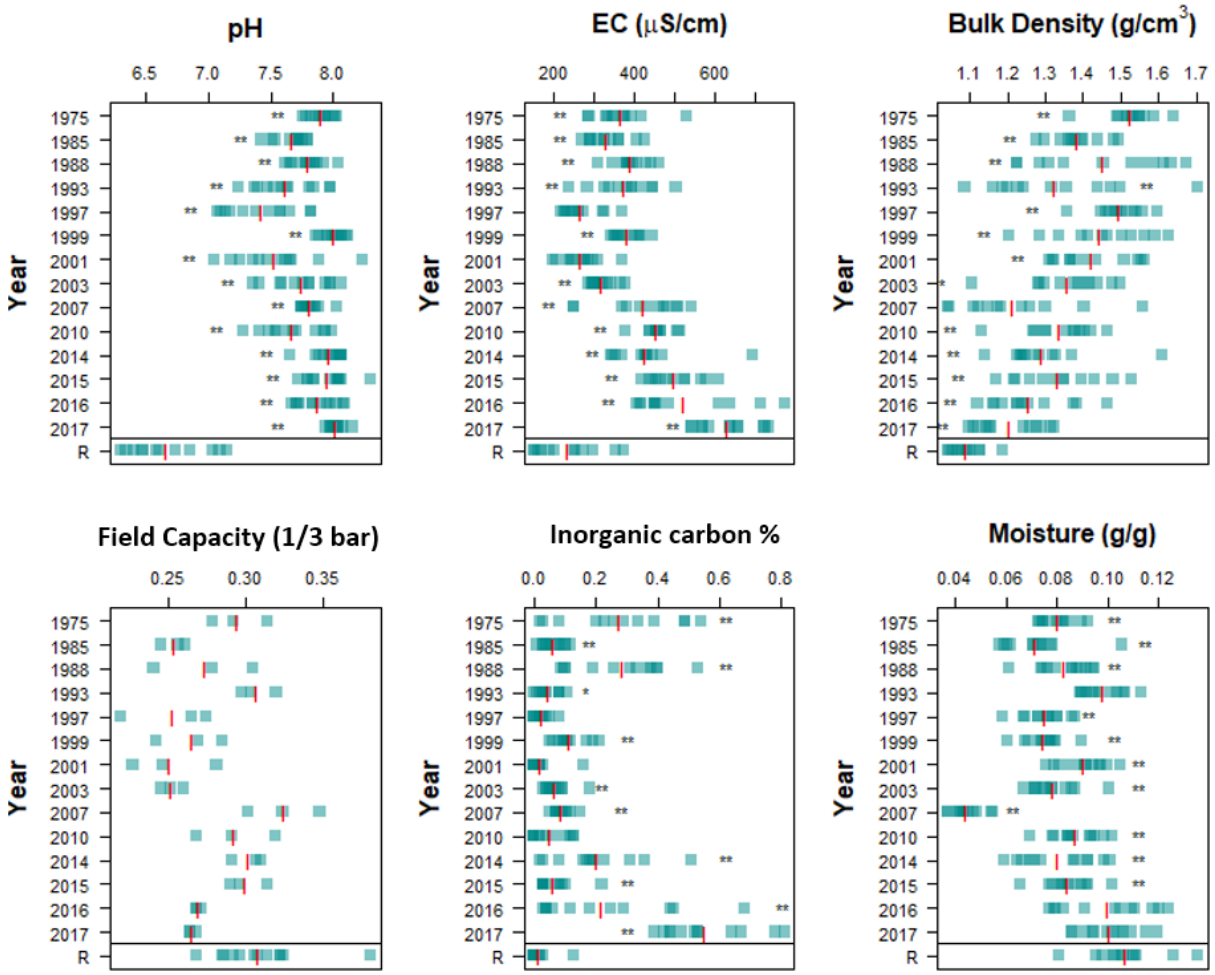

Figure 2.2. Strip charts with treatment mean (red line) and statistical significance level ($p<0.05$= *, $p<0.005$ = **) of edaphic properties at each year compared to the reference site, showing that pH and bulk density were the most negatively affected by the mining and reclamation process. Soil samples taken during summer of 2017 from BNI Coal mine near Center, ND, USA.

All soil organic carbon pools in all reclaimed soils were less ($p<0.005$) than the reference pools (Fig 2.3). Microbial biomass carbon was consistently less in reclaimed soils compared to the reference, ranging from 27 to 77% less than the mean reference (Appendix B, Table 3). Similarly, POXC and soil organic carbon ranged 36 to 75% and 34 to 56% respectively less than the reference site. Labile carbon, measured via POXC, was not higher in soils that had longer recovery times. The oldest two sites had

similar levels of POXC, with a mean of 471 mg C kg^{-1} soil for the 1975 treatment and a mean of 470 mg C kg^{-1} soil for the 1985 treatment (Table 2.1) compared to the reference site mean of 1100 mg C kg^{-1} soil. Soil organic carbon in the oldest site had values roughly half (2.5 %) of the reference site (4.0 %).

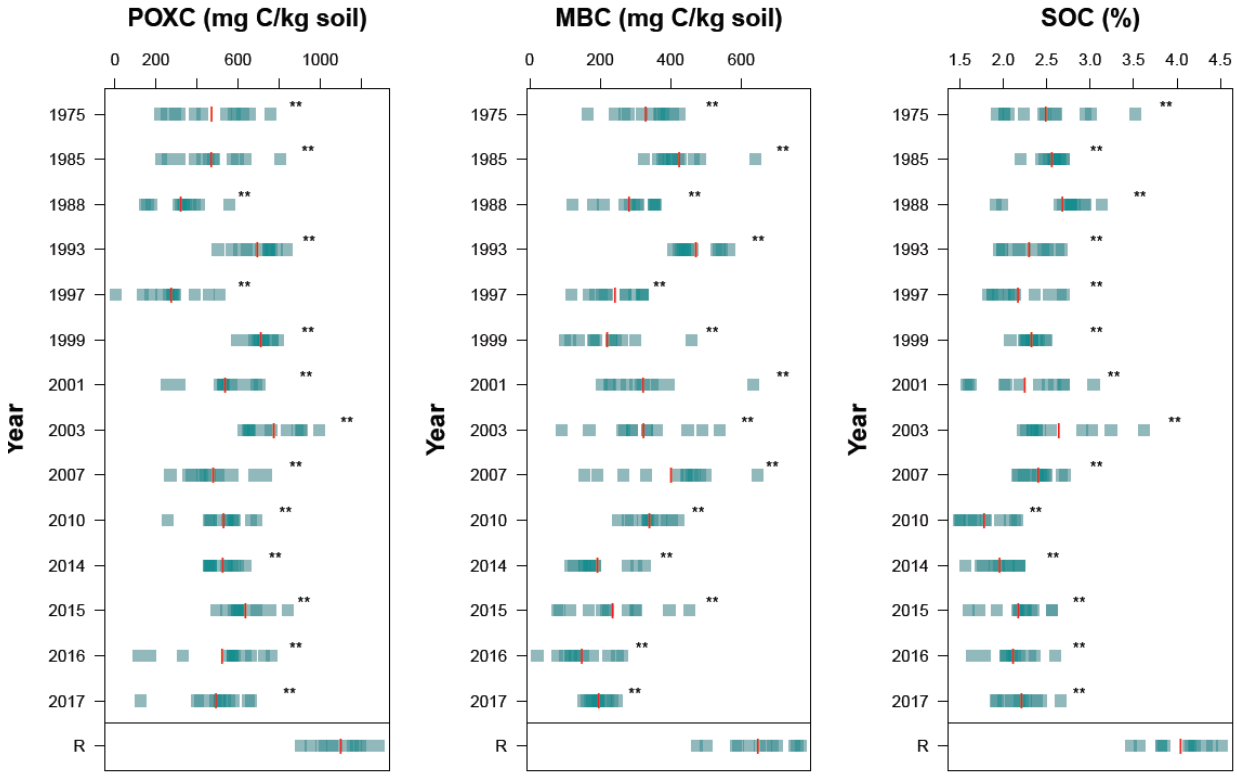

Figure 2.3. Strip charts with treatment mean (red line) and statistical significance level ($p<0.005$ = **) of soil carbon properties of each year compared to the reference site. All carbon pools are lower in reclaimed years. Decreased rhizosphere and microbial habitat are likely the largest contributors to the smaller C pools observed in this study. POXC – permanganate oxidizable carbon, MBC – microbial biomass carbon, SOC – soil organic carbon, R – reference site. Soil samples taken during summer of 2017 from BNI Coal mine near Center, ND, USA.

Table 2.1. Soil carbon pool means ± standard deviation in each treatment. Reclaimed strips sampled during the summer of 2017 from BNI Coal mine near Center, ND, USA. POXC – permanganate oxidizable carbon, MBC – microbial biomass carbon, SOC – soil organic carbon, R – reference site

Year	POXC	MBC	SOC
1975	471 ± 178	329 ± 77	2.49 ± 0.49
1985	470 ± 167	424 ± 81	2.56 ± 0.14
1988	320 ± 117	282 ± 76	2.68 ± 0.37
1993	694 ± 100	472 ± 56	2.3 ± 0.26
1997	274 ± 138	242 ± 67	2.17 ± 0.31
1999	711 ± 55	220 ± 96	2.33 ± 0.12
2001	536 ± 137	322 ± 115	2.25 ± 0.49
2003	774 ± 128	323 ± 127	2.64 ± 0.45
2007	479 ± 133	401 ± 140	2.4 ± 0.18
2010	529 ± 113	340 ± 52	1.78 ± 0.25
2014	525 ± 63	193 ± 70	1.96 ± 0.2
2015	636 ± 96	235 ± 117	2.17 ± 0.3
2016	522 ± 208	148 ± 71	2.11 ± 0.25
2017	492 ± 140	196 ± 29	2.21 ± 0.22
R	1100 ± 116	647 ± 96	4.04 ± 0.33

The combined linear discriminant analysis explained 92.6% of the variability in multivariate properties across treatments with 72, 15 and 5% of the variability explained via LD1, LD2, and LD3 functions respectively. Vegetative cover (leaf litter, combined cover) and lack-there-of (bare ground) provided the greatest separation between treatments, and these properties were highly correlated with LD1 (Fig 2.4). Bare ground had the strongest negative correlation out of all properties, (-0.99) with LD1 (Table 2.2). Bare ground and leaf litter were able to separate the most recently reclaimed sites from each other, with 2014 being somewhat of a transition year. Inorganic carbon (IC) and EC were also negatively correlated with LD1. IC and EC were both higher in the younger reclamation years, with 2017 having the largest values. Soil physical and physiochemical properties were important separating factors between reclamation sites and the reference site on LD2. The bulk density and pH were both positively correlated with LD2, indicating their higher values in reclaimed soils compared to the reference soil (Table 2.2). The carbon pools (POXC, MBC, and SOC) all had strong negative correlations (-0.72, -0.58, and -0.76 respectively) with the same discriminant function (LD2), reflecting their low abundance in reclaimed soils relative to the reference soil. The third discriminant function (LD3) contributed only slightly more explanatory power, and it was negatively correlated with IC.

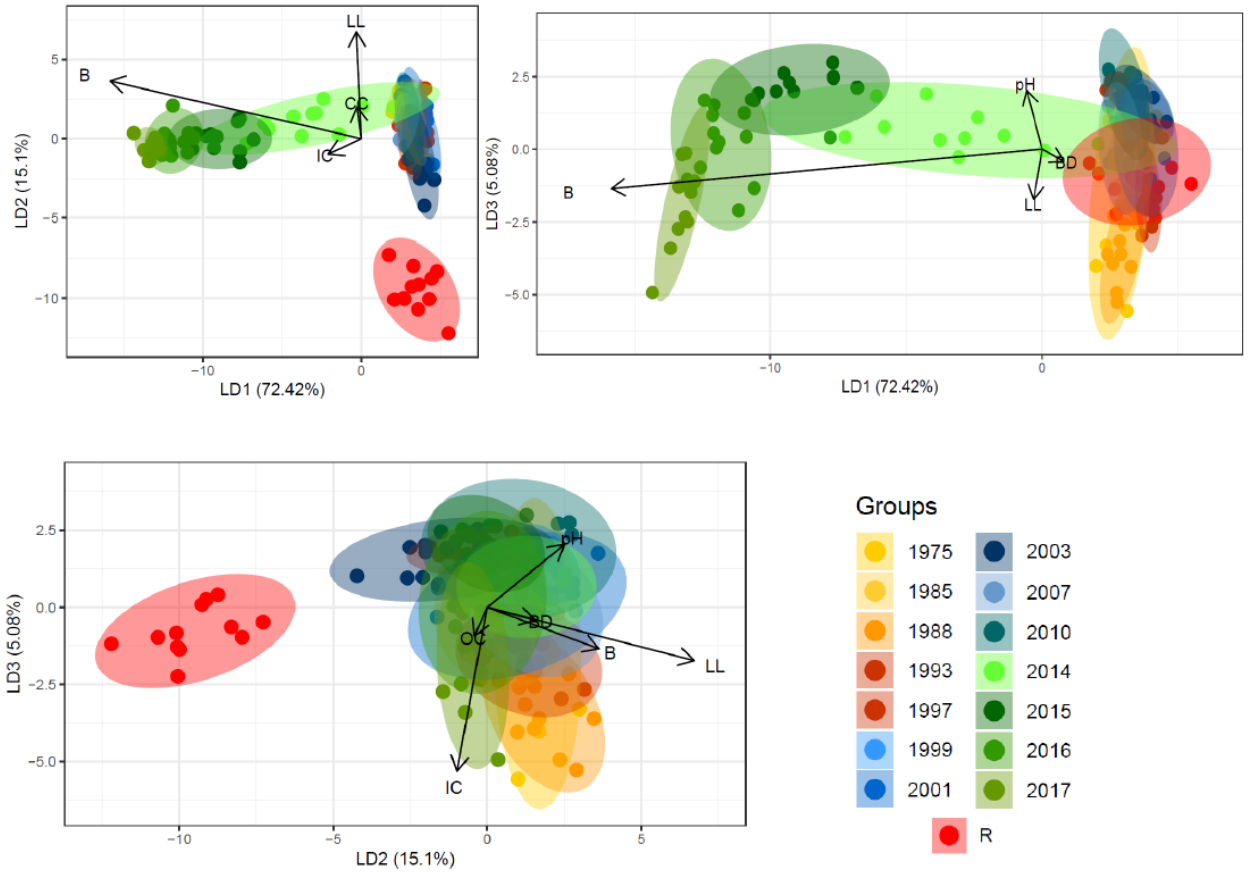

Figure 2.4. Linear discriminant biplots, including the first three discriminant functions (LD1, LD2, and LD3). Ellipses represent confidence intervals around the mean of each group (alpha=0.05). (BD- bulk density, IC – inorganic carbon, OC—soil organic carbon, B- bare, LL- leaf litter, CC- combined cover). Reclaimed strips sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

Table 2.2. Correlations between properties and linear discriminant functions (axes). Bare ground had the strongest negative correlation out of all properties with LD1. (POXC – permanganate oxidizable carbon, MBC—microbial biomass carbon). Correlations greater than ± 0.50 are bold. Reclaimed strips sampled during the summer of 2017 from BNI Coal mine near Center, ND, USA.

Explanatory variable	LD1 – 72.42%	LD2 – 15.1%	LD3 – 5.08%
pH	-0.39	**0.74**	0.14
Electrical conductivity	**-0.71**	0.27	0.08
Bulk density	0.28	**0.54**	-0.17
Inorganic carbon	**-0.51**	0.18	**-0.51**
POXC	0.08	**-0.72**	0.44
MBC	0.48	**-0.59**	0.03
Soil organic carbon	0.27	**-0.76**	-0.31
Bare ground	**-0.99**	-0.08	0.01
Leaf litter	**0.62**	**0.52**	-0.18
Combined cover	**0.57**	0.13	0.13

Discussion

It is important to monitor changes in disturbed soil as ecosystems recover, in order to facilitate full ecosystem recovery. We found disturbance affected all soil properties at all stages of recovery over a 42-year time-since-reclamation chronosequence. We found microbial biomass carbon in disturbed soil was consistently less than the reference site. Moreover, the soil of the oldest reclaimed year contains almost half the microbial biomass carbon of the reference soil. Microbial necromass is a major contributor to stable SOM (Kallenbach et al., 2015), and our results indicate a lack of recovery of all carbon pools (MBC, POXC, SOC) measured over a 42-year time span. SOM may require decades to centuries to recover to reference levels with limited microbial necromass entering the soil. Lack of recovery of the soil microbial community can lead to several issues, most importantly perhaps being the disruption of enzymatic processes, produced by microbes (Schimel et al., 2012; Tischer et al., 2015). Without these enzymes, soil functionality may be reduced or slowed resulting in limited ecosystem recovery.

We found soil organic carbon in reclaimed soils was consistently lower ($p < 0.005$) compared to the reference site, the mean soil organic carbon of all reclaimed sites ranged from 1.78-2.68% compared to the reference site 4.04%. Low soil organic carbon is likely due to low MBC, and limited diverse vegetation (Lange et al., 2015). Our results demonstrate that despite regulations of separation between top and subsoil, effects of the mining and reclamation process reduced soil organic carbon in reclaimed soils. Soil mixing within horizons results in disruption of aggregate-protected SOM, allowing SOM mineralization to increase (Ingram, 2005; Rustad et al., 2001; Williamson et al., 1990). This has been found in other mine reclamation studies (Ussiri et al., 2005), along with other highly disruptive soil disturbances (i.e. tillage). The lack of increase in all carbon parameters measured, and in particular soil organic carbon is an indicator that SOM is not on its way to recovery within a reasonable time frame without management intervention.

We found that POXC of the oldest reclaimed site (471 mg C/kg soil) was less than half that of the reference site (1100 mg C/kg soil). POXC is part of the labile carbon pool and reflective of management changes—meaning it shows change more quickly than the total SOC pool (Culman et al., 2012). Compared to soil organic carbon, POXC has shown to be a sensitive indicator of changes in the labile carbon pool (Haynes, 2005). The fact that POXC did not increase with time-since-reclamation implies that

without a change in management, POXC will likely stay low. Low POXC may be due to lack of diverse vegetation combined with heavily compacted soils that do not allow for as much carbon to be exuded via varied rooting depths (Lange et al., 2015).

Vegetation in older reclaimed sites was dominated by exotic grasses and showed limited native grasses or forb species. These results are similar to other reclamation studies (Bauman et al., 2015) that found limited native vegetative recruitment post reclamation (Chambers et al., 1994). Kentucky bluegrass, a shallow-rooted grass, was the most abundant exotic grass species across all reclaimed sites. The vegetation found on reclaimed sites in this study indicates that lack of root penetration and diverse rooting depths may be directly affecting soil microbial activity and potentially microbial diversity (Steinauer et al., 2016). Diversity of root-exudates influences the community composition of soil microbes and our study indicates a probable link between homogeneity of vegetation and limited microbial activity. Moreover, compaction can exacerbate shallow rooting depths by increasing resistance to root penetration and lead to even less nutrient availability via decreased root biomass (Panayiotopoulous et al., 1994).

In this study, bulk density is not only higher in all reclaimed soils ($p < 0.005$) but also highest in the oldest reclaimed area indicating that time alone is not alleviating compaction. Previous research at these sites also found no improvement of compaction measured via root penetrometer resistance (Bohrer et al., 2017). Due to the relatively fine particle size of soils in the region of this study, they are particularly prone to compaction because smaller particles are able to fill in pore space—as observed in this study via high levels of bulk density. Compaction is an inevitable part of both the mining and reclamation practice. However, compaction is significantly increased by repeated traffic via heavy machinery specified by the need to grade soil to a pre-defined slope (OSM, 1977). The benefit of decreasing compaction outweighs the need for grade and slope specification in regard to soil quality. However, in terms of watershed processes (erosion, water retention, sub-surface flow) this might not always be the case. Implications of high levels of BD are well established (Simojoki et al., 1991; Taylor et al., 1991), with limitations to water movement, and therefore, nutrient access being some of the most detrimental consequences. Limited gas and nutrient exchange in soil has been shown to reduce turnover of microbial biomass (Breland et al., 1996), and because compacted soils have less porosity, and therefore, less rhizospheric volume – the

microbial habitat is decreased. Decreased rhizosphere and microbial habitat are likely the largest contributors to the smaller C pools observed in this study.

The goal of reclamation is to return an ecosystem to pre-disturbance productivity levels. This study found that at this particular location, soil organic carbon is roughly half that compared to reference soil carbon levels even 42 years post-reclamation. We recommend the incorporation of belowground monitoring during the post reclamation, pre-bond release phase. Agronomic systems have demonstrated the capability of POXC as an inexpensive and informative tool (Hurisso, 2016); mines could incorporate this measurement into their monitoring in order to measure soil health and make management decisions based off the results. For example, if low POXC levels were found in year three post-reclamation, mines could change the management strategy of that area to increase vegetation (i.e. native seeding after mulching, manure spreading, mycorrhizal fungi inoculation) (Hurisso et al., 2018; Nadeem et al., 2014). Our results also demonstrate that incorporating more robust soil monitoring (pH, electrical conductivity, inorganic carbon, POXC, microbial biomass carbon, soil organic carbon, bulk density) into the post-reclamation monitoring program will be indicative of change because this study found that almost all soil measurements of reclaimed sites were different than that of the reference site.

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APPENDIX A. APPENDIX MATERIAL FOR CHAPTER 1

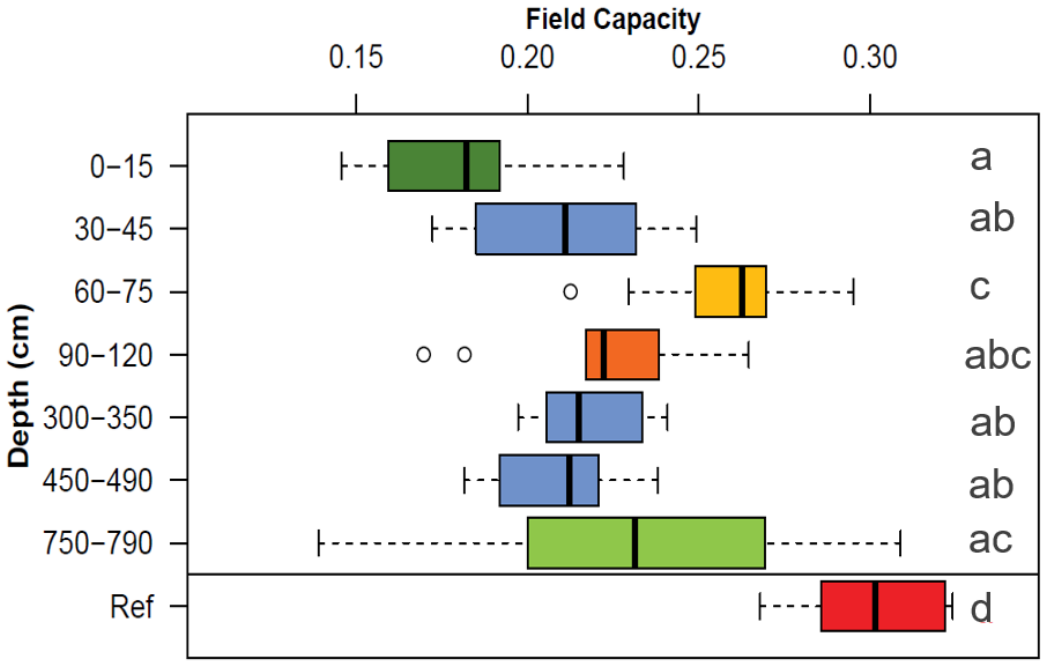

Figure A1. Box-and-whisker plots of field capacity across depths. Colors and letters indicate significant differences ($p < 0.05$) according to ANOVA with Tukey's posthoc HSD test. Stockpile sampled during August of 2017 from BNI Coal mine near Center, ND, USA.

APPENDIX B. APPENDIX MATERIAL FOR CHAPTER 2

Table B1. Mean percent cover using a ¼ x ¼ m quadrat for each reclamation year and reference site, categorized by vegetative groupings in order to maximize explanatory vegetative variables. Reclaimed strips sampled during growing season of 2017 from BNI Coal mine near Center, ND, USA.

Year	Exotic Grass	Exotic Forb	Native Grass	Native Forb	Bare Ground	Leaf Litter
1975	29.8	10.7	0.8	0.6	0.0	58.5
1985	37.3	23.3	1.1	2.1	0.0	36.2
1988	16.9	6.4	0.7	0.0	0.0	76.3
1993	43.4	8.0	0.5	2.6	0.0	45.5
1997	19.4	35.1	1.3	0.0	0.0	44.6
1999	22.8	19.6	0.8	0.0	0.0	57.1
2001	21.3	32.2	0.4	0.0	0.0	46.3
2003	52.7	16.3	0.9	0.0	0.0	30.3
2007	23.2	19.0	0.5	0.3	0.0	57.2
2010	28.4	17.8	0.9	0.0	0.0	53.4
2014	5.3	17.2	11.2	7.6	35.7	23.2
2015	4.2	0.6	10.8	5.1	77.7	1.7
2016	1.4	0.6	2.9	0.0	94.5	0.7
2017	3.0	0.0	0.1	0.0	96.9	0.0
R	13.1	0.0	22.1	9.4	0.0	62.0

Table B2. Non-parametric means comparison using the Mann-Whitney U test. Calculated p-values are comparing each reclamation year vegetative groupings to the reference site. Reclaimed strips sampled during growing season of 2017 from BNI Coal mine near Center, ND, USA.

Year	Exotic Grass	Exotic Forb	Native Grass	Native Forb	Bare Ground	Leaf Litter	Combined Cover
1975	<0.001	<0.001	<0.001	0.001	NA	0.93	0.33
1985	<0.001	<0.001	<0.001	0.011	NA	0.69	0.15
1988	0.34	<0.001	<0.001	<0.001	NA	0.033	0.01
1993	<0.001	0.003	<0.001	0.016	NA	0.01	0.005
1997	0.04	<0.001	<0.001	<0.001	NA	0.046	0.06
1999	0.01	<0.001	<0.001	<0.001	NA	0.39	0.183
2001	0.04	<0.001	<0.001	<0.001	NA	0.04	0.009
2003	<0.001	<0.001	<0.001	<0.001	NA	0.004	0.30
2007	0.01	<0.001	<0.001	<0.001	NA	0.18	0.032
2010	<0.001	<0.001	<0.001	<0.001	NA	0.30	0.34
2014	0.06	<0.001	0.02	0.28	0.004	<0.001	0.37
2015	0.01	0.36	0.02	0.16	0.0005	<0.001	0.01
2016	<0.001	0.17	<0.001	<0.001	0.002	<0.001	<0.001
2017	<0.001	NA	<0.001	NA	0.002	NA	<0.001

Table B3. Percent variance between each variable per year against the reference. (EC – electrical conductivity, BD – bulk density, IC – inorganic carbon, POXC – permanganate oxidizable carbon, MBC – microbial biomass carbon, SOC – soil organic carbon). Reclaimed strips sampled during growing season of 2017 from BNI Coal mine near Center, ND, USA.

	% Variance							
Year	pH	EC	BD	IC	Moisture	POXC	MBC	SOC
1975	18.55	55.99	40.07	1498.10	24.88	-57.17	-49.13	-38.37
1985	14.97	41.70	26.93	252.38	32.95	-57.28	-34.46	-36.69
1988	16.95	66.67	33.27	1547.62	22.62	-70.88	-56.43	-33.66
1993	14.28	60.52	15.70	161.91	8.45	-36.90	-27.11	-43.12
1997	11.31	13.90	37.04	42.86	29.76	-75.05	-62.55	-46.28
1999	20.06	62.85	32.65	566.67	30.00	-35.36	-66.08	-42.46
2001	12.92	13.94	30.45	38.10	15.46	-51.22	-50.28	-44.36
2003	16.17	36.37	24.63	300.00	26.32	-29.62	-50.12	-34.65
2007	17.25	79.73	11.41	414.29	58.23	-56.41	-38.05	-40.52
2010	14.98	94.37	22.70	195.24	18.39	-51.93	-47.51	-55.93
2014	19.46	82.73	18.46	1057.14	25.04	-52.25	-70.26	-51.56
2015	19.33	112.83	22.34	276.19	21.11	-42.17	-63.70	-46.26
2016	18.10	122.17	15.33	1147.62	6.61	-52.54	-77.21	-47.70
2017	20.26	169.07	10.65	3066.67	5.91	-55.29	-69.67	-45.33