RECOVERY OF CARBON AND NITROGEN CYCLING AND MICROBIAL COMMUNITY FUNCTIONALITY IN A POST- LIGNITE MINING REHABILITATION CHRONOSEQUENCE IN EAST TEXAS

A Dissertation

by

JUSTIN PARK HO NG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2012

Major Subject: Soil Science

Recovery of Carbon and Nitrogen Cycling and Microbial Community Functionality in a Post-Lignite Mining Rehabilitation Chronosequence in East Texas

RECOVERY OF CARBON AND NITROGEN CYCLING AND MICROBIAL COMMUNITY FUNCTIONALITY IN A POST-LIGNITE MINING REHABILITATION CHRONOSEQUENCE IN EAST TEXAS

A Dissertation

by

JUSTIN PARK HO NG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Co-Chairs of Committee,	Terry J. Gentry
	Frank M. Hons
Committee Members,	Sam Feagley
	Thomas W. Boutton
	Elizabeth Pierson
Head of Department,	David D. Baltensperger

August 2012

Major Subject: Soil Science

ABSTRACT

Recovery of Carbon and Nitrogen Cycling and Microbial Community Functionality in a Post-Lignite Mining Rehabilitation Chronosequence in East Texas. (August 2012)

Justin Park Ho Ng, B.S., Trinity University;

M.S., University of Texas at San Antonio

Co-Chairs of Advisory Committee: Dr. Terry J. Gentry
Dr. Frank M. Hons

Surface mining for coal alters the original soil profile characteristics and the associated physical, chemical, and biological conditions. Our objectives were to compare soil characteristics and the distribution of nutrients to 1 m depth over a chronosequence of 40 years to determine when a reclaimed mine soil (RMS) returned to premined conditions. We sampled 5 sites aged 0 to 20 years reclaimed by the crosspit spreader technique (CP) and 3 sites aged 20 to 40 years reclaimed by the mixed overburden technique (MO). An unmined site (UM) served as a control.

Changes in soil texture (sand to clay loam) after mining corresponded with increased macroaggregation (>2 mm) and enhanced C sequestration up to ~250 Mg C ha^{-1} at the MO20 site. Soil chemical [pH, electrical conductivity (EC), and sodium adsorption ratio (SAR)] and physical properties [bulk density (BD) and texture] met or exceeded reclamation and revegetation standards. Most soil C was associated with organic matter, but a small amount of lignitic C was detected in some samples. Soil organic C and N reached or exceeded premined concentrations after 0 and 10 years, respectively. Soil NO_3^--N and P did not reach premined conditions, but soil K, Ca, Mg and S exceeded premined conditions and stratified

after 10-15 years. Micronutrients exceeded premined concentrations. Soil microbial biomass and mineralization rates recovered after 16 years of reclamation. *Bacteria* and *fungi* recovered to premined levels after 20 years. The CP20 site was most closely related to the UM site, but sites 10 years and older were comparable. Dominant phyla (*Actinobacteria*, *Acidobacteria* and *Proteobacteria*; 70% of all sequences) returned to premined levels after 10 years, which correlated with soil quality indicators, suggesting the importance of these phyla in soil health. Community-level physiological profiles did not differ between sites and metabolic diversity peaked at CP15 and CP20. GeoChip showed separation between the UM sites and reclamation sites. Soil microbial functionality appeared to recover faster than taxonomic composition of the soil microbial community. Further analysis of functional genes will expand upon this research so that we may better quantify soil quality in RMS.

DEDICATION

This dissertation is dedicated to my family and friends past and present, all of whom have touched my life in some way and contributed to me becoming the person I am today and will be in the future. The words of these pages are the fruits of your support of my education.

ACKNOWLEDGEMENTS

I would like to thank my committee chairs, Dr. Terry J. Gentry and Dr. Frank M. Hons, and my committee members, Dr. Sam Feagley, Dr. Thomas W. Boutton, and Dr. Elizabeth Pierson, for their guidance throughout the course of this research.

Thanks also go to my colleagues in the Soil and Aquatic Microbiology Laboratory and Soil Fertility Laboratory and the faculty and staff at the Department of Soil and Crop Sciences for making my time at Texas A&M University a memorable experience.

A special thanks to Luminant for the financial and technical support during my doctoral research.

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

LIST OF TABLES

TABLE Page

TABLE Page

CHAPTER I

INTRODUCTION: THE MOTIVATION FOR RESEARCH

Burning lignite coal to generate steam-powered electricity is a significant source of energy production in the state of Texas. Lignite coal mining has a history of over 100 years in Texas, with Luminant (formerly TXU Corp.) leading operations in East Texas since the late 1800s. By 2030, it is expected that 180 million tons of coal per year will be consumed, a rate that is double the amount consumed in 1990 (RRC, 1993). The primary method of removing lignite is surface mining, which is efficient, but can be detrimental to land, air, and water quality. Surface mining causes dramatic disturbances to the land, including the removal of the overlying vegetation and disturbing native soil conditions and surface hydrologic function. In the United States, surface mines must be reclaimed to the standards of its prior use as mandated by the Surface Mining Control and Reclamation Act (SMCRA) of 1977. Proper reclamation practices are capable of returning post-mined sites to a productive state within a few years through basic steps, such as replacing the overburden, the topsoil (in some instances), and the vegetation over excavated pits (Hons, 1978; Peach, 2001). As part of the reclamation process at Texas' largest and one of the oldest lignite producing mines, Big Brown Mine, a mixed overburden technique replaced topsoiling as the primary way to cover mined areas by providing a material with better soil chemical and physical properties to enhance revegetation compared to native topsoil (Bearden, 1984; Dixon et al., 1980; Hons,

---

This dissertation follows the style of Soil Science Society of America Journal.

1978). A cross pit spreader replaced the mixed overburden with its improved speed and overburden segregation 20 years. Using Big Brown Mine as a research site provides a unique opportunity to compare the progress of reclamation on post-mined land that has been carefully managed over 40 years. The completion of land reclamation is dependent on the return of soil quality to an undisturbed or improved condition, which allows for the restoration of area vegetation and surface hydrologic properties and possible economic productivity. While land reclamation is accomplished with the minimal intention of returning land to a pre-mined state, sometimes post-mine quality is improved to prime farmland criteria.

Soil quality can be evaluated by biological, chemical, and physical properties, which are each important and interlinked. The objective of this research was to assess the recovery of nutrient distribution, cycling, and the microbial community in a chronosequence of post-mined sites. Studying the change of soil nutrients, soil structure, and soil microbial communities during the surface mining and reclamation process at Big Brown Mine is important to the improvement of post-mine land reclamation practices and understanding microbial community succession.

EFFECTS ON SOIL NUTRIENTS

Reclaiming land on post-mined areas should be conducted with the goal of enhancing the availability and cycling of soil nutrients in order to increase the potential for revegetation and biological activity. Nutrients are commonly lost during the mining process as soil aggregates are disrupted and carbon becomes available for decomposition (Adu and Oades, 1978; Ussiri and Lal, 2008). In addition, nutrients can be diluted during the mixing process

and eroded as spoil banks are left exposed before the backfilling and regrading stage (Ingram et al., 2005; Lal et al., 1998). The reclamation process at Big Brown Mine and other surface mines has resulted in post-mined land that has concentrations of nutrients (i.e. total and organic carbon, nitrogen, phosphorus) and nutrient cycling rates that have increased over time. Essential plant nutrients were found in greater concentrations in reclaimed soils, and were able to sustain row crops, such as sorghum, given proper fertilization and cultural practices (Angel, 1973; Askenasy, 1977). Toups (1986) provided data that supported the abundance of soil and foliar plant nutrients for loblolly pine plantations in reclaimed soils. In addition to row crops and trees, mined soils can provide support for various grasses and legumes, which are important for maintaining pasturelands (Hons, 1978). Besides data showing the beneficial effects of reclaimed soils on vegetation, it was discovered that the age of soils in restored wetlands was related to an increase in the carbon storage pools (Stapleton, 2004). A study by Waggoner (1993) suggested that reclaimed mine soils can recover and exceed certain previous nitrogen cycling activities (i.e. mineralization and nitrification) within just a year. Due to the continued mining and reclamation operations at Big Brown Mine, the number and age of reclaimed sites are always increasing, which allows for an in-depth chronosequence to be investigated.

The first area of focus for my research was to analyze mine soils of different ages ranging from 0 to 40 years based on the amount of soil nutrients and chemical characteristics. The concentrations and vertical distribution of soil nutrients are very important for the revegetation of the land. The main focus of the nutrient analysis will be measuring the levels of carbon pools and macronutrients important in land reclamation, such as organic, inorganic carbon and total nitrogen in the soil (Ingram et al., 2005). Organic carbon is important for

many soil processes, like water and nutrient holding capacity. On a larger scale, soil carbon sequestration is important for mitigating increasing atmospheric CO_2 concentrations. Nitrogen is commonly the most deficient plant available nutrient and required for protein and nucleic acid synthesis. Concentration ratios of carbon to nitrogen are important in determining net mineralization and immobilization activity, which relate to whether nutrients are becoming plant available. While nutrients such as carbon, nitrogen, and phosphorus, are essential for plant growth, there are many nutrients required in smaller amounts that must be taken into consideration for revegetation. For example, calcium is important for many leguminous species and sulfur levels may indicate the presence of acid-forming materials. In addition to concentration, distribution by depth must be measured to understand where nutrients are available or unavailable for plant uptake and microbial use. This will also indicate the movement of mobile compounds and elements over time.

Nutrient turnover is a great indicator of soil fertility by measuring potential microbial activity in associated with carbon and nitrogen. Turnover is the combination of the conversion rate and holding capacity of organic and inorganic nutrients by microorganisms. This activity is important on local and global scales for terrestrial ecologists and scientists who are attempting to account for nutrient budgets. To document turnover, microbial biomass and respiration rates can be compared to total carbon levels (Banning et al., 2008; Harris, 2003; Ingram et al., 2005). This is a currently undocumented topic at Big Brown, but is important in determining the microbially-driven portion of ecosystem recovery and its influence on soil carbon sequestration. For the nitrogen side of the organic matter equation, mineralization rates were evaluated to determine nitrogen turnover rates. Mineralization is the process of converting organic nutrients from decaying plants, animals, and

microorganisms into inorganic forms, such as ammonium and nitrate, which are available for plant use. Higher rates coupled with increased carbon mineralization would indicate a healthier soil population (Banning et al., 2008). However, the goal of studying these processes in a chronosequence was to see if post-mined lands developed a conservative (increased capacity to retain nutrients) nutrient cycle that was capable of supporting revegetation for extended periods of time (Banning et al., 2008; Odom, 1969). These results should provide insight into the carbon and nitrogen turnover rates of a chronological sequence of mine soils.

Chemical characteristics of soils are equally important as nutrient concentrations for revegetation and overall land reclamation. pH is arguably the most important characteristic of soils due to the information it gives on the availability of many micronutrients and some macronutrients. Many plant essential nutrients, as well as microbial activity, are often greatest at or near found at near neutral pH (7.0). A major qualification of soils deemed usable as topsoil and completely reclaimed is pH, which must fall between 5.0 and 8.4. Besides pH, cation exchange capacity (CEC) was measured. CEC is a measure of the ability for a soil to hold nutrient cations (Mg, K, Ca) in a readily plant available form. Increased CEC is commonly found in soils with greater amounts of organic matter and soils with layered silicates (e.g. smectite). Two studies indicated a relationship between the cation exchange capacity in soils and organic matter and tree growth in aged mine soils (Stewart, 1996; Toups, 1986). Determination of both soil nutrients and chemical characteristics in mined and post-mined sites and how they change by age are important in evaluating the success of reclamation and future productivity.

EFFECTS ON SOIL STRUCTURE

Soil physical characteristics are also very important in the reclamation of mine spoils. Soil physical qualities (e.g. texture, structure, moisture content, bulk density, and water-holding capacity) determine the permeability of water, gases, and roots through the soil as well as its water retention. Soil physical features also play important roles in soil biological activity. Within the soil biosphere, root proliferation and penetration, and microbial activity are essential for revegetation during the early stages of reclamation. How these properties change and influence successful surface mine reclamation over time is a topic that has garnered interest in Texas. In previous environmental research conducted at Big Brown Mine, it was found that certain chemical and physical characteristics for sustaining biological activity were adequate in the overburden. Barth (2002) showed that land capability classes were improved in reclaimed sites because of the reduction of slopes and root-zone limitations, which resulted in improved permeability, rooting depth and available water capacity. Willett (1978) observed a correlation between age, improved moisture holding capacity, permeability, and aeration with loblolly pine stand height. These previous studies lead to the next area of focus for the proposed research, documenting how soil texture and aggregation and their associated nutrients and chemical characteristics change in reclaimed mine soils.

Soil texture is the distribution of sand, silt, and clay in the soil. This distribution of particles gives information on water holding capacity and soil fertility. For example, higher percentages of sand may allow better movement, but less holding capability. Soil texture is one of the most stable soil properties and is mostly unaffected by practices such as cultivation. Banning et al. (2008) found little difference in soil texture between sites over a

26 year chronosequence. Yet, there may be an interesting change between reclamation practices by cross pit spreader in younger sites and mixed overburden in sites aged 20 years and greater. To improve soil fertility indication on post-mined sites, analysis of particle size distribution was coupled with a study on aggregation, which is the grouping of two or more primary particles (i.e. sand, silt, or clay). This grouping was mainly due to organic matter and biological activity holding the particles together, and represents a good indicator of soil health because it improves rooting, nutrient storage, and gas diffusion. Aggregation was found to improve over 28 years mine soils in Ohio (Shrestha and Lal, 2008). In addition to aggregate distribution, I analyzed nutrient concentration among the aggregates, which play an important role as a nutrient reservoir. The drastic disturbance of mining likely breaks apart aggregates and exposes organic matter. However, after reclamation practices are installed and revegetation continues unabated, nutrients can be returned to the soil and aggregates will form. As seen in total nutrient levels over time, I would expect both the percentage of aggregate size and associated nutrient concentrations to increase with age (Wick et al., 2009). Physical properties are an important area of research for soil analysis, yet have not been fully examined at Big Brown Mine.

EFFECTS ON THE SOIL MICROBIAL COMMUNITY

The main focus of the research was to observe the interrelationships that occur between soil structure, organic and inorganic nutrient dynamics, and microbial communities over time following surface mine reclamation. Microbial communities play an important role in maintaining many ecosystem processes. For example, microorganisms are closely involved in soil carbon and nitrogen cycles from their activity in decomposition and plant

symbioses. For physical characteristics, the microbial production of glomalin, also known as microbial glue, is a major component of soil aggregate formation, which is one determinant of soil health. Disturbance by surface mining initially has a detrimental effect on the soil microbial community, and has been the topic of a few microbial studies at Big Brown. One study found that *Rhizobium* and microbial populations in general can recover relatively quickly after disturbance (Mott, 1984; Peach, 2001). More interesting was the discovery of Harris (1985), who found that increasing the number and diversity of *Rhizobium* organisms in the soil increased biomass and nitrogen and phosphorus yields, resulting in stimulated vegetation growth. Swanson (1996) came to a similar conclusion when he found increasing levels of soil microbial biomass with age, which correlated well with the growth of coastal bermudagrass. Waggoner (1993) studied microbial rates of nitrification in fertilized mined soils and observed elevated numbers and activity correlated with age. Jackson (1979) observed a positive correlation between the acidity of soil and the index of chemoautotrophic activity. These previous studies show the intimate relationship between the stability and diversity of the microbial community, nutrient cycling and availability, soil properties, and successful revegetation. It is, therefore, imperative that microbial studies be continued to better understand their role in the health of reclaimed ecosystems.

My research expanded upon this previous work by including a biodiversity analysis of microbial communities to track compositional and long-term successional changes using newly developed molecular techniques that enable unprecedented levels of soil microbial characterization. The objective of this microbial study was to analyze the microbial community from both an ecological (functional) and an identification (taxonomic) standpoint, while combining this data in the context of soil edaphic properties. Microbial

taxonomic and functional diversity was measured using several different methods: community-level physiological profiling (CLPP), GeoChip, quantitative polymerase chain reaction (qPCR), and *16S rRNA* (bacteria) and *ITS* (fungi) rRNA gene sequencing. The CLPP measures sole-carbon-source-utilization in entire microbial communities by using a Biolog Ecoplate™, and was a primary way to measure functional diversity in soil microbial communities. GeoChip is a functional gene array in which genes can be quantified, correlated to soil edaphic parameters, and then identified to the phyla to which they belong (He et al., 2007; Pastorelli et al., 2011; Reeve et al., 2010). Taxonomic diversity was analyzed with a method that exceeds traditional culturing procedures of community analysis. The *16S rRNA* gene of prokaryotic organisms, which is a conserved region in the genomes of all microorganisms, can be extracted and sequenced for identification. qPCR is a method that measures the number of organisms in soil based on the amount of specific genes in community DNA. These methods have enhanced community analysis by circumventing the need to culture microorganisms, which was a lengthy and inefficient way to characterize soil microbial communities.

CHAPTER II

RECOVERY OF CARBON AND NITROGEN POOLS AND CYCLING IN A POST-LIGNITE MINING REHABILITATION CHRONOSEQUENCE IN EAST TEXAS

INTRODUCTION

Surface mining for coal results in the destruction of the original soil profile characteristics, and therefore has altered physical, chemical, and biological conditions at sites across the U.S. and world (McSweetney and Jansen, 1984). Carbon and N can be lost when topsoil is removed and stored, and also when soil aggregates are disrupted, exposing organic matter for mineralization (Adu and Oades, 1978; Six et al., 2000a; Ussiri and Lal, 2008). Soil C and N concentrations may also decrease when soil horizons are diluted during mixing or eroded when spoil banks are left exposed prior to backfilling and regrading (Ingram et al., 2005; Lal et al., 1998). Unsuccessful revegetation of reclaimed minesoils (RMS) is often associated with deteriorated soil conditions, arising from low soil organic matter content, high salinity, poor soil structure, and reduced soil fertility (Kleeberg, 2008; Sencindiver and Ammons, 2000; Ussiri et al., 2006). While vegetation plays a major role in improving RMS over time, the initial reclamation steps of topsoil backfilling, reconstruction, and regrading control the distribution of soil C and N, and other nutrients and characteristics in the soil profile (Bradshaw, 1987; Merrill et al., 1998) and are crucial for early survival of vegetation and microbial activity.

Reclamation of post-surface-mined areas should be conducted with the goal of returning disturbed sites to a premined or enhanced state (SMCRA, 1977). Reclamation

specialists have improved RMS quality by enhancing the availability and cycling of soil nutrients through a number of methods (Barnhisel and Hower, 1997; Bradshaw, 1997; Palmer et al., 2010; Hons, 1978; Lorenz and Lal, 2007; Whitford, 1988; Coyne et al., 1998; Ingram et al., 2005). One important characteristic, soil organic carbon (SOC), has been previously identified as an indicator of RMS quality because it influences soil chemical (e.g. nutrient concentrations) and physical properties (e.g. aggregation) and microbial activity (Franzluebbers et al., 2000; Ingram et al., 2005). Soil OC usually decreases upon initiation of mining (Abdul-Kareem and McRae, 1984; Ingram et al., 2005; Lal et al. 1998; Ussiri et al., 2006), but normally increases over time after reclamation with enhanced biomass production from revegetation and root development (Akala and Lal, 2000; 2001; Haering et al., 1993; Lal et al., 1998; Sourkova et al, 2005). Surface soil quality criteria mandated by the Surface Mining Control and Reclamation Act (SMCRA) include physical (e.g. percentages of clay and sand) and chemical properties [e.g. pH and sodium absorption ratio (SAR)], but not SOC. Soil OC is an important component of soil quality and influences numerous soil properties including nutrient cycling and availability, water holding capacity, many chemical properties, and enhances soil structure through aggregation (Ingram et al., 2005) Because of these relationships, SOC and N pools throughout the soil profile and within aggregate size classes should be quantified when determining the quality of RMS.

Successful reclamation efforts have been reported in Ohio (Lorenz and Lal, 2007; Ussiri et al., 2006), Kentucky (Coyne et al., 1998), Wyoming (Anderson et al., 2008; Ingram et al., 2005), and elsewhere. The Big Brown Mine in eastern Texas contains RMSs that have successfully supported cropland and pasture since the late 1960s (Askenasy, 1977; Hons, 1978). Previous soil studies at Big Brown compared physical properties (Willett, 1978),

chemical properties (Stewart and Hossner, 2001), nutrient concentrations (Angel, 1973; Toups, 1986), SOC storage (Stapleton, 2004), and N cycling rates between reclaimed sites (Waggoner, 1993). The link between soil properties and SOC sequestration in RMS has been extensively studied as chemical and physical factors can limit C accrual (Jacinthe and Lal, 2007). No studies have been conducted at Big Brown on the presence of residual lignite, which can artificially influence the SOC sequestration of RMS, and may alter soil dynamics due to its aromaticity and porous nature (Schobert, 1990; Ussiri and Lal, 2008). Since the number and ages of reclaimed sites at Big Brown Mine are continually increasing with time (currently >40 years), a chronosequence investigation of changes in soil quality parameters over time following surface mining and reclamation was conducted.

The first objective of this research was to determine physical and chemical characteristics of RMS in a chronosequence through 40 years, and to determine if or when conditions returned to a premined state following lignite surface mining. We expanded upon previous research at Big Brown Mine by measuring different soil C fractions and mineralization rates. Our hypothesis was that soil quality indicators would decrease after mining and increase over time before reaching a maximum capacity (Odum, 1969). Past studies of RMS have also shown the importance of measuring nutrient stratification in the soil (Anderson et al., 2008; Ussiri et al., 2006), leading to our second objective, to quantify change within 1 m depth of profile. We hypothesized that C and N would decrease with depth and resemble an undisturbed soil profile as biological activity in the topsoil increased with age (Jobbagy and Jackson, 2001; Lorenz and Lal, 2005). Our study will serve as crucial data in evaluating the success of management strategies in land reclamation and in predicting future productivity of RMS.

MATERIALS AND METHODS

Site Description and Soil Sampling

The Big Brown Mine is a lignite surface mine located east of Fairfield, TX and opened in 1971 (Table 1). The predominant soil series in the area of the mine are Axtell, Edge, and Tabor, and are mostly loamy or sandy soils over clayey subsoils that limit water and gas movement (Peach, 2001). Fairfield has a gently rolling to hilly topography (100-270 m above sea level) and is located within the Post Oak Savannah vegetation region of Texas (Gould, 1975). The regional mean annual temperature is 18.9 °C with July and August being the hottest months of the year with daily average maximum temperature of 35.6 °C as measured from 1962 to 1990. Annual average precipitation is approximately 970 mm, with the highest monthly rainfall normally occurring in May (120 mm) and the lowest in July (50 mm) as measured from 1941 to 1990. During the early reclamation era (1970-1980s), the mixed overburden technique (MO) was utilized where RMS were backfilled and regraded with a mixed combination of topsoil and overburden. The MO was shown to provide a material with better chemical and physical properties that enhanced revegetation compared to native topsoil alone (Bearden, 1984; Dixon et al., 1980; Hons, 1978). In 1986, the crosspit spreader (CP) technique replaced MO. The CP technique improved the speed of material segregation by separating the topsoil/topsoil substitute material from overburden and generally results in more uniform mixing of these materials. This method was used until the CP burned in 2009.

Table 1. List of sampling sites at Big Brown Mine, Fairfield, TX including locations, method of overburden removal, and years since reclaimed. UM, CP, and MO indicate unmined, cross pit spreader, and mixed overburden, respectively.

Site Name	Treatment	Age	GPS coordinates
UM	None	NA	N 31°48’4.36”/W 96°5’32.13”
CP0	Cross Pit Spreader	0	N 31°50’37.72”/W 96°3’28.05”
CP5	Cross Pit Spreader	5	N 31°50’51.84”/W 96°3’28.01”
CP10	Cross Pit Spreader	10	N 31°50’59.69”/W 96°3’42.94”
CP15	Cross Pit Spreader	15	N 31°51’24.07”/W 96°3’46.05”
CP20	Cross Pit Spreader	20	N 31°51’29.85”/W 96°4’10.14”
MO20	Mixed Overburden	20	N 31°46’48.17”/W 96°8’23.48”
MO30	Mixed Overburden	30	N 31°46’1.32”/W 96°9’19.47”
MO40	Mixed Overburden	40	N 31°45’4.23”/W 96°9’47.82”

Selected sampling sites had minimal slope and were on tops of hills to minimize effects from runoff, erosion, and siltation. Five CP sites were selected with post-reclamation ages of 0, 5, 10, 15, and 20 years (CP0, CP5, CP10, CP15, and CP20). Three MO sites were selected with post-reclamation ages of 20, 30 and 40 years (MO20, MO30, and MO40) (Table 1). For comparison of reclamation success, in which land is returned to a pre-mined or improved state, a nearby control site that was unmined (UM) with an undetermined age was selected.

All sites were vegetated primarily with coastal bermudagrass (*Cynodon dactylon L. pers.*) and managed as grassland and pastureland. Soil at the UM site was classified as the Padina series and was additionally vegetated with Bahia grass (*Paspalum notatum*). On this site, hay was baled (~two 1200 kg bales/ha) twice a year before being grazed (~1 cow/2 ha) every year and fertilized with 40-0-0 at 140 kg/ha. The CP0 and CP5 sites were initially revegetated with wheat (*Triticum L.*) and ryegrass (*Lolium spp.*) for temporary cover before coastal bermudagrass was sprigged. The CP0 site was classified as a Nahatche-Hatliff soil

association and fertilized with 13-13-13 at 280 kg/ha during seeding. The CP5 received the same fertilizer treatment as CP0. The CP10, 15, and 20 sites were fertilized with 17-17-17 at 280 kg/ha (during their initial revegetation). The CP5 and CP10 sites were originally classified as part of the Tabor series. Sites CP10 to 20 were hayed (~three 1100 kg bales/ha) and grazed yearly at a density of ~1 cow/ha. CP15 was initially classified as the Edge series, while CP20 was classified as the Silawa series. Sites CP15 and CP20 were also revegetated with two legume species, Yuchi Arrowleaf clover (*Trifolium vesiculosum*) and Crimson clover (*Trifolium incarnatum*). The MO20 site was grazed and hayed similar to the CP sites from ages 10 to 20, but was fertilized additionally with chicken litter at 4.5 t/ha. The MO30 site was grazed (~1 cow/ha), and was fertilized with 44-0-0 at 140 kg/ha. The MO40 site differed slightly from the other reclamation sites in that Johnsongrass [*Sorghum halepense (L.) pers.*] encroached upon the coastal bermudagrass at the site. The site was hayed (~three 1100 kg bales/ha) and fertilized with 17-17-17 at 280 kg/ha. All MO sites were classified as part of the Bigbrown soil series. Lime was applied at 9.9-12.3 t/ha for pH maintenance (pH < 5).

Soils were sampled on 22 June 2009 and 26 June 2010, times when peak plant biomass levels were expected (Swanson, 1996). Samples in 2009 were analyzed for total C (TC), organic C (OC), inorganic C (IC), lignitic C (LC), non-lignitic soil OC (SC), total N (TN), pH, electrical conductivity (EC), cation exchange capacity (CEC), texture, and bulk density (BD). Samples in 2010 were analyzed for aggregation, aggregate TC and TN, C and N mineralization, and soil microbial biomass C and N. For the 2010 data, sites were labeled as UM, CP1, CP6, CP11, CP16, CP21, MO21, MO31, and MO41.

At each site, a spot was randomly chosen as the center of the sampling plot, with measurements made 15 m in each cardinal direction (N to S, and E to W) as well as in bisecting directions (SE to NW, and SW to NE), forming four 30-m transects. Ten 1-m cores (5 on each side of the center to avoid repeat samples at the center) were taken across the diameter of the circle with roughly 3 m between each sample core. The soil core (3.0 cm diameter) was taken using a truck-mounted hydraulic probe, and sectioned into five depth segments: 0-15, 15-30, 30-45, 45-75, and 75-100 cm. From each transect and depth sections, the cores were composited into bags, and placed in coolers. A field weight was obtained shortly after sampling. Samples were then transported back to the laboratory within 12 hours and air-dried at 4°C on brown paper, broken with a combination of mortar/pestle and by hand, and passed through a 4-mm sieve.

Soil Chemical and Physical Analyses

Texture was determined using 50.0 g of soil and the hydrometer procedure. This method was performed at room temperature using deionized (DI) water in a 1-L graduated cylinder (Day, 1965). Bulk density (BD) was calculated as the dry weight of the soil core divided by the volume of the soil core interval as determined by the probe. Soil pH was determined in 1:2 soil:water extracts using DI water. Samples were stirred and allowed to equilibrate for a minimum of 30 minutes after adding DI water before determination with a hydrogen selective combination electrode (Schofield and Taylor, 1955). Cation exchange capacity (CEC) was determined according to Soil Survey Staff (1996) and Holmgren et al. (1977). Each 2.5-g subsample of soil was placed in a syringe and repeatedly extracted with pH 8.2 sodium acetate (NaOAc), pH 7.0 ammonium acetate (NH_4OAc), and ethanol. Flame

emission on an atomic absorption spectrometer was used to determine the final concentration of Na.

The combustion procedure was used to determine OC, TC and TN at 650 °C and 900 °C, respectively, with a vario MAX C/N analyzer (Elementar, Germany) (McGeehan and Naylor, 1988; Schulte and Hopkins, 1996; Storer, 1984). Two to five g of dried and sieved soil were pulverized using a ring and puck mill, with 250-mg subsamples weighed into aluminum capsules (Alpha Resources, Inc., Stevensville, USA). Inorganic carbon (IC) was determined as the difference between TC and OC. To determine the amount of lignite C in soil samples, a chemi-thermal method was used (Ussiri and Lal, 2008). Briefly, soil samples were extracted three times with 1M HCl and 0.5M NaOH to remove soil inorganic C and recent, more labile OC. Highly recalcitrant OC was removed with 50% HNO_3, and 10% HF was used to release mineral bound organic matter (OM). After oven drying at 60 °C and placing in a muffle furnace at 340 °C for 3 hr, TC in samples was determined by combustion analysis using a vario MAX C/N analyzer (Elementar, Germany). Non-lignitic soil OC (SC) was determined as the difference between OC and lignitic C. Carbon stocks were determined as BD x soil depth x C% x 100 (Cheng and Kimble, 2001; Pearson et al., 2007; Robertson et al., 1999).

Soil Aggregation

Dry-sieving was performed on soils from the 0-15 cm depth interval. A 200 g subsample of soil was placed on a nest of sieves and shaken using a Ro-Tap Shaker (W.S. Tyler, Mentor, Ohio) at 278 opm for 2 min (Schutter and Dick, 2002). Aggregates were defined as macroaggregates (>2 mm), small macroaggregates (2 mm – 250 μm),

microaggregates (250 μm – 53 μm), and silt and clay fractions (<53 μm). Soil from aggregates was analyzed for TC and TN as described previously. The percent contribution of each aggregate fraction to the total whole soil carbon was calculated on a weight basis.

Soil Microbial Biomass

Ten grams of air-dry soil was wetted to 50% water holding capacity as calculated by the van Genuchten equation (van Genuchten, 1980) and incubated at 25 °C for 5 days in a glass jar in the dark. Soils were subsequently fumigated with ethanol-free $ClCH_3$ (Ricca Lab, Arlington, Texas) for 24 hr in the dark at room temperature alongside a non-fumigated set of soils. Soils were vented after the incubation and extracted with 40 mL of 0.5 M K_2SO_4 by shaking for 1 hr. The solutions were centrifuged (3250 x g) for 5 min, and gravity-filtered through 2.5-μm pore-size filter paper (Fisher Scientific). The filtrate was frozen prior to analysis on a Shimadzu DOC analyzer. Soil microbial biomass was calculated using the formula of Paul et al. (1999). A k_{EC} and k_{EN} of 0.45 and 0.54, respectively, were used in the calculations (Appuhn and Joergensen, 2006; Beck et al., 1997; Brookes et al., 1985; Pothoff et al., 2009; Wu et al., 1990).

Carbon Mineralization

Forty grams of air-dry soil were wetted to 50% water holding capacity (van Genuchten, 1980). Soils were placed in glass jars in the dark with 20 mL 1M KOH and DI water to maintain soil moisture. Jars with no soil were used as controls. Soil and jars were incubated at 30°C. After 3, 7, 14, 21, 28, and 35 days, the KOH was titrated with 0.5 M HCl to the phenolphthalein endpoint following precipitation of carbonates with $BaCl_2$ (Zibilske,

1994). On those days, all jars were vented. CO_2-C trapped was calculated as ($HCl_{blank\ avg}$ − HCl_{sample})/2*22. After titration, soils were dried, pulverized, and analyzed for TC and TN as described previously.

Nitrogen Mineralization

Nitrogen mineralization was determined based on the concentrations of nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) in each soil sample after CO_2 titrations minus the concentrations in air-dry samples prior to incubation. Five grams of dried soil were shaken for 1 hr with 25 mL of 1 M KCl. Samples were then centrifuged (3250 x g) for 5 min and filtered through 2.5 µm cellulose filter paper. The filtrate was stored at 4 °C until analysis. Concentrations of NH_4^+-N and NO_3^--N were subsequently determined spectrophotometrically with a FIAlab-2500 instrument (FIAlab Instruments Inc., Bellevue, WA) equipped with a cadmium column for the reduction of NO_3^--N to nitrite (NO_2^--N).

Statistical Analysis

Analysis of variance (ANOVA) and student's t-tests were used to determine significant differences between ages and depths and CP and MO using JMP 8 (SAS Institute, 2008). A p-value of 0.05 was used as the cutoff value for determining significant differences.

RESULTS

Soil Physical and Chemical Characteristics

The UM site had sandy texture, which was much coarser than that of all reclaimed sites. All CP sites contained much finer-textured soils (Table 2). At CP0 and CP10, textures

were finer than those at CP5, CP15, and CP20. Few textural differences were observed throughout the profile for CP sites. Sites reclaimed using MO had near uniform clay loam texture, with only MO20 having a coarser texture in the 0-15 cm portion (Table 3).

Bulk density (BD) of UM samples increased with depth (Table 3). Soil profiles at CP sites had BD that was mostly uniform (Table 3). From 0-15 cm, BD of CP sites increased relative to the UM site before returning to premined conditions at 20 years. Bulk densities of CP samples below 15 cm were similar or lower than those from the UM site. The BD of 0-15 cm samples from the MO sites was near pre-mined conditions and displayed a trend of increased BD with age and depth. The CP20 BD was similar to that of MO20.

The UM profile had near neutral pH in the surface that became more acidic with depth (Table 2). CP0 had a uniform pH through the profile and was more alkaline than the UM site (Table 2). Soils of all CP sites of 5 to 20 years through all depths were more acidic than the UM site and became more acidic with depth. The pH of MO sites was similar to those of the UM site, and displayed decreasing pH with depth. CP20 was more acidic than MO20.

Cation exchange capacities (CEC) of UM samples were low and decreased with depth (Table 3). All reclaimed sites had significantly higher CEC than the UM site, with CP0 being the highest of all CP sites and MO40 the highest of the MO sites. No trend was observed in the CP sites with age. Little change with depth was also noted for CP. For MO sites, little difference in CEC was observed with either depth or age. Soils from MO20 had CEC values that were higher than for CP20. A sample of pure lignite exhibited a CEC of 111.1 cmol (+) kg^{-1}.

Table 2. Soil chemical and physical characteristics of an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas as affected by site. Data represent the average of 4 transects at each site.

	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
pH	0-15	6.70b	7.78a	6.63bc	5.30d	5.40d	6.15c	6.38bc	6.73b	6.88b
	15-30	6.70b	7.68a	5.30d	5.35d	5.35d	4.98d	6.00c	6.10bc	6.33bc
	30-45	6.65b	7.68a	5.00de	5.20de	5.10de	4.68e	6.23bc	5.60cd	5.95c
	45-75	6.45b	7.68a	5.30cd	5.13de	4.88de	4.48e	5.90bc	5.43cd	6.33bc
	75-100	6.23b	7.70a	5.63bcd	5.33cd	5.05de	4.28e	5.73bcd	5.35bcd	5.93bc
CEC	0-15	2.90f	22.93c	12.45e	16.38d	14.68de	16.78d	26.15b	33.23a	28.50b
(cmol	15-30	2.08e	23.93b	12.78d	16.73c	13.03d	14.78cd	24.05b	28.08a	26.98a
(+) kg^{-1})	30-45	1.88d	22.43b	12.03c	16.10c	13.20c	14.55c	23.30b	30.25a	29.18a
	45-75	1.50e	24.25b	11.05d	16.03c	12.78cd	14.18cd	22.33b	30.00a	28.25a
	75-100	1.40e	25.23ab	12.20d	17.43c	11.95d	15.40cd	22.23b	28.18a	28.58a
BD	0-15	1.37b	1.66a	1.61a	1.66a	1.66a	1.34b	1.27b	1.28b	1.38b
(g cm^{-3})	15-30	1.85a	1.86a	1.81ab	1.79ab	1.83ab	1.72bc	1.56d	1.62cd	1.69bc
	30-45	1.99a	1.80bc	1.80bc	1.80bc	1.86b	1.73cd	1.66de	1.62e	1.70de
	45-75	2.00a	1.77b	1.70bc	1.67bc	1.66c	1.62c	1.62c	1.63cd	1.63c
	75-100	2.68a	1.88b	1.68bc	1.62b	1.80b	1.64b	1.82b	1.77b	1.69bc
Sand	0-15	92.8a	44.3c	57.0b	35.0d	60.5b	56.5b	38.8cd	25.0e	40.0cd
(%)	15-30	94.3a	40.3d	62.3b	39.3de	60.0bc	53.5c	32.3e	22.3f	33.0de
	30-45	94.0a	38.3cd	60.8b	39.8c	56.3b	56.5b	32.2e	24.8e	31.5de
	45-75	92.0a	37.8c	58.8b	39.3c	58.8b	54.3b	34.3c	21.8d	37.0c
	75-100	89.7a	42.3cd	60.8b	39.3d	62.3b	48.8c	37.5d	28.8e	37.0d
Silt	0-15	2.5e	24.7cd	21.5d	37.0ab	18.0d	23.0cd	34.0b	44.0a	30.5bc
(%)	15-30	2.5f	27.7cd	18.5e	34.7bc	19.5e	23.0de	38.5b	46.2a	35.0bc
	30-45	2.0e	30.7bc	20.5d	35.2b	22.5cd	22.0cd	34.5b	44.8a	35.0b
	45-75	3.0f	29.2cd	22.5e	36.7b	19.5e	25.5de	34.0bc	45.2a	30.8bcd
	75-100	3.3e	25.7cd	19.5d	33.3bc	19.0d	30.5bc	34.0b	41.8a	30.8bc
Clay	0-15	5.3c	31.0a	21.5b	28.0a	21.5b	20.5b	27.2a	31.0a	29.5a
(%)	15-30	3.2f	32.0a	19.2e	26.0bc	20.5de	23.5bc	29.2ab	31.5a	32.0a
	30-45	4.0d	32.0a	18.7c	25.0b	21.2bc	21.5bc	33.3a	30.5a	33.4a
	45-75	5.0d	33.0a	18.7c	24.0b	21.7bc	20.2c	31.7a	33.0a	32.2a
	75-100	7.0d	32.0a	19.8c	27.4b	18.7c	20.7c	28.5ab	29.4ab	32.2a
Textural	0-15	s	Cl	scl	cl	scl	scl	scl	cl	cl
Class	15-30	s	Cl	sl	l	scl	scl	cl	cl	cl
	30-45	s	Cl	sl	l	scl	scl	cl	cl	cl
	45-75	s	Cl	sl	l	scl	scl	cl	cl	cl
	75-100	s	Cl	sl	l	sl	l	cl	cl	cl

Means in the same row followed by the same letter are not significantly different by at P<0.05. UM=unmined pasture. CP = crosspit spreader. MO = mixed overburden. CEC = cation exchange capacity. BD = bulk density. s = sandy. c = clay. l = loam.

Table 3. Depth differences of chemical and physical characteristics of an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas. Data represent the average of 4 transects at each site.

	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
pH	0-15	6.70A	7.78A	6.63A	5.30A	5.40A	6.15A	6.38A	6.73A	6.88A
	15-30	6.70A	7.68A	5.30B	5.35A	5.35A	4.98B	6.00A	6.10B	6.33AB
	30-45	6.65A	7.68A	5.00B	5.20A	5.10AB	4.68BC	6.23A	5.60BC	5.95B
	45-75	6.45A	7.68A	5.30B	5.13A	4.88B	4.48BC	5.90A	5.43C	6.33AB
	75-100	6.23A	7.70A	5.63AB	5.33A	5.05AB	4.28C	5.73A	5.35C	5.93B
CEC	0-15	2.90A	22.93A	12.45A	16.38A	14.68A	16.78A	26.15A	33.23A	28.50A
(cmol	15-30	2.08B	23.93A	12.78A	16.73A	13.03B	14.78AB	24.05A	28.08B	26.98A
(+) kg^{-1})	30-45	1.88C	22.43A	12.03A	16.10A	13.20B	14.55B	23.30A	30.25B	29.18A
	45-75	1.50D	24.25A	11.05A	16.03A	12.78BC	14.18B	22.33A	30.00B	28.25A
	75-100	1.40D	25.23A	12.20A	17.43A	11.95C	15.40AB	22.23A	28.18B	28.58A
BD	0-15	1.37C	1.66C	1.61B	1.66C	1.66B	1.34B	1.27C	1.28C	1.38B
(g/cm^{-3})	15-30	1.85B	1.86AB	1.81A	1.79AB	1.83AB	1.72A	1.56B	1.62B	1.69A
	30-45	1.99B	1.80AB	1.80A	1.80AB	1.86A	1.73A	1.66AB	1.62B	1.70A
	45-75	2.00B	1.77B	1.70B	1.67BC	1.66B	1.62A	1.62AB	1.63B	1.63A
	75-100	2.68A	1.88A	1.68B	1.62C	1.80AB	1.64A	1.82A	1.77A	1.69A
Sand	0-15	92.8AB	44.3A	57.0A	35.0A	60.5A	56.5A	38.8A	25.0A	40.0A
(%)	15-30	94.3A	40.3AB	62.3A	39.3A	60.0A	53.5A	32.3AB	22.3A	33.0A
	30-45	94.0A	38.3AB	60.8A	39.8A	56.3A	56.5A	32.2B	24.8A	31.5A
	45-75	92.0AB	37.8B	58.8A	39.3A	58.8A	54.3A	34.3AB	21.8A	37.0A
	75-100	89.7B	42.3AB	60.8A	39.3A	62.3A	48.8A	37.5AB	28.8A	37.0A
Silt	0-15	2.5A	24.7A	21.5A	37.0A	18.0A	23.0A	34.0A	44.0A	30.5A
(%)	15-30	2.5A	27.7A	18.5A	34.7A	19.5A	23.0A	38.5A	46.2A	35.0A
	30-45	2.0A	30.7A	20.5A	35.2A	22.5A	22.0A	34.5A	44.8A	35.0A
	45-75	3.0A	29.2A	22.5A	36.7A	19.5A	25.5A	34.0A	45.2A	30.8A
	75-100	3.3A	25.7A	19.5A	33.3A	19.0A	30.5A	34.0A	41.8A	30.8A
Clay	0-15	5.3AB	31.0A	21.5A	28.0A	21.5A	20.5B	27.2A	31.0AB	29.5A
(%)	15-30	3.2B	32.0A	19.2A	26.0A	20.5AB	23.5A	29.2A	31.5AB	32.0A
	30-45	4.0AB	32.0A	18.7A	25.0A	21.2AB	21.5AB	33.3A	30.5AB	33.4A
	45-75	5.0AB	33.0A	18.7A	24.0A	21.7A	20.2B	31.7A	33.0A	32.2A
	75-100	7.0A	32.0A	19.8A	27.4A	18.7B	20.7AB	28.5A	29.4B	32.2A
Text-	0-15	s	cl	scl	cl	scl	scl	scl	cl	cl
ure	15-30	s	cl	sl	l	scl	scl	cl	cl	cl
Class	30-45	s	cl	sl	l	scl	scl	cl	cl	cl
	45-75	s	cl	sl	l	scl	scl	cl	cl	cl
	75-100	s	cl	sl	l	sl	l	cl	cl	cl

Means in the same column and characteristic followed by the same letter are not significantly different at $P<0.05$. UM=unmined pasture. CP = crosspit spreader. MO = mixed overburden. CEC = cation exchange capacity. BD = bulk density. s = sandy. c = clay. l = loam.

Carbon Stocks

Carbon stocks in the top 1 m of soil generally increased over time (Fig. 1). Carbon stocks in the UM site (38 Mg C ha^{-1}) were lower than for all reclaimed sites. Carbon stocks at CP0 (190 Mg C ha^{-1}) increased after mining compared to UM, with roughly equal amounts in each section by depth. A decrease in carbon stock compared to CP0 was observed for the CP5 site (61 Mg C ha^{-1}). However, a greater proportion of carbon stock was found in the top 15 cm compared to CP0. Increased carbon stock was observed in CP10 (102 Mg C ha^{-1}) followed by a decrease for CP15 (80 Mg C ha^{-1}). Carbon stock in CP20 elevated to 186 Mg C ha^{-1}, and was more evenly distributed through the 1-m profile. Carbon stocks in MO sites were similar (286 Mg C ha^{-1}) and exceeded those in CP sites.

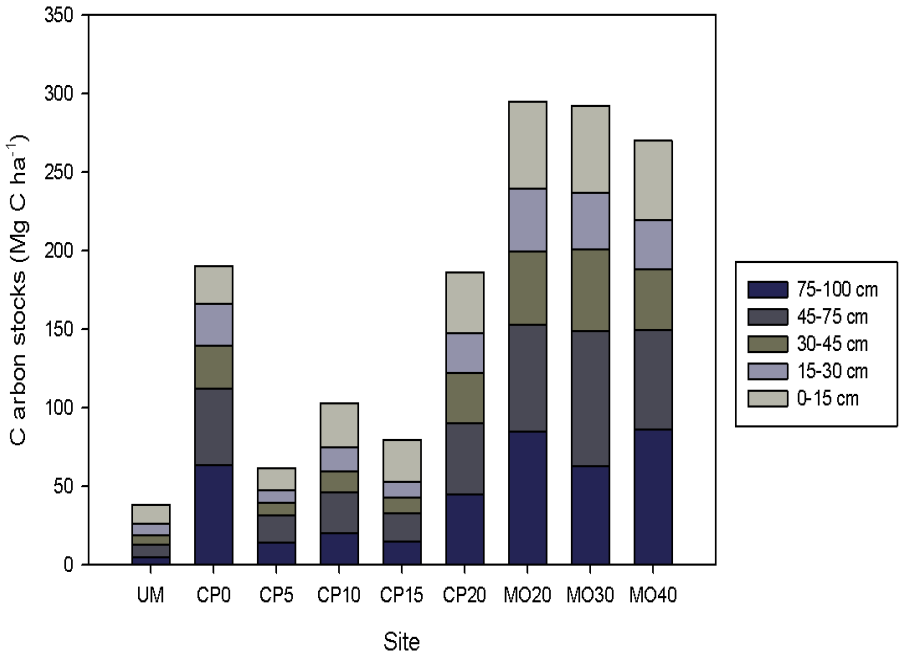

Fig. 1. Total carbon stocks (Mg C ha^{-1}) in a post-surface mining chronosequence. Unmined soil (UM) and mined soils were sampled at five depth intervals: 0-15, 15-30, 30-45, 45-75, and 75-100 cm and compared by crosspit spreader (CP) from 0 to 20 years and mixed overburden (MO) from 20 to 40 years.

Carbon Pools

Total (TC), organic (OC), and non-lignitic organic soil C (SC) pools had similar trends with age and depth (Tables 4 and 5). The lowest carbon concentrations for all pools were consistently found in the UM and CP5 sites. While TC in the UM site decreased uniformly with depth, the CP5 site decreased below 0-15 cm, but then exhibited similar concentrations with depth. The next highest carbon concentrations were generally about 1.5-2.0 times higher than for the UM site and were found in CP0, CP10, and CP15. Total carbon distribution in the CP0 profile was generally uniform, except for an increase at the 75-100-cm depth. Similar to CP5, the distribution of TC in CP10 and CP15 profiles decreased after 0-15 cm, but was similar throughout the remaining 15-100 cm. This trend of uniformity of TC through 15-100 cm was observed for all reclaimed sites after 5 years. Total C in the 1-m profile increased more than two-fold from CP15 to CP20. All MO sites had greater TC than any CP site. MO20 and MO30 were similar to each other, while MO40 showed a small decrease in TC at 0-15 cm.

Inorganic C was highly variable, and tended to be the smallest of the measured carbon pools (0.01 to 2.65 g kg^{-1}), with no consistent pattern based on reclamation age or depth (Tables 4 and 5). The lowest IC concentrations were found in the UM site. Inorganic carbon concentrations in CP sites were mostly not statistically different by age, but were greater than for the UM site. Mixed overburden sites had greater IC concentrations than CP sites, but exhibited no pattern with depth. Increases in IC were observed in 30-45 cm and 75-100 cm depths of MO sites.

Table 4. Site differences in soil carbon pools: total, inorganic, organic, lignitic, and non-lignitic organic carbon, in profile samples of an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas from 2009. Data represent the average of four transects at each site.

g kg^{-1}	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
TC	0-15	5.72e	9.64d	5.75e	11.31d	10.92d	19.14c	28.99a	28.87a	24.34b
	15-30	2.78d	9.64bc	2.82d	5.77cd	3.54d	9.81bc	17.40a	14.98ab	12.48ab
	30-45	2.03d	9.96bcd	3.07d	4.83cd	3.64cd	12.58abc	18.85ab	21.06a	15.04ab
	45-75	1.15d	9.32abcd	3.43cd	5.26bcd	3.53cd	9.35abcd	13.79ab	17.69a	12.96abc
	75-100	0.75d	13.48abc	3.26d	4.86cd	3.32d	10.91bcd	17.73ab	4.15abc	20.66a
IC	0-15	0.32b	0.94ab	0.33b	0.52ab	0.36b	0.61ab	1.47a	1.1ab	1.33a
	15-30	0.42b	0.96ab	0.14b	0.44b	0.10b	0.47b	1.77a	0.82b	0.46b
	30-45	0.04b	0.84b	0.05b	0.22b	0.13b	0.55b	5.55a	2.65ab	2.31ab
	45-75	0.01c	0.80abc	0.38bc	0.40bc	0.27bc	1.25ab	1.60a	1.32ab	1.02abc
	75-100	0.02b	0.80ab	0.10b	0.12b	0.19b	0.46b	2.68a	1.83ab	1.92ab
OC	0-15	5.39f	8.71e	5.41f	10.79d	10.56de	18.54c	27.52a	27.78a	23.01b
	15-30	2.36e	8.68cd	2.68e	5.33de	3.43e	9.35bcd	15.63a	14.16ab	12.01abc
	30-45	1.98d	9.12bcd	3.03d	4.61cd	3.51d	12.03abc	13.3ab	18.41a	12.74ab
	45-75	1.14e	8.52bc	3.05de	4.86cde	3.26de	8.1bcd	12.19ab	16.37a	11.95ab
	75-100	0.74e	12.68ab	3.16de	4.73cde	3.13de	10.46bcd	15.05ab	12.31abc	18.74a
LC	0-15	0.31e	0.81c	0.51de	0.91c	0.69cd	0.84c	1.40b	2.80a	1.34b
	15-30	0.27e	0.89c	0.53de	0.84cd	0.63cde	0.75cd	1.39b	1.89a	1.37b
	30-45	0.31d	0.91bcd	0.53d	0.75cd	0.75cd	0.73cd	1.60abc	2.32a	1.66ab
	45-75	0.33e	0.89cd	0.53de	0.77cde	0.72de	0.66de	1.21bc	2.24a	1.67b
	75-100	0.31d	1.13bc	0.58cd	0.83cd	0.72cd	0.81cd	1.62ab	1.65ab	1.96a
SC	0-15	5.08f	7.90e	4.90f	9.88d	9.87d	17.7c	26.13a	24.97a	21.68b
	15-30	2.08d	7.79bc	2.16d	4.49cd	2.81d	8.6bc	14.25a	12.27ab	10.65ab
	30-45	1.68d	8.20bcd	2.50d	3.86cd	2.76d	11.30ab	11.70ab	16.09a	11.08abc
	45-75	0.82d	7.63bc	2.52d	4.08cd	2.54d	7.44bc	10.98ab	14.13a	10.27ab
	75-100	0.43d	11.55a	2.59cd	3.90bcd	2.41d	9.65abc	13.43a	10.66ab	16.77a

Means in the same row followed by the same letter are not significantly different at $p<0.05$. UM=unmined pasture. CP = crosspit spreader. MO = mixed overburden.TC = total carbon. IC = inorganic carbon. LC = lignitic carbon. OC = organic carbon. SC = non-lignitic soil organic carbon.

Table 5. Depth differences between soil carbon pools: total, inorganic, organic, lignitic, and non-lignitic organic soil, in soil profiles of an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas from 2009. Data are displayed as an average of data from 4 transects at each site.

g kg^{-1}	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
TC	0-15	5.72A	9.64A	5.75A	11.31A	10.92A	19.14A	28.99A	28.87A	24.34A
	15-30	2.78B	9.64A	2.82B	5.77B	3.54B	9.81B	17.40A	14.98B	12.48B
	30-45	2.03C	9.96A	3.07B	4.83B	3.64B	12.58B	18.85A	21.06AB	15.04B
	45-75	1.15D	9.32A	3.43B	5.26B	3.53B	9.35B	13.79A	17.69B	12.96B
	75-100	0.75D	13.48A	3.26B	4.86B	3.32B	10.91B	17.73A	14.15B	20.66AB
IC	0-15	0.32A	0.94A	0.33AB	0.52A	0.36A	0.61A	1.47A	1.10AB	1.33A
	15-30	0.42A	0.96A	0.14BC	0.44AB	0.10A	0.47A	1.77A	0.82B	0.46A
	30-45	0.04A	0.84A	0.05C	0.22AB	0.13A	0.55A	5.55A	2.65A	2.31A
	45-75	0.01A	0.80A	0.38A	0.40AB	0.27A	1.25A	1.60A	1.32AB	1.02A
	75-100	0.02A	0.80A	0.10C	0.12B	0.19A	0.46A	2.68A	1.83AB	1.92A
OC	0-15	5.39A	8.71A	5.41A	10.79A	10.56A	18.54A	27.52A	27.78A	23.01A
	15-30	2.36B	8.68A	2.68B	5.33B	3.43B	9.35B	15.63AB	14.16B	12.01C
	30-45	1.98B	9.12A	3.03B	4.61B	3.51B	12.03B	13.30B	18.41B	12.74BC
	45-75	1.14C	8.52A	3.05B	4.86B	3.26B	8.10B	12.19B	16.37B	11.95C
	75-100	0.74C	12.68A	3.16B	4.73B	3.13B	10.46B	15.05AB	12.31B	18.74AB
LC	0-15	0.31A	0.81B	0.51A	0.91A	0.69AB	0.84A	1.40A	2.80A	1.34B
	15-30	0.27A	0.89AB	0.53A	0.84AB	0.63B	0.75A	1.39A	1.89A	1.37B
	30-45	0.31A	0.91AB	0.53A	0.75B	0.75A	0.73A	1.60A	2.32A	1.66AB
	45-75	0.33A	0.89AB	0.53A	0.77AB	0.72AB	0.66A	1.21A	2.24A	1.67AB
	75-100	0.31A	1.13A	0.58A	0.83AB	0.72AB	0.81A	1.62A	1.65A	1.96A
SC	0-15	5.08A	7.90A	4.90A	9.88A	9.87A	17.70A	26.13A	24.97A	21.68A
	15-30	2.08B	7.79A	2.16B	4.49B	2.81B	8.60B	14.25AB	12.27B	10.65BC
	30-45	1.68B	8.20A	2.50B	3.86B	2.76B	11.30B	11.70B	16.09B	11.08BC
	45-75	0.82C	7.63A	2.52B	4.08B	2.54B	7.44B	10.98B	14.13B	10.27C
	75-100	0.43C	11.55A	2.59B	3.90B	2.41B	9.65B	13.43B	10.66B	16.77AB

Means in the same column and characteristic followed by the same letter are not significantly different at $p<0.05$. UM = unmined pasture. CP = crosspit spreader. MO = mixed overburden.TC = total carbon. IC = inorganic carbon. LC = lignitic carbon. OC = organic carbon. SC = non-lignitic soil carbon.

Most of the TC was OC (85 to 99%) (Table 4). The distribution of OC in the soil profile was segregated by depth in the UM site. Like TC, all CP sites except CP0 showed greater OC in the top 15 cm, but no difference from 15-100 cm (Table 5). CP0 had increased OC compared to UM, but showed little difference throughout the soil profile. The trend of OC was similar to that of TC, in that OC decreased in CP5 compared to CP0 and was only greater at 0-15 cm. After CP5, OC increased significantly in CP10 and CP15 sites, and again in CP20. The MO sites had increased OC compared to UM and CP sites, but had similar distribution with depth. Organic carbon for all MO sites was greater in the surface and showed little depth trend. MO20 had higher OC than CP20 through the 1 m soil profile, but was only significant from 0-30 cm.

A small amount of LC was found in the UM site (Table 4), and these concentrations were smallest among all sites. Concentrations of LC tended to increase with age, with the exception of a decrease in CP5 and CP15. Lignitic carbon was mostly uniform in the soil profile throughout reclamation. Mixed overburden sites had higher LC concentrations (1.21 to 2.80 g kg^{-1}) than UM and CP sites (0.27 to 1.13 g kg^{-1}).

Non-lignitic OC (SC) at the UM site showed a significant decrease with depth (Table 3 and Fig. 2). Most CP sites showed greater concentrations of SC in the top 15 cm compared to the rest of the soil profile. Non-lignitic OC in CP0 increased compared to UM, but had no differences with depth. Non-lignitic OC decreased from CP0 to CP5, but showed stratification with depth (Table 5). At the 0-15 cm depth, SC in CP10 and CP15 increased compared to younger sites. CP20 had greater SC concentrations than all other CP sites through 0-30 cm. Concentrations of SC in MO samples were significantly higher than for UM and CP sites. The MO sites showed significant differences through the soil profile with

the highest concentrations observed at 0-15 cm. From 20 to 40 years, SC decreased in the MO treatment from 0-30 cm. Similar to the trend of OC, MO20 had higher SC than CP20 throughout the 1-m soil profile.

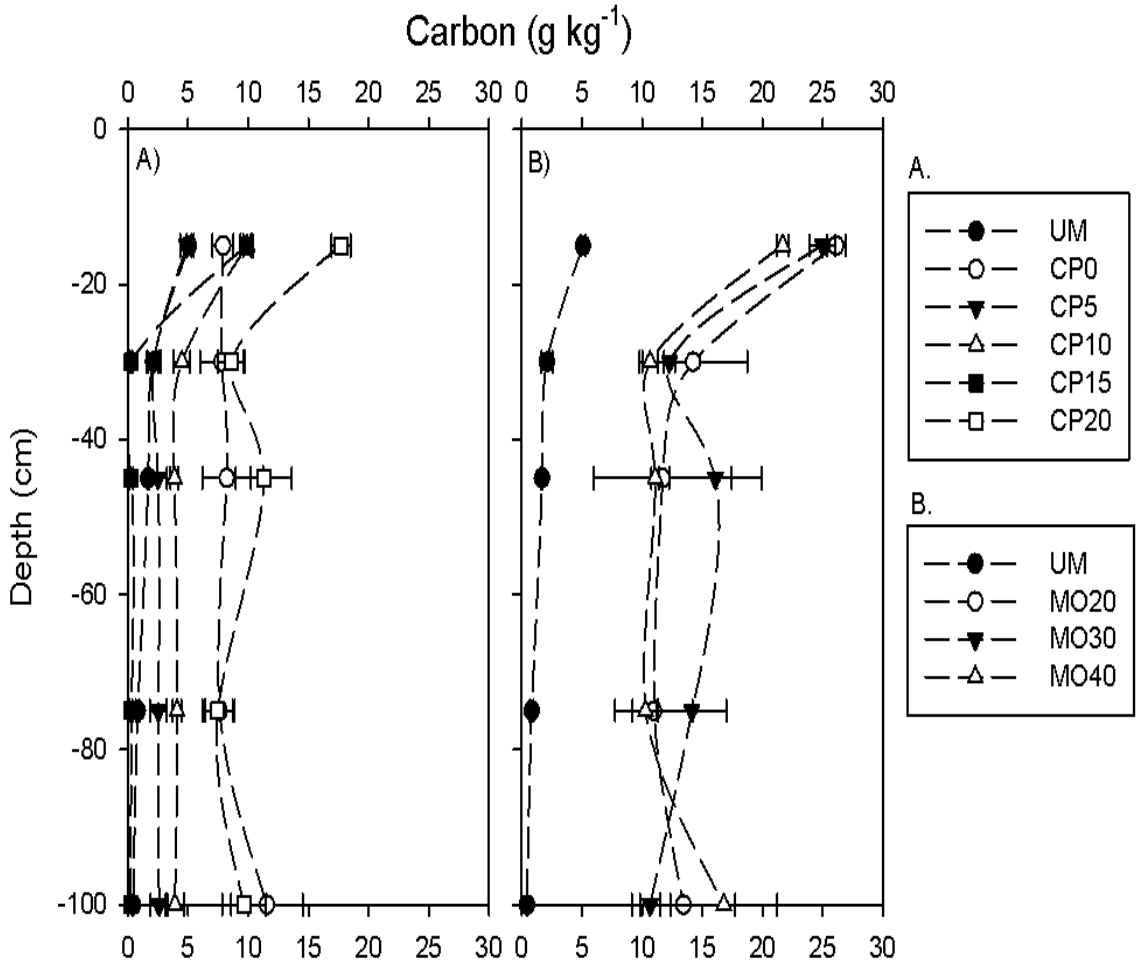

Fig. 2. Non-lignitic soil organic carbon depth trends in a post-lignite mining rehabilitation chronosequence. Unmined soil (UM) and mined soils were sampled at five depth intervals: 0-15, 15-30, 30-45, 45-75, and 75-100 cm and compared by (A) crosspit spreader (CP) from 0 to 20 years and (B) mixed overburden (MO) from 20 to 40 years. Horizontal bars indicate standard error of the mean.

Total Nitrogen

Total nitrogen (TN) in UM samples was highest at 0-15 cm, decreased significantly to the next depth interval, and was similar in remaining depths (Figs. 3A and B). For CP0 to CP5, no significant differences between depth intervals were observed, but all older reclamation sites exhibited higher TN in 0-15 cm samples (Figs. 3A and B). The highest TN concentrations for CP samples were associated with CP10 through all depths. The MO sites generally exhibited significantly higher TN concentrations throughout the profile than UM. Between MO sites, few differences were found between ages with depth. MO20 TN was mostly higher than CP20.

Aggregate-associated Carbon and Nitrogen

Unmined samples did not contain detectable levels of macroaggregates (Ma) (> 2 mm) (Fig. 4). The proportion of whole soil from CP sites as Ma, showed a greater range (15.3 to 29.0%) than did MO (20.7 to 27.1%). Small macroaggregate (Sma) (2 mm – 250 μm) proportions were similar to the trend for Ma. The proportion of whole soil as Sma in CP samples (33.1 to 43.8%) and MO samples (44.4 to 52.4%) were higher than in UM samples (10.0%). Aggregates in UM soil were mostly microaggregates (Mi) (250 μm – 53 μm). The proportion of whole soil as Mi in CP samples (24.3 to 45.8%) was higher than in MO (11.2 to 29.0%). Silt and clay fractions (S + C) (< 53 μm) in UM samples were smaller (2.0%) than in CP (5.6 to 6.9%) and MO samples (5.9 to 9.3%).

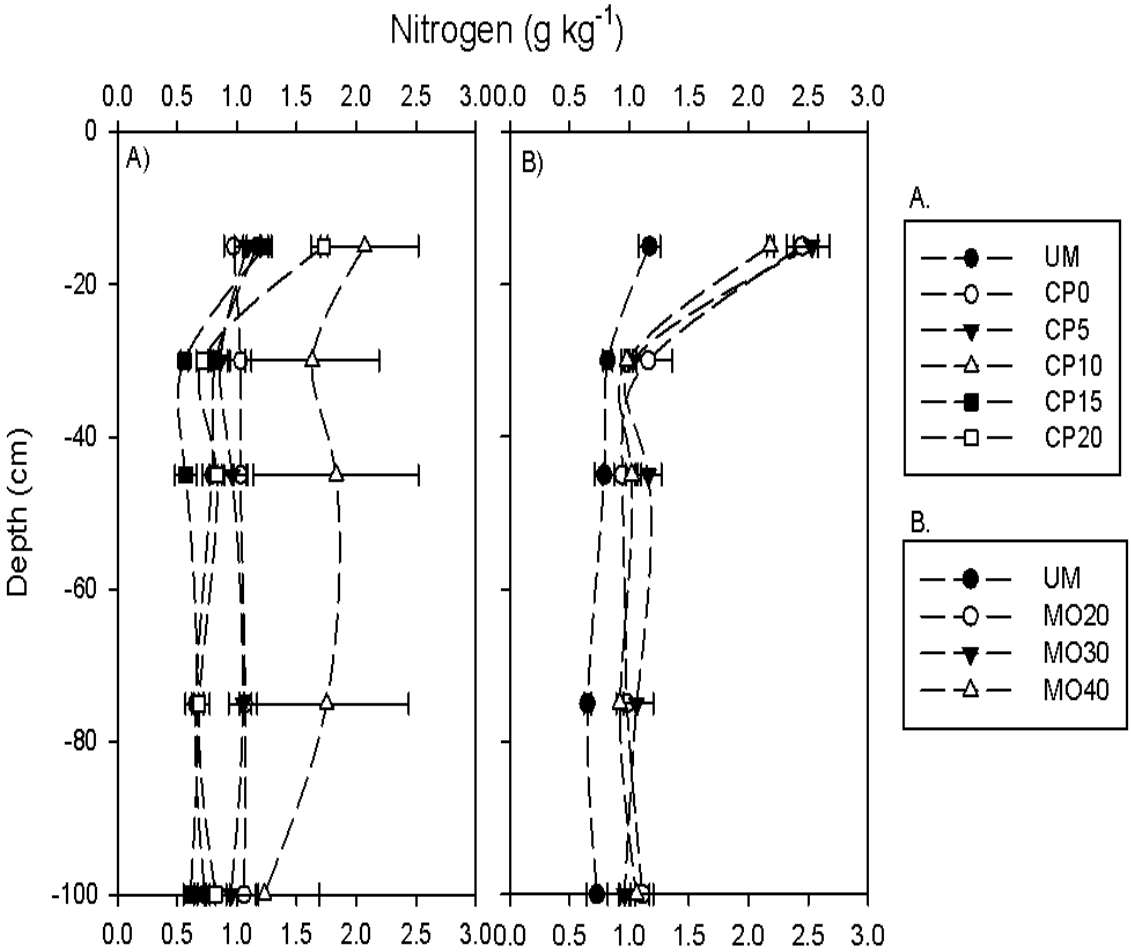

Fig. 3. Total soil nitrogen depth trends in a post-lignite mining rehabilitation chronosequence. Unmined soil (UM) and mined soils were sampled at five depth intervals: 0-15, 15-30, 30-45, 45-75, and 75-100 cm and compared by (A) crosspit spreader (CP) from 0 to 20 years and (B) mixed overburden (MO) from 20 to 40 years. Horizontal bars indicate standard error of the mean.

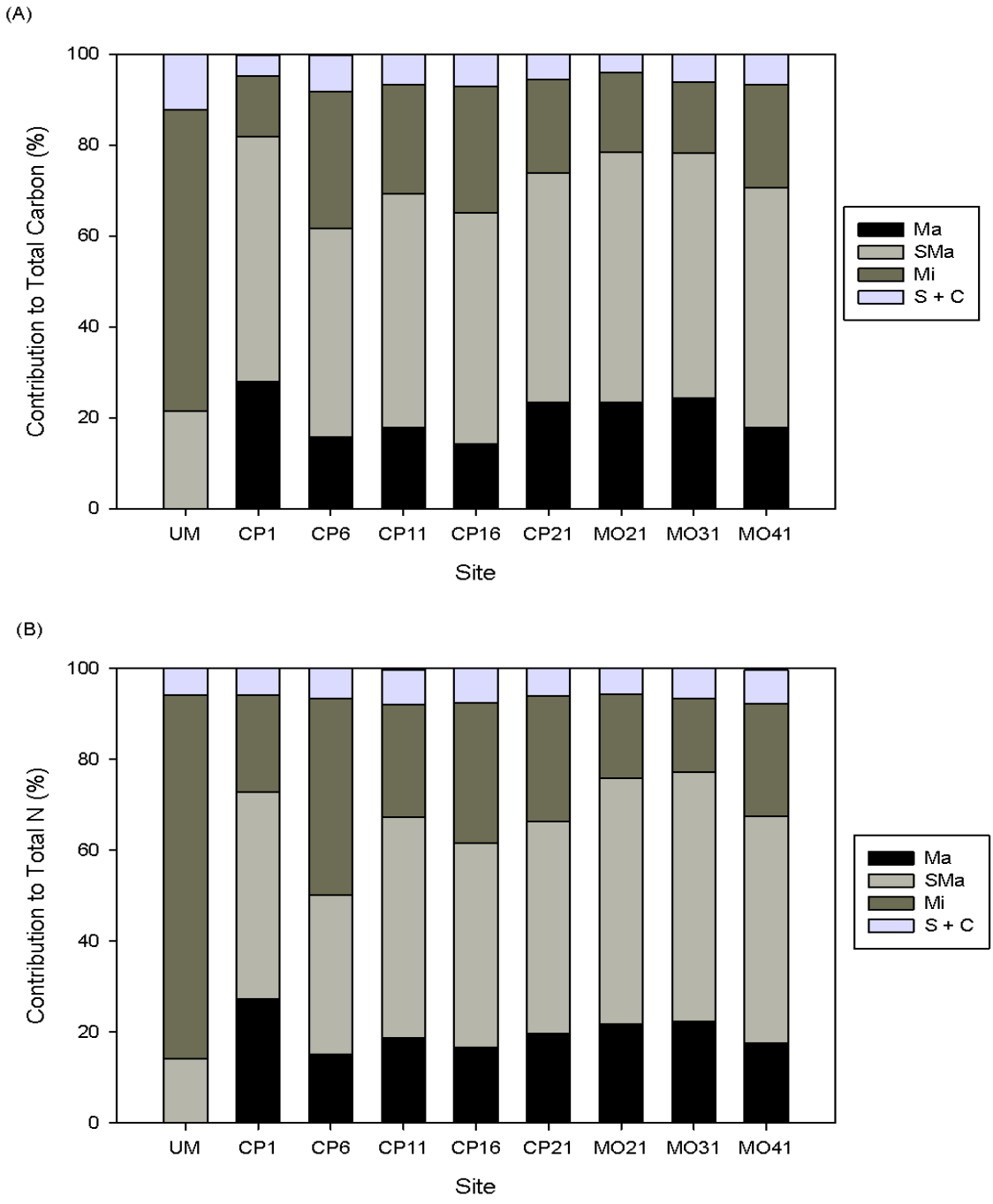

Fig. 4. Contribution of aggregate fractions sampled in 2010: macroaggregate (Ma), small macroaggregates (SMa), microaggregates (Mi), and silt and clay (S+C) in the 0-15 cm depth interval in a post-lignite mining rehabilitation chronosequence composed of unmined site (UM), crosspit spreader sites (CP) aged 1 to 21 years, and mixed overburden (MO) sites aged 21 to 41 years. The four aggregate-size fractions were measured for their contributions to (A) total carbon and (B) nitrogen.

In CP samples, the concentration of TC in Ma was similar through 16 years, but was highest for CP21 (20.3 g kg^{-1}) (data not shown), and contributed ~20% of whole soil TC (Fig. 4A). In MO samples, the concentration of TC in Ma (16.2 to 24.6 g kg^{-1}) decreased with age and contributed ~22% to whole soil TC. For the UM site, TC concentration in Sma (9.7 g kg^{-1}) was similar to that in Sma from CP1 to CP16 (7.5 to 12.2 g kg^{-1}), but lower than that in Sma of CP21 samples (22.7 g kg^{-1}). Unmined Sma contributed less to whole soil TC (~21.5%) than did those from CP sites (50.3%). At MO sites, concentrations of TC in Sma declined with age, but contributed 53.8% of whole soil TC. The TC concentration in Mi of UM samples (3.4 g kg^{-1}) accounted for 80.0% of whole soil TC, compared to ~23.2% of TC in CP sites and ~18.6% of TC in MO sites. The concentration of TC in UM S + C (28.3 g kg^{-1}), contributed 12.3% to whole soil TC, while TC in S + C increased with age in CP sites (5.3 to 16.2 g kg^{-1}) and contributed up to 6.3% of whole soil TC. The TC concentrations (14.7 to 21.4 g kg^{-1}) in S + C fractions of MO samples increased with age, but generally contributed the least of all sites to TC (~5.7%).

The concentration of TN in Ma of CP soils increased significantly with age (0. 7 to 1.4 g kg^{-1}) (data not shown), and contributed ~19.6% of whole soil TN (Fig. 4B). The concentration of TN in MO samples decreased with age, but was higher than in CP Ma, contributing ~20.7% of whole soil TN. Concentrations of TN in Sma in CP samples were lower than in Sma from the UM site (1.1 g kg^{-1}) from 0 to 5 years (0.8 g kg^{-1}), but greater from CP11 to CP21 (1.5 g kg^{-1}). For the UM site, Sma contributed ~14.2% of whole soil TN, compared to ~40.0% in CP sites, and ~52.7% of TN of MO. Due to the much greater proportion of Mi in UM samples, this size fraction contributed the highest portion of whole soil TN (66.2%) in these samples. In CP samples, Mi TN increased with age, contributing

~29.5% compared to ~19.8% of TN in MO samples. In UM samples, S + C (3.2 g kg^{-1} TN concentration) contributed 7.7% of whole soil TN. Concentration of TN in S + C (0.8 to 1.8 g kg^{-1}) generally increased with age in CP sites, contributing ~7.2% of soil TN. In MO samples, TN concentrations of S + C increased with age (1.5 to 2.3 g kg^{-1}), and contributed ~6.4% to whole soil TN.

Soil Microbial Biomass – Carbon, Nitrogen

Surface soil from the UM site exhibited a soil microbial biomass – carbon (SMB-C) concentration of 107 mg C kg^{-1}, decreased after mining at CP1 (70 mg C kg^{-1}), but then increased with age (Fig. 5). SMB-C in CP16 samples exceeded UM levels, and again increased significantly at 21 years. Samples from all MO sites had higher SMB-C levels than those from CP sites, with the highest concentration observed at 21 years. Soil microbial biomass – N (SMB-N) was similar to SMB-C, decreasing immediately after reclamation and increasing over time, where SMB-N in CP16 and CP21 samples exceeded those from the UM site. SMB-N was higher in MO21 samples compared to CP21. The SMB-C/N (4.79 to 10.38) was highest at CP1 and lowest at CP11.

Carbon and Nitrogen Mineralization

Cumulative carbon mineralization during 35 d ($Cmin_{0-35}$) decreased initially after mining, but recovered with time (Fig. 6A). The lowest $Cmin_{0-35}$ was associated with CP1 samples, but exceeded that from UM at 11 years. $Cmin_{0-35}$ from CP16 soil was most similar to that of the UM site. Greatest $Cmin_{0-35}$ for all CP sites was observed in CP21 samples. Soils

from MO sites had higher $Cmin_{0-35}$ rates than UM and CP samples, but decreased from 21 to 41 years. $Cmin_{0-35}$ of MO41 soil was similar to that of CP21.

Nitrogen mineralization ($Nmin_{0-35}$) followed a similar trend to $Cmin_{0-35}$ (Fig. 6B). $Nmin_{0-35}$ of UM samples was greater compared to that of CP1 and CP6 samples. $Nmin_{0-35}$ of CP11 samples exceeded UM, but decreased to near UM rates in CP16 soil. The $Nmin_{0-35}$ of CP21 samples exceeded that of UM and all other CP sites. Nitrogen mineralization in MO21 and 31 samples was greater than for all other sites. Between 21 and 35 days, $Nmin_{0-35}$ for MO21 dipped below MO31 due to a decrease in NO_3^--N. In MO41samples, $Nmin_{0-35}$ was lower than for CP11, but higher than for CP16.

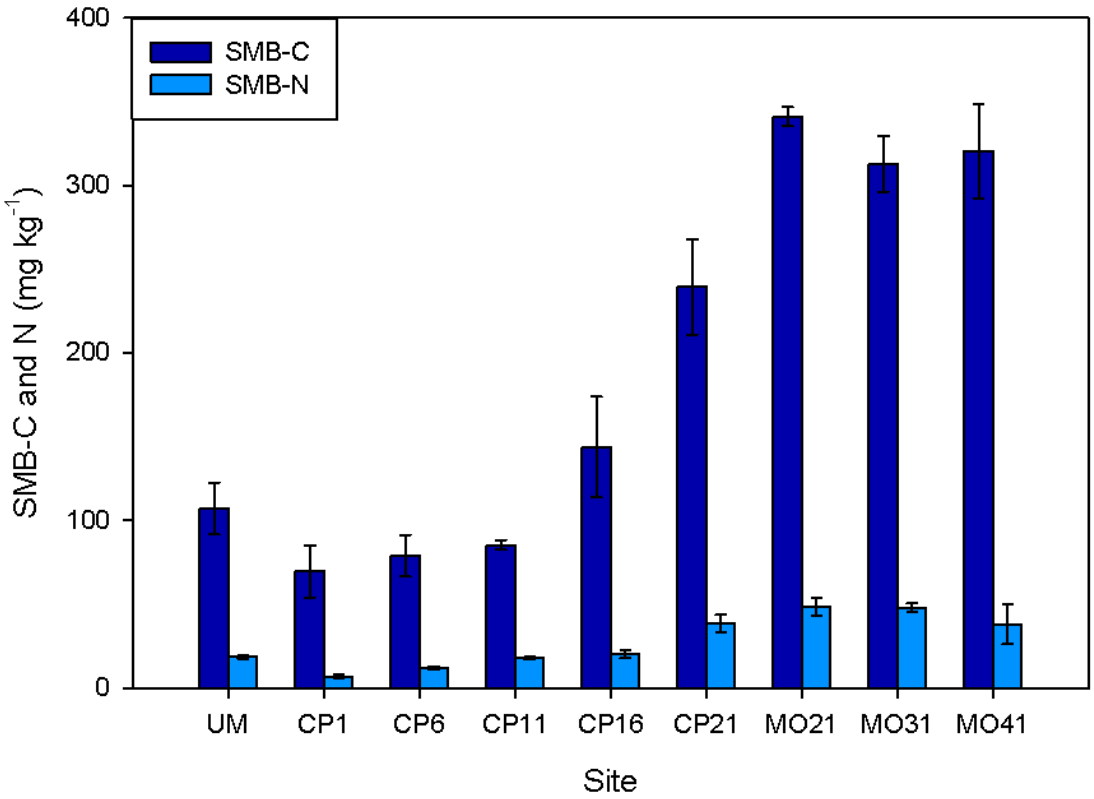

Fig. 5. Soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) in a post-lignite mining rehabilitation chronosequence. Unmined (UM) and mined soils [crosspit spreader (CP) from 1 to 21 years and mixed overburden (MO) from 21 to 41 years] were sampled. Vertical bars indicate standard error of the mean.

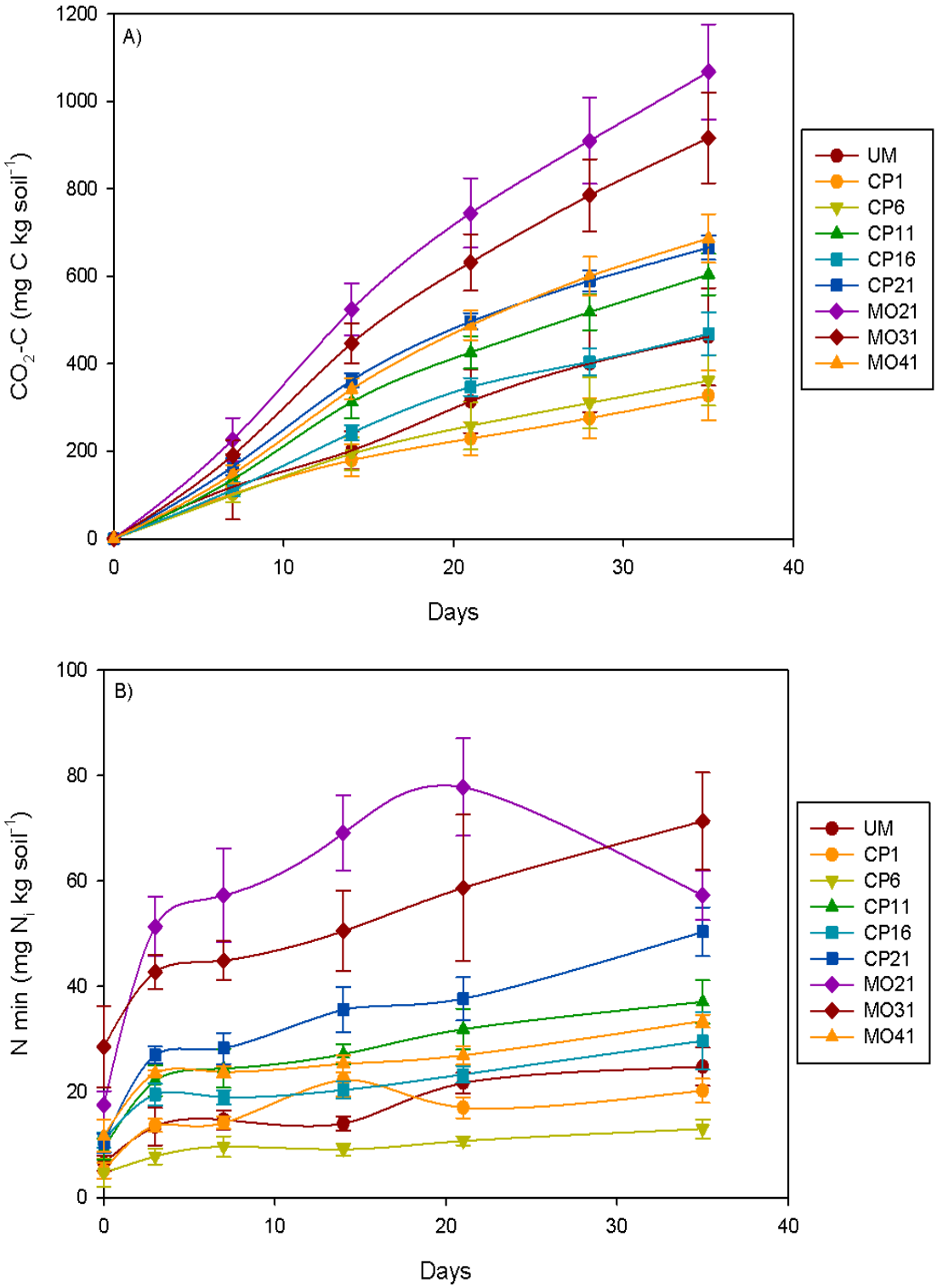

Fig. 6. A) Carbon and B) nitrogen mineralization rates during 35 days of incubation in a post-lignite mining rehabilitation chronosequence. Unmined (UM) and mined soils [crosspit spreader (CP) from 1 to 21 years and mixed overburden (MO) from 21 to 41 years] were sampled. Vertical bars indicate standard error of the mean.

DISCUSSION

SMCRA states that the goal of reclamation is to return disturbed lands to a pre-mined or improved condition (1977). However, the law has limited soil criteria for these reclaimed lands, which measure reclamation success based on contouring, hydrology and vegetative productivity. The criteria for backfilled surface soil suitability include: < 80% sand, < 40% clay, pH 5.0-8.4, > 0.0 acid/base (T $CaCO_3$/1000 T soil), < 4.0 EC (mmhos/cm), and < 13.0 sodium adsorption ratio (SAR). These criteria result in an interesting problem when returning a disturbed land to a pre-mined condition. Table 1 showed that the texture of all the reclaimed sites (CP and MO) did not match the texture of the unmined site (UM), which was not an uncommon feature of other reclaimed mine soil (RMS) studies (Ingram et al., 2005; Schroeder et al., 2010). Derived from reclamation law, this crucial change of soil texture in a post-mined site makes its exact return to a premined condition impossible. While a site with a sandy texture is not an intrinsically unproductive soil, there are some secondary properties of a high sand site that could possibly hinder vegetation establishment or the rate of revegetation. Given the rich history of mine reclamation in Texas (+40 yrs), the established soil criteria ensure effective and efficient vegetative productivity for improved land conditions in favor of a return to premined conditions.

All soil parameters returned to or exceeded premined conditions (Table 6). Total soil carbon accumulated over the duration of the reclamation chronosequence (Fig. 1), increasing by 9.50 g kg^{-1} over 20 years in CP sites and by 14.70 g kg^{-1} in MO sites. Most of this carbon was in the form of non-lignitic organic carbon (SC) tied to Ma and Sma (> 2 mm, 2 mm – 250 μm) (Table 2, Fig. 3A), with IC coming from deeper in the soil during the respreading process or lime application. However, an absence of Ma and/or lower amounts of Sma in the

UM site was a direct result of the minimal amounts of S + C, which lead to lower carbon levels sequestered in the top 0-15 cm (Elliott and Coleman, 1988; Lorenz and Lal, 2007; Oades, 1984; Six et al., 2000a; 2000b). Shresta and Lal showed that the opposite held true if coarser particles are mixed with finer particles during reclamation (2008). Slightly better overburden segregation in texture was observed in CP sites compared to MO sites (Table 3). Carbon in the UM site was mainly relegated to Mi (>60%), while carbon in RMS was most associated with Ma and Sma (60-77%), similar to that seen in Ussiri et al. (2006) (Fig. 4). Differences in SC by depth between 20 year old RMS (CP and MO) were significant, with higher concentrations in the MO site. This was probably due to the increase in clay content and Ma formation. Our aggregation study was relegated to the 0-15 cm soil interval. It would be interesting to know how aggregates from deeper in the soil profile changed between our reclamation sites (Lorenz and Lal, 2005). Ussiri et al. (2006) showed that the carbon concentration in Ma and Sma decreased with depth, but does the same hold true for the contribution of these aggregates to the total carbon content? The change in small macro-aggregates and reclamation technique (CP vs. MO) probably had an effect on the amount of carbon that could potentially be sequestered over time. While we could not see a plateau effect in the CP sites, there seemed to be a ceiling of ~300 Mg C ha^{-1} in the MO sites. Further studies and/or time will tell if carbon will continue to be sequestered. Aside from their importance in physical properties and in water and gas movement and rooting depth, aggregation studies will be important to the energy industry because of C sequestration and C credit balancing. As lignite contains about 25-35% carbon, the amount of SC sequestered over the course of reclamation replaced ~2.6 to 3.6% of lignite carbon burned (KET, 2012).

Table 6. List of soil quality parameters: physical and chemical characteristics, carbon pools, nitrogen, and biological properties equivalent to unmined conditions or exceeded through 1 m.

	Soil Property											
Yr	pH	CEC	BD	TC	IC	OC	SC	TN	SMB-C	SMB-N	Cmin	Nmin
0 CP	X	X	X	X	X	X	X					
5 CP	X	X	X	X	X	X						
10 CP		X	X	X	X	X	X	X			X	X
15 CP		X	X	X	X	X	X		X	X	X	X
20 CP		X	X	X	X	X	X		X	X	X	X
20 MO	X	X	X	X	X	X	X	X	X	X	X	X
30 MO	X	X	X	X	X	X	X	X	X	X	X	X
40 MO	X	X	X	X	X	X	X	X	X	X	X	X

X = the soil quality parameter recovered to premined conditions. CEC = cation exchange capacity. BD = bulk density. TC = total carbon. IC = inorganic carbon. OC = organic carbon. SC = non-lignitic organic carbon. TN = total nitrogen. SMB-C = soil microbial biomass carbon. SMB-N soil microbial biomass nitrogen. Cmin = carbon mineralization. Nmin = nitrogen mineralization.

While all reclamation sites increased soil carbon stocks compared to UM, there was not a linear increase (Fig. 1). Carbon stocks were calculated as a combination of carbon concentrations, soil depth and BD. Since BD in the CP0 site was not significantly higher than other sites in the chronosequence and comparable with other bulk densities from RMS in past studies, we looked to other factors that might explain the relatively high carbon stock of CP0 (Ussiri et al., 2006) (Table 1). The CP0 site had unexpectedly high amounts of total carbon (TC) that contradicted what was found in young reclamation sites of other post-mining reclamation carbon studies (Abdul-Kareem and McRae, 1984; Ingram et al., 2005; Lal et al., 1998; Sourkova et al., 2005; Ussiri et al., 2006). The age of this site suggested that the spoil material used for backfilling was already high in TC. Table 4 revealed that some carbon fractions were not uniform throughout the chronosequence and could have influenced this unexpectedly higher carbon pool in this early stage of reclamation. As OC was the major fraction of TC, we noticed that there was a higher amount of lignitic carbon in CP0 (Table 4). It is interesting to note that LC also was observed in the UM site, suggesting that another

highly recalcitrant form of carbon might also be present in the reclaimed sites. High IC in the CP0 site suggests either carbonate materials (ex. siderite) in the original overburden or an early undocumented application of lime as observed in pH (Table 2). One final answer might be divulged in our selection of chronosequence sites and their soil series. We attempted to select sites that were higher in elevation to avoid the effects of runoff, but CP0 was classified as a Nahatche-Hatliff association. If we sampled from the Nahatche soil of this association, then the higher carbon amount could derive from the poor drainage that is characteristic of a frequently flooded soil, and not the better drained Hatliff soil series. With IC and LC subtracted from TC, SC in the CP0 was only higher than the CP5 site. If the CP0 site was removed from the study, the remaining sites of the chronosequence would match carbon sequestration trends found in other studies (Akala and Lal, 2000; 2001; Haering et al., 1993; Lal et al., 1998; Sourkova et al., 2005).

The secondary objective of this study was to observe changes in physical and chemical properties with depth and determine when a premined stratification was observed. Our analysis went to 1 m (3.28 ft) of soil depth, which is slightly shallower than 1.2 m (4 ft) normally required for reclamation of US surface mines. During the topsoil and overburden removal and storage, the greater concentration of carbon and nutrients present in topsoil was mixed with materials from deeper in the profile, creating a dilution effect that results in relatively lower SOC and nutrient concentrations in the reclaimed surface and affects the heterogeneity of soil respiration across sites (Ingram et al., 2005). Table 5 illustrated that despite the enhanced topsoil and overburden separation of CP reclamation compared to MO, the CP0 site showed carbon concentration uniformity through 1 m. The same soil profile uniformity might not be assumed 20 to 40 years earlier for MO sites as the MO reclamation

process was inherently more variable. Stratification was observed over time for carbon in CP and MO sites, but one key difference was that MO sites were not as well segregated as the CP sites. This result may indicate faster segregation from 5 to 20 years for CP, or slower segregation in MO sites. Yet given the limited time of our chronosequence, our RMS did not show the same carbon stratification as the UM site. Tables 3 and 5 showed that depth differences in carbon in the near surface soil occurred faster than soil deeper than 30 cm. Ingram et al. (2005) were able to detect smaller changes in carbon deeper in soil cores (2.5-15 cm) compared to soil from 0-2.5 cm. The cause of this difference in depth is mainly due to vegetation and root penetration, which removes nutrients at shallower depths earlier in reclamation and in deeper depths over time (Akala and Lal, 2000; 2001; Jobbagy and Jackson, 2001; Lal et al., 1998). While nutrient concentration and stratification are not regulated in surface mine reclamation, it is important that nutrient distribution by both age and depth be studied so that the functionality of reclaimed sites moves towards a premined or improved stage. From a management standpoint, reclaimed land must focus primarily on revegetation, erosion prevention, and carbon sequestration (Lorenz and Lal, 2005). If one is aiming to rehabilitate a soil profile to unmined conditions, there are many factors involved in the distribution of these parameters (e.g. vegetation, temperature, texture, and time). If one is using time as the only factor, Ussiri et al. (2006) found that even sites older than 70 years reclaimed as a forest did not reach undisturbed levels of stratification. Yet decisions must be balanced with economic and environmental sustainability as seen with the replacement of the MO technique with the CP method that was more efficient and resulted in better nutrient segregation.

Given its importance to revegetation, nitrogen should be available in sufficient quantities to support plant growth, which would lead to more added soil carbon via plant and root development. We observed a recovery of soil TN that matched UM levels immediately after reclamation (CP0), and exceeded UM levels after 10 years of reclamation (Fig. 3). The recovery of TN in all reclamation sites was not a surprise given the differences in fertilizer that were applied to sites. In addition, CP15 and CP20 were revegetated with two legume species. Aggregate contributions to TN were similar to those observed for TC, but a slightly higher contribution of microaggregates (Mi) to TN was observed in reclaimed sites compared to Mi contribution to TC (Fig. 4). Given the smaller diameter size of Mi (250 μm – 53 μm) compared to Ma and Sma, we might speculate about the sequestration potential of aggregates with organic matter (OM) that have different carbon to nitrogen ratios. A study by Cambardella and Elliot (1993) measured carbon and nitrogen from cultivated and native grassland soils. They found that while carbon to nitrogen ratios were similar, aggregate stability was higher in native soils and aggregates had higher carbon and nitrogen concentrations. As for management concerns, our data could provide the basis for more chronosequence aggregation research, and associated OM and nutrient cycling rates.

Soil microbial biomass pools and mineralization (Cmin and Nmin) did follow a trend that agreed with past literature with a decrease after mining followed by a steady increase with reclamation age (Figs. 2 and 4). Given the change in texture having such a large effect on the trend of TC and TN, it was refreshing to see two previously identified soil quality indicators follow similar trends from other studies regardless of texture (Akala and Lal, 2001; Chodak and Niklinska, 2010; Ingram et al., 2005; Stroo and Jenks, 1982). The recovery of mineralization and SMB required 15 years (Figs. 5 and 6). The discrepancy between our

study and previous literature may suggest a change in the microbial community or environmental variables (e.g. pH, moisture, temperature) that influence microbial activity (Machulla et al., 2005; Waggoner, 1993). Our mineralization experiment was conducted the same year as the aggregation study, giving us insight into dynamics of SOC within soil aggregates which usually has a lower decomposition rate than the more labile carbon located outside aggregates (Elliott and Coleman, 1988; Oades, 1984; Six et al., 2000b). Abdul-Kareem and McRae (1984) stated that rapid mineralization of carbon from destroyed aggregates would result in a decline in TC. Conversely, a decline in mineralization from aggregate formation could result in an increase in SC (Cambardella and Elliot, 1993; Elliot and Coleman, 1988; Golchin et al., 1994; Gregorich et al., 1991; Oades, 1984; Schlesinger, 1997; Six et al., 2000b; van Veen and Kuikman, 1990). More research would have to be completed to determine the exact mineralization contributions of each aggregate fraction, and which types of labile and recalcitrant carbon were present in the chronosequence. Finally, we believe that it is important that carbon to nitrogen ratios be maintained over time to stabilize mineralization rates. Past RMS studies show that a narrowing of the carbon to nitrogen ratio too suddenly may result in potential NO_3^- leaching after nitrification (Banning et al., 2008; Davidson et al., 1992; Schimel and Bennett, 2004).

CONCLUSION

Studies of RMS have shown the importance of measuring soil carbon and nitrogen accumulation and distribution in the soil profile. Reclamation managers at Big Brown Mine have successfully restored productivity to post-mined lands using the relatively new crosspit spreader technique. We conclude that the crosspit spreader technology was able to return

carbon and nitrogen concentrations to premined conditions within 5 years, and that texture, aggregation and age were important factors in soil quality indicators in reclaimed minesoils. We also found the return of carbon and nitrogen pools in biologically-associated fractions after 16 years. The practice of mixed overburden exceeded TC and TN concentrations beyond UM and CP levels. More research should be conducted on the biological and chemical properties of soil aggregates. Additionally, other nutrients should be analyzed to determine their effect on revegetation and subsequent reclamation of surface mines. Our analysis to 1 m depth of soil will be important not only for environmental restoration, but for grassland ecosystem research in which the detection of nutrient movement over shorter periods of time would be crucial for shallower root systems.

CHAPTER III

RECOVERY OF MACRO- AND MICRONUTRIENTS IN A POST-LIGNITE MINING REHABILITATION CHRONOSEQUENCE IN EAST TEXAS

INTRODUCTION

Surface mining for coal usually drastically disturbs the original soil profile, and alters soil physical, chemical, and biological conditions (McSweetney and Jansen, 1984). A goal of land reclamation on post-surface mined areas should be to return disturbed sites to a premined or enhanced state (SMCRA, 1977). While vegetation plays a major role in improving reclaimed mine soils (RMS) over time, the initial reclamation steps of topsoil backfilling, reconstruction, and regrading control the distribution of soil nutrients and other characteristics in the soil profile (Bradshaw, 1987; Merrill et al., 1998) and are crucial for early vegetational success and microbial activity. Chemical and physical properties [clay, sand, pH, electrical conductivity (EC), sodium adsorption ratio (SAR), and acid/base ratio] are regulated during soil backfilling at mined sites, but plant essential nutrients are not, despite their importance in successful revegetation and the recovery of nutrient cycles. Nutrients are lost as topsoil is removed and stored, and also when soil aggregates are disrupted, freeing nutrients for mineralization (Adu and Oades, 1978; Six et al., 2000a; Ussiri and Lal, 2008). Additionally, nutrient concentrations decrease when soil horizons are diluted during mixing or eroded when spoil banks are left exposed before the backfilling and regrading stages occur (Ingram et al., 2005; Lal et al., 1998). Impediments to improving the quality of RMS can stem from a lack of revegetation from deteriorated soil conditions,

including low soil organic matter content, high salinity, poor soil structure, and reduced soil fertility (Kleeberg et al., 2008; Sencindiver and Ammons, 2000; Ussiri et al., 2006).

Reclamation specialists improve soil quality by enhancing the availability and cycling of soil nutrients through a variety of methods (Barnhisel and Hower, 1997; Bradshaw, 1997; Coyne et al., 1998; Hons 1978; Ingram et al., 2005; Lorenz and Lal, 2007; Palmer et al., 2010; Whitford, 1988). Soil organic carbon (SOC) is a well-documented indicator of RMS quality because it influences both soil chemical (e.g. nutrient concentrations) and physical properties (e.g. aggregation). Being a good gauge of soil quality, SOC usually decreases after mining begins (Abdul-Kareem and McRae, 1984; Ingram et al., 2005; Lal et al., 1998; Ussiri et al., 2006) and increases over time with enhanced biomass production and root development from vegetation (Akala and Lal, 2000; 2001; Haering et al., 1993; Lal et al., 1998;). The presence of SOC has been linked to the availability of plant essential nutrients. Macronutrients have been studied most extensively on RMS, as N (Anderson et al., 2008; Banning et al., 2008; Schimel and Bennett, 2004; Ussiri et al., 2006), and P (Komnitsas et al., 2010; Sourkova et al., 2005; Zipper et al., 2011) are taken up in significant quantities by plants, and are important to hydrologists and environmentalists because of impacts in runoff. As an important nutrient for grasses, K recovery is also significant (Kleeberg, 2008; Komnitsas et al., 2010). Secondary nutrients, such as S, have mostly been reported in relation to acid-forming materials (AFM) (Cummins et al., 1965; Pugh et al., 1984), while less data exists on Ca and Mg as liming is a heavily utilized reclamation practice on RMS (Chambers et al., 1987; Thorne and Cardina, 2011). Similarly, micronutrients such as Mn have only been considered when occurring in toxic levels and have been less extensively studied (Barnhisel and Massey, 1969; Cummins et al., 1965). Despite their importance in vegetative success and

in managing sites for future productivity, changes in soil chemical characteristics and their relations with nutrient concentrations have not been studied in detail in RMSs.

Successful reclamation efforts have been reported not only in the US [Ohio (Lorenz and Lal, 2007; Ussiri et al., 2006), Kentucky (Coyne et al., 1998), and Wyoming (Anderson et al., 2008; Ingram et al. 2005)], but also around the world, including China (Komnitsas et al., 2010) and Spain (Aguilar et al., 2004). The Big Brown Mine in eastern Texas includes successfully rehabilitated RMS sites that have supported croplands and pastures since the late 1960s (Angel, 1973; Askenasy, 1977; Hons, 1978; Toups, 1986). Since the number and ages of the reclaimed sites at Big Brown Mine are increasing with time (currently >40 years), we designed an investigation to determine how soil chemical parameters changed over time following surface mining and reclamation. The first objective of this research was to quantify the chemical characteristics of RMS in a chronosequence that ranged from 0 to 40 years, and to determine when conditions returned to a premined state. While past studies of RMS have shown the importance of measuring nutrient stratification in the soil profile (Anderson et al., 2008; Ussiri et al., 2006), our second objective was to determine if we could improve on this parameter by sampling deeper to 1 m. This is especially important for post-mined sites managed as pasturelands vegetated with grass species or sites that have been freshly seeded, where the detection of shallower soil nutrient movements is critical (Lorenz and Lal, 2005). Our hypothesis was that soil quality indicators would decrease after mining and increase over time before reaching a maximum capacity (Odum, 1969), and that nutrient concentrations would decrease with depth.

MATERIALS AND METHODS

Site Description and Soil Sampling

The Big Brown Mine is a lignite surface mine located east of Fairfield, TX and opened in 1971 (Table 1). The predominant soil series in the area of the mine are Axtell, Edge, and Tabor, and are mostly loamy or sandy soils over clayey subsoils that limit water and gas movement (Peach, 2001). Fairfield has a gently rolling to hilly topography (100-270 m above sea level) and is located within the Post Oak Savannah vegetation region of Texas (Gould, 1975). The regional mean annual temperature is 18.9 °C with July and August being the hottest months of the year with daily average maximum temperature of 35.6 °C as measured from 1962 to 1990. Annual average precipitation is approximately 970 mm, with the highest monthly rainfall normally occurring in May (120 mm) and the lowest in July (50 mm) as measured from 1941 to 1990. During the early reclamation era (1970-1980s), the mixed overburden technique (MO) was utilized where RMS was backfilled and regraded with a mixed combination of topsoil and overburden. Mixed overburden was shown to provide a material with better chemical and physical properties that enhanced revegetation compared to native topsoil alone (Bearden, 1984; Dixon et al., 1980; Hons, 1978). About 20 years ago (1986), the crosspit spreader (CP) technique replaced MO. The CP technique improved the speed of material segregation by separating the topsoil/topsoil substitute material from overburden and generally results in more uniform mixing of these materials.

Selected sampling sites had minimal slope and were on tops of hills to minimize effects from runoff, erosion, and siltation. Five CP sites were selected with post-reclamation ages of 0, 5, 10, 15, and 20 years (CP0, CP5, CP10, CP15, and CP20). Three MO sites were selected with post-reclamation ages of 20, 30 and 40 years (MO20, MO30, and MO40)

(Table 1). For comparison of reclamation success, in which land is returned to a pre-mined or improved state, a control site that was unmined (UM) with an undetermined age was selected.

All sites were revegetated primarily with coastal bermudagrass (*Cynodon dactylon L. pers.*) and managed predominately as grassland and pastureland. The UM site was classified as part of the Padina series and was additionally vegetated with Bahia grass (*Paspalum notatum*). Hay on the UM site was baled (~two 1200 kg bales/ha) twice a year before being grazed (~1 cow/2 ha) yearly and fertilized with 40-0-0 at 140 kg/ha. The CP0 and CP5 sites were initially revegetated with wheat (*Triticum L.*) and rye grass (*Lolium spp.*) for temporary cover before coastal bermudagrass was sprigged. The CP0 site was classified within the Nahatche-Hatliff soil association and fertilized with 13-13-13 at 280 kg/ha during seeding. The CP5 site received the same fertilizer treatment as CP0. The CP10, 15, and 20 sites were fertilized with 17-17-17 at 280 kg/ha (during their initial revegetation). Both the CP5 and CP10 sites were originally classified as Tabor series. Sites CP10, CP15, and 20 were hayed (~three 1100 kg bales/ha) and grazed yearly at a density of ~1 cow/ha. CP15 was initially classified as part of the Edge series, while CP20 was classified as part of the Silawa series. Sites CP15 and CP20 were also revegetated with two legume species: Yuchi Arrowleaf clover (*Trifolium vesiculosum*) and Crimson clover (*Trifolium incarnatum*), that were overseeded into coastal bermudagrass. The MO20 site was grazed and hayed similar to the CP 10, 15, and 20 sites, but was fertilized additionally with chicken litter at 4.5 t/ha. The MO30 site was grazed (~1 cow/ha), and fertilized with 44-0-0 at 140 kg/ha. The MO40 site also differed slightly from the other reclamation sites in that Johnson grass [*Sorghum halepense (L.) pers.*] encroached upon the coastal bermudagrass at this site. The site was

hayed (~three 1100 kg bales/ha) and fertilized with 17-17-17 at 280 kg/ha. All MO sites were classified as part of the Bigbrown soil series. Limestone was applied at 9.9-12.3 t/ha for pH maintenance (pH < 5).

Soils were sampled on 22 June 2009 when peak plant growth was expected (Swanson 1996). At each sampling site, a spot was randomly chosen as the center of the sampling plot. Fifteen m were measured in each cardinal direction from the center (N to S, and E to W), as well as in bisecting directions (SE to NW, and SW to NE), forming a 30-m diameter circular area with a total of four 30-m transects. Ten 1-m deep cores (5 on each side of the center to avoid repeat samples at the center) were taken across the diameter of the circle with roughly 3 m between each sample core. Care was taken to avoid sampling obvious manure from cattle. Soil cores (15-mm radius) were taken using a hydraulic probe mounted on a weighted truck. Each 1-m core was then divided into 5 depths: 0-15, 15-30, 30-45, 45-75, and 75-100 cm. Soil from individual depth increments and transects was composited, placed in polyethylene bags in coolers and weighed shortly after sampling to obtain a field moist weight. Samples were then transported back to the Texas A&M University campus (College Station, TX) within 12 hours and air-dried at 4 °C on brown paper, broken with a combination of mortar/pestle and hand, and passed through a 4-mm sieve.

Soil Chemical and Physical Analysis

Subsamples of soil were analyzed for texture, pH, EC, SAR and extractable nutrient concentrations. Texture was determined using 50.0 g of soil and the hydrometer procedure. This method was performed at room temperature using deionized (DI) water in a 1-L graduated cylinder (Day, 1965). Soil pH was determined in a 1:2 soil:water extract of the soil

using DI water. Samples were stirred and allowed to equilibrate for a minimum of 30 minutes after adding DI water before determination with a hydrogen selective combination electrode (Schofield and Taylor, 1955). Soil EC was determined in a 1:2 soil:water extract of the soil using DI water. Samples were stirred and allowed to equilibrate for a minimum of 30 minutes after adding the water. The actual determination was made using a conductivity probe fitted with a 10 mm dipping electrode and reported in µmol/cm (Rhoades, 1982).

Nitrate-N (NO_3^--N) was extracted from soils using a 1 N KCl solution and a 1:10 soil to extractant ratio. Nitrate was determined by reduction of NO_3^- to nitrite-N (NO_2^--N) using a cadmium column followed by spectrophotometric measurement (FIALab 2500) (Bellevue, Washington, USA) (Keeney and Nelson 1982). Additional nutrients (P, K, Ca, Mg, Na, and S) were extracted using the Mehlich III extractant (1:10 soil:extractant) and were analyzed using a Genesis Radial Inductively Coupled Plasma (ICP) (Spectro, Germany). The Mehlich III extractant is a dilute acid-fluoride-EDTA solution at pH 2.5 that consists of 0.2 N CH_3-COOH, 0.25 N NH_4NO_3, 0.015 N NH_4F, 0.013 N HNO_3, and 0.001 M EDTA (Mehlich, 1984). Sodium adsorption ratio was calculated by dividing Na (meq/L) by the square root of Ca + Mg (meq/L) divided by 2. Micronutrients (Cu, Fe, Mn and Zn) were extracted (1:2 soil:extractant) using a 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.10 M triethanolamine solution (TEA) (Lindsay and Norvell, 1978). The analytes were determined using an Arcos Axial ICP (Spectro, Germany).

Statistical Analysis

Analysis of variance (ANOVA), Student's t-test, pairwise (r^2), and Spearman's ρ tests were used to determine significant differences in measured characteristics between ages and

correlations using JMP 9 (SAS Institute, 2011). Statistical tests were conducted between the UM, CP, and MO sites. A p-value of 0.05 was used as a minimum for determining significant differences.

RESULTS

Soil pH, Electrical Conductivity, Sodium Adsorption Ratio and Texture

Unmined pasture (UM) soil had near neutral pH, acidifying slightly with depth (Tables 7 and 8). The CP0 site had a uniform pH through the soil profile that was significantly more alkaline than the UM site. Soil pH in 5 to 20 year CP sites through 1-m depth ranged from 4.28 to 6.63 and were acidic relative to the UM and CP0 sites. All CP sites became more acidic with depth, especially in sites 5, 15 and 20 years old. Soil pH of MO sites for all ages and depths were similar to the UM site. A trend of increased pH with age was observed for MO sites from 0-30 cm of depth. All MO sites displayed slight acidification with depth. Soils from CP20 were more acidic than those from MO20. Both sites displayed acidification with depth, but the changes were greater for the CP site.

Table 7. Soil chemical and physical characteristics of an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by site. Data are displayed as an average of data from 4 transects at each site.

	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
pH	0-15	6.70b	7.78a	6.63bc	5.30d	5.40d	6.15c	6.38bc	6.73b	6.88b
	15-30	6.70b	7.68a	5.30d	5.35d	5.35d	4.98d	6.00c	6.10bc	6.33bc
	30-45	6.65b	7.68a	5.00de	5.20de	5.10de	4.68e	6.23bc	5.60cd	5.95c
	45-75	6.45b	7.68a	5.30cd	5.13de	4.88de	4.48e	5.90bc	5.43cd	6.33bc
	75-100	6.23b	7.70a	5.63bcd	5.33cd	5.05de	4.28e	5.73bcd	5.35bcd	5.93bc
EC	0-15	0.045e	0.439a	0.343ab	0.210cd	0.178d	0.171d	0.333ab	0.339ab	0.294bc
(dS m^{-1})	15-30	0.024d	0.821a	0.515b	0.303bc	0.118cd	0.104cd	0.249c	0.270c	0.238c
	30-45	0.021e	0.787a	0.648a	0.449bc	0.122de	0.105de	0.278cd	0.304cd	0.284cd
	45-75	0.019f	0.853a	0.680ab	0.553abc	0.162def	0.127ef	0.401bcde	0.478bcd	0.338cdef
	75-100	0.018d	0.877a	0.773a	0.824a	0.178cd	0.267bcd	0.509abc	0.882a	0.679ab
SAR	0-15	1.13a	0.83b	0.67c	0.50d	0.46d	0.32e	0.35e	0.28e	0.31e
	15-30	1.34a	1.15b	1.36a	0.88c	0.39d	0.44d	0.34d	0.32d	0.34d
	30-45	1.54a	1.26b	1.72a	1.26b	0.46c	0.51c	0.34c	0.36c	0.43c
	45-75	1.59a	1.28b	1.72a	1.61a	0.58c	0.54cd	0.41cd	0.38d	0.43cd
	75-100	2.04a	1.16c	1.70b	2.09a	0.54d	0.54d	0.50d	0.43d	0.39d
Sand	0-15	92.8a	44.3c	57.0b	35.0d	60.5b	56.5b	38.8cd	25.0e	40.0cd
(%)	15-30	94.3a	40.3d	62.3b	39.3de	60.0bc	53.5c	32.3e	22.3f	33.0de
	30-45	94.0a	38.3cd	60.8b	39.8c	56.3b	56.5b	32.2e	24.8e	31.5de
	45-75	92.0a	37.8c	58.8b	39.3c	58.8b	54.3b	34.3c	21.8d	37.0c
	75-100	89.7a	42.3cd	60.8b	39.3d	62.3b	48.8c	37.5d	28.8e	37.0d
Silt	0-15	2.5e	24.7cd	21.5d	37.0ab	18.0d	23.0cd	34.0b	44.0a	30.5bc
(%)	15-30	2.5f	27.7cd	18.5e	34.7bc	19.5e	23.0de	38.5b	46.2a	35.0bc
	30-45	2.0e	30.7bc	20.5d	35.2b	22.5cd	22.0cd	34.5b	44.8a	35.0b
	45-75	3.0f	29.2cd	22.5e	36.7b	19.5e	25.5de	34.0bc	45.2a	30.8bcd
	75-100	3.3e	25.7cd	19.5d	33.3bc	19.0d	30.5bc	34.0b	41.8a	30.8bc
Clay	0-15	5.3c	31.0a	21.5b	28.0a	21.5b	20.5b	27.2a	31.0a	29.5a
(%)	15-30	3.2f	32.0a	19.2e	26.0bc	20.5de	23.5bc	29.2ab	31.5a	32.0a
	30-45	4.0d	32.0a	18.7c	25.0b	21.2bc	21.5bc	33.3a	30.5a	33.4a
	45-75	5.0d	33.0a	18.7c	24.0b	21.7bc	20.2c	31.7a	33.0a	32.2a
	75-100	7.0d	32.0a	19.8c	27.4b	18.7c	20.7c	28.5ab	29.4ab	32.2a
Tex-	0-15	s	cl	scl	cl	scl	scl	scl	cl	cl
tural	15-30	s	cl	sl	l	scl	scl	cl	cl	cl
Class	30-45	s	cl	sl	l	scl	scl	cl	cl	cl
	45-75	s	cl	sl	l	scl	scl	cl	cl	cl
	75-100	s	cl	sl	l	sl	l	cl	cl	cl

Means in the same row followed by the same letter are not significantly different at P<0.05. UM=unmined pasture. CP = crosspit spreader. MO = mixed overburden. EC = electrical conductivity. SAR = sodium adsorption ratio. s = sandy. c = clay. l = loam.

Table 8. Soil chemical and physical characteristics of an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by depth. Data are displayed as an average of data from 4 transects at each site.

	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
pH	0-15	6.70A	7.78A	6.63A	5.30A	5.40A	6.15A	6.38A	6.73A	6.88A
	15-30	6.70A	7.68A	5.30B	5.35A	5.35A	4.98B	6.00A	6.10B	6.33AB
	30-45	6.65A	7.68A	5.00B	5.20A	5.10AB	4.68BC	6.23A	5.60BC	5.95B
	45-75	6.45A	7.68A	5.30B	5.13A	4.88B	4.48BC	5.90A	5.43C	6.33AB
	75-100	6.23A	7.70A	5.63AB	5.33A	5.05AB	4.28C	5.73A	5.35C	5.93B
EC	0-15	0.045A	0.439B	0.343A	0.210D	0.178A	0.171AB	0.333AB	0.339B	0.294B
(dS m^{-1})	15-30	0.024B	0.821AB	0.515A	0.303CD	0.118B	0.104B	0.249B	0.270B	0.238B
	30-45	0.021B	0.787AB	0.648A	0.449BC	0.122B	0.105B	0.278AB	0.304B	0.284B
	45-75	0.019B	0.853AB	0.680A	0.553B	0.162AB	0.127B	0.401AB	0.478B	0.338B
	75-100	0.018B	0.877A	0.773A	0.824A	0.178A	0.267A	0.509A	0.882A	0.679A
SAR	0-15	1.13D	0.83B	0.67C	0.50E	0.46AB	0.32B	0.35B	0.28C	0.31C
	15-30	1.34CD	1.15A	1.36B	0.88D	0.39B	0.44AB	0.34B	0.32BC	0.34BC
	30-45	1.54BC	1.26A	1.72A	1.26C	0.46AB	0.51A	0.34B	0.36AB	0.43A
	45-75	1.59B	1.28A	1.72A	1.61B	0.58A	0.54A	0.41B	0.38AB	0.43A
	75-100	2.04A	1.16A	1.70A	2.09A	0.54A	0.54A	0.50A	0.43A	0.39AB
Sand	0-15	92.8AB	44.3A	57.0A	35.0A	60.5A	56.5A	38.8A	25.0A	40.0A
(%)	15-30	94.3A	40.3AB	62.3A	39.3A	60.0A	53.5A	32.3AB	22.3A	33.0A
	30-45	94.0A	38.3AB	60.8A	39.8A	56.3A	56.5A	32.2B	24.8A	31.5A
	45-75	92.0AB	37.8B	58.8A	39.3A	58.8A	54.3A	34.3AB	21.8A	37.0A
	75-100	89.7B	42.3AB	60.8A	39.3A	62.3A	48.8A	37.5AB	28.8A	37.0A
Silt	0-15	2.5A	24.7A	21.5A	37.0A	18.0A	23.0A	34.0A	44.0A	30.5A
(%)	15-30	2.5A	27.7A	18.5A	34.7A	19.5A	23.0A	38.5A	46.2A	35.0A
	30-45	2.0A	30.7A	20.5A	35.2A	22.5A	22.0A	34.5A	44.8A	35.0A
	45-75	3.0A	29.2A	22.5A	36.7A	19.5A	25.5A	34.0A	45.2A	30.8A
	75-100	3.3A	25.7A	19.5A	33.3A	19.0A	30.5A	34.0A	41.8A	30.8A
Clay	0-15	5.3AB	31.0A	21.5A	28.0A	21.5A	20.5B	27.2A	31.0AB	29.5A
(%)	15-30	3.2B	32.0A	19.2A	26.0A	20.5AB	23.5A	29.2A	31.5AB	32.0A
	30-45	4.0AB	32.0A	18.7A	25.0A	21.2AB	21.5AB	33.3A	30.5AB	33.4A
	45-75	5.0AB	33.0A	18.7A	24.0A	21.7A	20.2B	31.7A	33.0A	32.2A
	75-100	7.0A	32.0A	19.8A	27.4A	18.7B	20.7AB	28.5A	29.4B	32.2A
Text-	0-15	s	cl	scl	cl	scl	scl	scl	cl	cl
ure	15-30	s	cl	sl	l	scl	scl	cl	cl	cl
Class	30-45	s	cl	sl	l	scl	scl	cl	cl	cl
	45-75	s	cl	sl	l	scl	scl	cl	cl	cl
	75-100	s	cl	sl	l	sl	l	cl	cl	cl

Means in the same column and characteristic followed by the same letter are not significantly different at $P<0.05$. UM=unmined pasture. CP = crosspit spreader. MO = mixed overburden. EC = electrical conductivity. SAR = sodium adsorption ratio. s = sandy. c = clay. l = loam.

Electrical conductivity (EC) decreased with depth and was much lower in the UM site for all depths compared to the mined chronosequence sites (Tables 7 and 8). For CP sites, EC was highest in the year 0 site, and decreased with time before stabilizing from 15 to 20 years of reclamation. In all CP reclamation sites, EC increased with depth. In CP sites >15 years old, EC slightly decreased from 0-15 to 15-30 cm. MO sites had significantly greater EC than the unmined site, with the highest values at age 30. Distribution of soil EC by depth in all MO sites exhibited a slight decrease from 0 to 30 cm before increasing with depth to 100 cm. Electrical conductivity of the surface soil of MO20 was higher than that of CP20.

Sodium adsorption ratio (SAR) throughout the 1-m soil profile in the UM site was higher than that of most soil intervals from reclamation sites (Tables 7 and 8). For the UM site, SAR increased with depth. Surface CP soils (0-15 cm) exhibited decreasing SAR with age. At CP soil depths from 15-75 cm, SAR fluctuated by increasing in the first 5 years, and then decreasing to 15 years and stabilizing. All MO sites had lower SAR than CP sites, but MO20 remained statistically similar to CP20.

The UM site had a very sandy texture compared to reclaimed sites (Table 7). Soil texture in CP0 and CP10 sites varied from loams to clay loam, while that of CP5, CP15, and CP20 were sandy loam and sandy clay loam. Few textural differences were observed through the profile among CP sites. MO sites had near uniform clay loam texture, with only MO20 having more sand in the topsoil. In comparing the two 20-yr old sites, the surface soils of both were sandy clay loam, but the CP site was sandier with depth, while the MO site had more silt and clay.

Macronutrients: Nitrogen, Phosphorus, and Potassium

Nitrate-N (NO_3-N) was higher in the UM site than all reclamation sites and stratified with depth (Fig. 7A), though concentrations were low to very low in all depths and sites. The UM site had higher concentrations (~4 mg/kg) in the 0-30 cm interval compared to deeper soil. The CP0 site had NO_3-N concentrations lower than all sites (0.3 to 1.1 mg kg^{-1}) with the exception of the 30-45 cm interval. Compared with CP0, soil NO_3^--N through 1 m increased in all older CP sites. From 0-45 cm, NO_3^--N concentrations in CP sites were lower than the UM site, but NO_3-N had statistically similar concentrations from 45-100 cm in CP sites 10 years and older. Nitrate-N in MO sites was greater in the topsoil than most CP sites in the same depth interval (2.0 to 3.0 mg kg^{-1}), but wasn't as high as nor exhibited a stratification trend similar to the UM site (Fig. 7B). MO20 had higher NO_3^--N concentration in the surface depth than the 30 and 40 year old sites, but had lower concentrations in deeper depths than the older sites. MO20 was higher in NO_3^--N compared to CP20, but only in the 0-15 and 30-45 cm intervals.

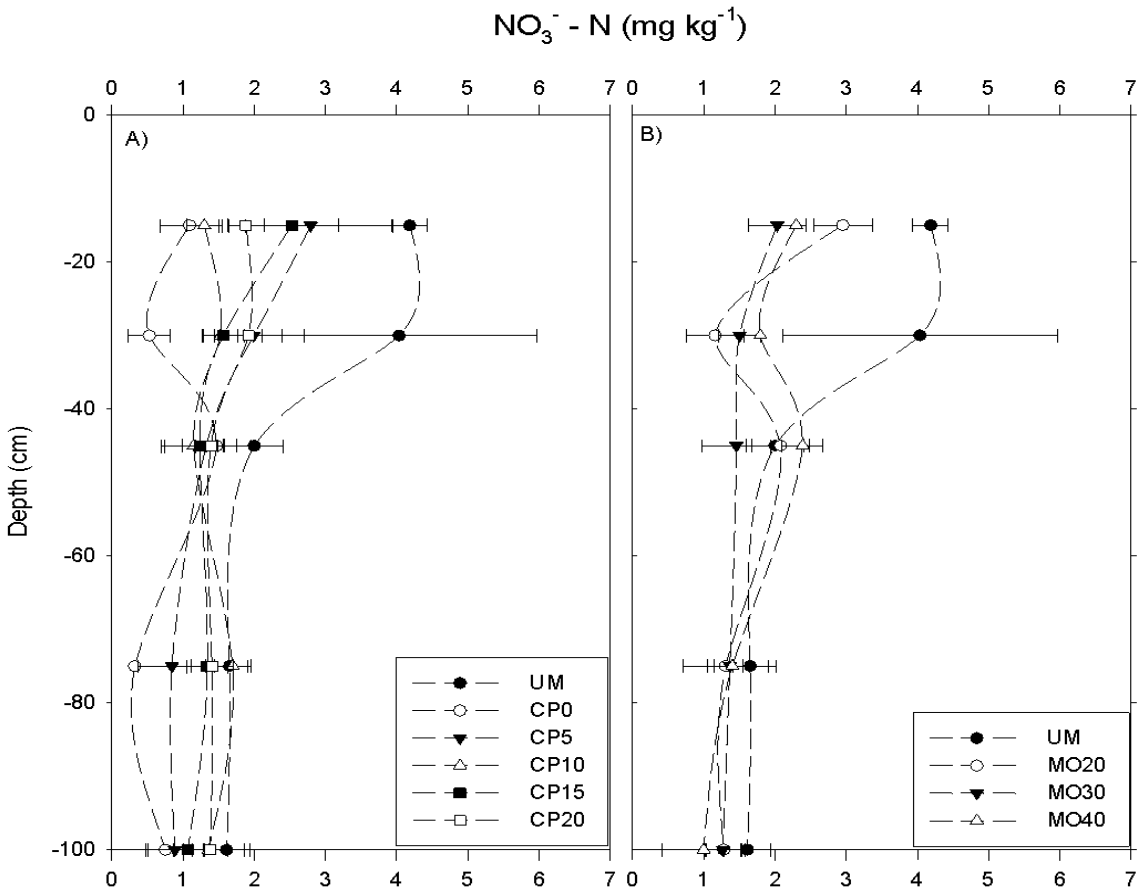

Fig. 7. Soil NO_3^--N concentrations with depth in a post-lignite mining rehabilitation chronosequence. Unmined soil (UM) and mined soils were sampled at five depth intervals: 0-15, 15-30, 30-45, 45-75, and 75-100 cm and compared by (A) crosspit spreader (CP) from 0 to 20 years and (B) mixed overburden (MO) from 20 to 40 years. Horizontal bars indicate standard error of the mean.

Extractable P concentrations of samples from the UM site were higher than those from all reclaimed sites from 0-75 cm of depth and showed stratification at soil depths below 30 cm (Fig. 8A, B). Soil P decreased significantly following mining and reclamation relative to the UM site. After initial reclamation, soil P increased significantly up to 10 years in surface soil (9.6 to 26.2 mg/kg), and up to 15 years at 45-100 cm deep. In surface soil, CP20 P concentrations decreased compared to CP15 (28.2 to 18.2 mg/kg), but were similar at other depths. All CP sites had higher surface soil P concentrations compared to lower depths. Like

CP sites aged 5 to 20 years, extractable soil P from MO samples exhibited differences in surface soil compared to lower depths (Fig. 8B). Although all MO sites were similar (23.9 to 32.4 mg/kg), MO20 had higher P levels in the 0-30 cm depth. The MO20 site had significantly higher P compared to CP20 at 0-15 cm, but had lower concentrations from 15-100 cm.

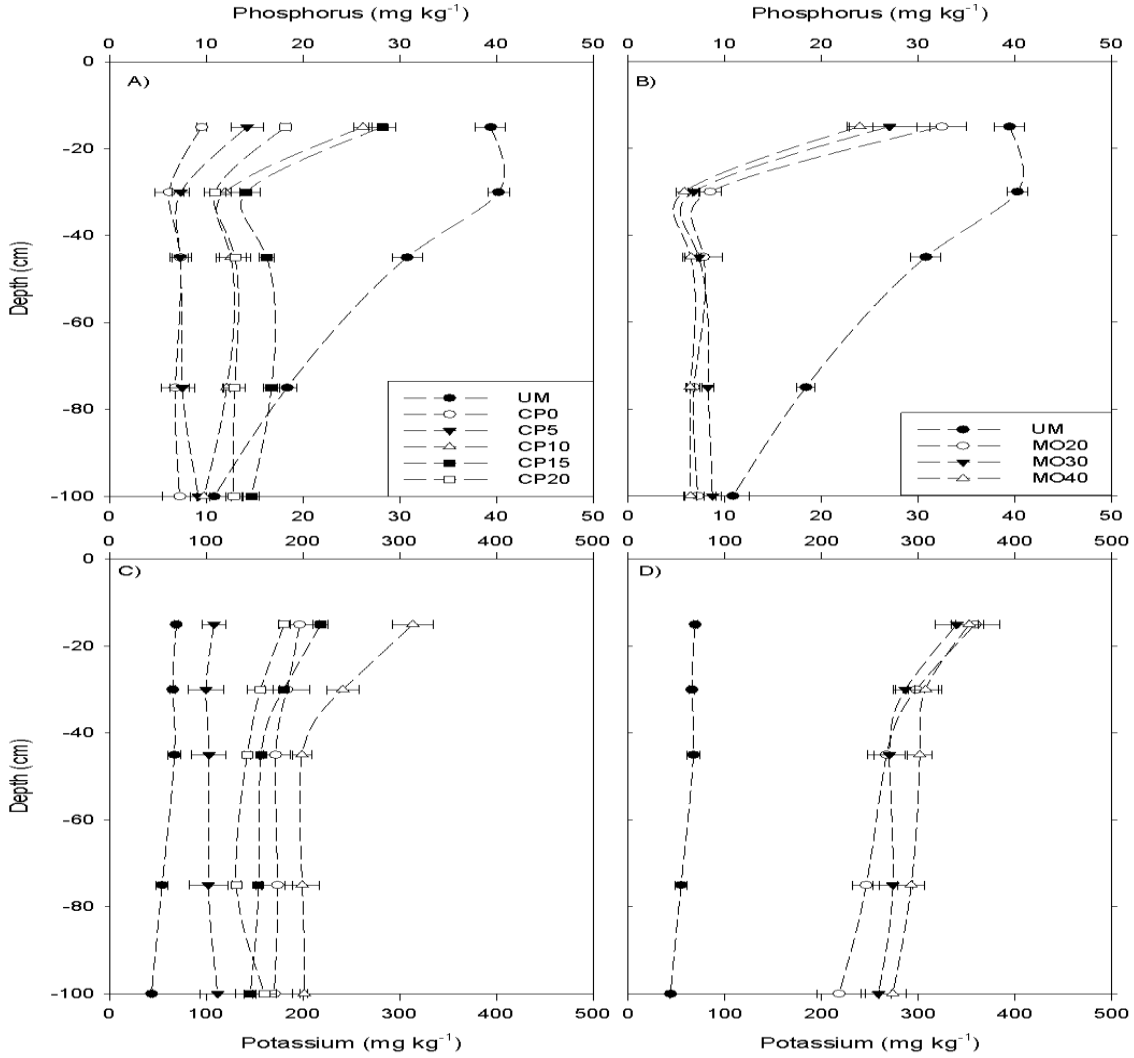

Fig. 8. Soil phosphorus and potassium concentrations with depth in a post-lignite mining rehabilitation chronosequence. Unmined soil (UM) and mined soils were sampled at five depth intervals: 0-15, 15-30, 30-45, 45-75, and 75-100 cm and compared by (A, C) crosspit spreader (CP) from 0 to 20 years and (B, D) mixed overburden (MO) from 20 to 40 years. Horizontal bars indicate standard error of the mean.

As opposed to trends with soil P, lower extractable soil K concentrations were found in the UM site compared to all reclaimed soils (Fig. 8C, D). The UM site showed greater soil K levels at 0-45 cm before decreasing slightly with depth. The soil K level in surface soil of CP0 (197 mg kg^{-1}) was significantly greater compared to UM (69 mg kg^{-1}) before decreasing in CP5 (108 mg kg^{-1}). Samples from CP10 exhibited the highest soil K concentrations through all depths (199 to 314 mg kg^{-1}) for the CP treatment, but concentrations decreased in CP15 samples and stabilized at the 20-year site. Soil K concentrations (246 to 359 mg kg^{-1}) in MO samples were greater compared to the UM and CP sites (Fig. 8D). Soil K concentrations in 0-45 cm depth samples from MO sites were not significantly different by age. Soil K in MO sites increased with age at depths of 45-100 cm. Soil K concentrations of MO20 samples uniformly decreased with depth compared to those from CP20, which showed an increase in soil K at 75-100 cm. At all depths, except 75-100 cm, soil K in MO20 samples was significantly higher than in CP20.

Secondary Nutrients: Calcium, Magnesium, and Sulfur

Secondary macronutrients (Ca, Mg, and S) occurred in much lower concentrations in UM soils than in those from all reclaimed sites (Table 9). Extractable soil Ca in UM samples declined with depth (387 to 92 mg kg^{-1}) and was lower than that in samples from all reclaimed sites at all depths (Tables 9 and 10). Soil Ca increased with depth in samples from the CP0 site (4332 to 5260 mg kg^{-1}). Soil Ca concentrations at the CP0 site were greater than corresponding concentrations at all the other CP and MO sites, except for the 0-15 cm depth of MO30 and MO40. Soil Ca in CP sites never exhibited a decreasing concentration with depth trend similar to that of the UM site. Soil Ca concentrations with CP tended to decrease

with time. Soil Ca concentrations at shallower depths were higher compared to lower soil intervals for CP5, CP15, and CP20. Soil Ca in MO sites appeared to increase with age, with the surface soil concentrations peaking at MO30. Soil Ca for MO was greater than for CP, with the exception of the CP0 site. At MO20 and MO30 sites, extractable soil Ca was highest in the surface soil and then decreased with depth. With MO40, soil Ca decreased below 15 cm, but then increased after 30 cm. Soil Ca concentrations for CP20 showed greater stratification with depth, but lower concentrations than for MO20.

Extractable soil Mg concentrations from reclaimed sites were several fold greater than from the UM site (Table 9). Soil Mg concentrations in UM samples decreased with depth, whereas concentrations in reclaimed soils tended to increase or at least were similar with depth (Table 10). Soil Mg ranged from 518 to 1010 mg kg^{-1} in CP samples, which was significantly greater than soil Mg in UM samples. The highest soil Mg concentrations were observed in CP10 samples, followed by CP0. Soil Mg concentrations at CP5, CP15, and CP20 sites were similar. With the exception of CP10, soil Mg in CP sites had a trend of decreasing with age. Soil Mg levels in MO samples were also significantly higher than those from the UM site, but similar to CP samples. At MO sites, soil Mg increased with depth. The highest Mg concentrations at all depth intervals among MO sites were observed with MO40. Concentrations at the MO20 site were similar to those of the CP20 site through 30 cm, but showed slightly more stratification with depth and higher Mg concentrations from 45-100 cm.

Table 9. Concentrations of extractable soil secondary and micronutrients from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by site. Data are displayed as an average of data from 4 transects at each site.

mg kg^{-1}	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
Ca	0-15	387d	4331a	1950bc	2010bc	1596c	2275b	3977a	4477a	4072a
	15-30	251e	4657a	1348d	2107c	1669d	1552d	2940b	3128b	3212b
	30-45	177f	5192a	1351e	1989d	1517e	1272e	2936bc	2848c	3244b
	45-75	144f	5041a	1278e	1956d	1499e	1194e	2887c	3040c	3577b
	75-100	92f	5261a	1459e	2180d	1523e	1389e	2729c	2952c	3770b
Mg	0-15	53e	688b	547cd	778a	522cd	518cd	559c	488d	635b
	15-30	36e	725bc	650cd	902a	609d	638d	686bcd	736b	832a
	30-45	27e	770bc	689cd	872ab	581d	620d	790bc	879ab	951a
	45-75	22e	768bc	668cd	921a	579d	575d	844ab	933a	894ab
	75-100	21e	739bc	723bcd	1010a	568d	661cd	829b	1010a	1006a
S	0-15	11b	38b	85a	38b	18b	19b	39ab	24b	23b
	15-30	10c	161ab	249a	81bc	14c	18c	24c	18c	15c
	30-45	9c	215b	355a	196b	18c	22c	30c	44c	29c
	45-75	8d	233abc	316a	252ab	30cd	28cd	160abcd	237abc	51bcd
	75-100	8c	199bc	289abc	482ab	42c	78c	301abc	544a	502ab
Na	0-15	89d	222a	129b	104c	84d	65e	89d	75de	79d
	15-30	85d	218a	242b	192c	73d	83d	77d	76d	83d
	30-45	83c	367a	309b	269b	83c	88c	80c	86c	109c
	45-75	78c	369a	304b	342ab	104c	90c	97c	94c	111c
	75-100	81c	339b	315b	470a	98c	98c	116c	107c	104c
Fe	0-15	18.2de	13.7e	16.3de	51.5a	49.5ab	29.3cd	52.0a	34.3c	37.2bc
	15-30	15.6b	16.3b	20.3b	42.1a	33.4ab	39.2a	48.8a	48.8a	42.5a
	30-45	12.5d	14.6cd	22.3bcd	35.6bcd	37.8bc	40.9b	41.3b	66.1a	45.6ab
	45-75	13.8d	14.5d	18.1cd	37.6bc	36.2bc	36.3bc	44.1ab	62.1a	46.4ab
	75-100	13.1c	15.3c	14.7c	28.2bc	27.6bc	39.0ab	39.0ab	54.5a	56.5a
Zn	0-15	1.6e	1.0f	0.8f	2.5d	1.6e	2.2d	3.7b	4.4a	3.1c
	15-30	0.4e	1.6bc	0.7de	1.9b	1.2cd	1.8b	2.0b	3.4a	2.1b
	30-45	0.3d	1.5bc	0.8cd	2.2ab	1.3bc	2.0b	2.0b	2.9a	2.0b
	45-75	0.3f	1.3de	0.9ef	2.9a	1.3de	2.2bc	1.7cd	2.5ab	1.8cd
	75-100	0.1e	1.5cd	1.0de	2.8a	1.1d	2.4ab	2.1abc	2.0abc	1.8bcd
Mn	0-15	12.7ab	4.9e	7.4de	15.4a	11.7abc	10.5cd	10.4bcd	8.1cde	8.9bcde
	15-30	10.7abc	3.0e	15.7a	11.2ab	8.2bcde	10.2bcd	5.5cde	4.8e	5.3de
	30-45	8.4bcd	3.0d	17.3a	11.2abc	10.3abcd	12.1ab	4.5cd	6.6bcd	6.5bcd
	45-75	7.6bc	3.7c	11.7ab	12.3ab	15.4a	15.4a	4.9c	8.7bc	4.7c
	75-100	8.8bc	5.5c	8.6bc	9.4bc	12.9ab	17.1a	6.9c	6.0c	5.0c
Cu	0-15	0.2d	0.7c	0.7c	1.3b	0.8c	0.7c	1.2b	1.5a	1.6a
	15-30	0.2e	1.0d	0.9d	1.5c	1.0d	0.9d	1.5bc	1.9a	1.8ab
	30-45	0.1e	0.9d	0.8d	1.4bc	1.0cd	0.9d	1.6b	2.1a	1.8ab
	45-75	0.1f	0.9e	0.8e	1.6bc	1.0de	0.9e	1.3cd	2.0a	1.8ab
	75-100	0.1e	0.9cd	0.8d	1.4b	0.9cd	1.0cd	1.2bc	1.8a	1.8a

Means in the same row followed by the same letter are not significantly different at P<0.05. UM=unmined pasture. CP = crosspit spreader. MO = mixed overburden.

Table 10. Concentrations of extractable soil secondary and micronutrients from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by depth. Data are displayed as an average of data from 4 transects at each site.

mg kg^{-1}	Depth	Site								
	(cm)	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
Ca	0-15	387A	4331B	1950A	2010A	1596AB	2275A	3977A	4477A	4072A
	15-30	251B	4657A	1348B	2107A	1669A	1552B	2940B	3128B	3212B
	30-45	177C	5192A	1351B	1989A	1517B	1272C	2936B	2848B	3244B
	45-75	144CD	5041A	1278B	1956A	1499B	1194C	2887B	3040B	3577AB
	75-100	92D	5261A	1459B	2180A	1523B	1389BC	2729B	2952B	3770AB
Mg	0-15	53A	688B	547A	778C	522B	518B	559C	488C	635C
	15-30	36B	725AB	650A	902AB	609A	638A	686BC	736B	832B
	30-45	27BC	770A	689A	872BC	581A	620A	790AB	879AB	951AB
	45-75	22C	768A	668A	921AB	579A	575AB	844A	933A	894AB
	75-100	21C	739AB	723A	1010A	568A	661A	829AB	1010A	1006A
S	0-15	11A	38B	85A	38C	18C	19B	39A	24B	23B
	15-30	10AB	161A	249A	81BC	14C	18B	24A	18B	15B
	30-45	9BC	215A	355A	196BC	18C	22B	30A	44B	29B
	45-75	8BC	233A	316A	252B	30B	28B	160A	237AB	51B
	75-100	8C	199A	289A	482A	42A	78A	301A	544A	502A
Na	0-15	89A	222B	129C	104E	84AB	65A	89B	75B	79C
	15-30	85AB	218A	242B	192D	73B	83A	77B	76B	83BC
	30-45	83AB	367A	309A	269C	83AB	88A	80B	86B	109A
	45-75	78B	369A	304A	342B	104A	90A	97AB	94AB	111A
	75-100	81AB	339A	315A	470A	98A	98A	116A	107A	104AB
Fe	0-15	18.2A	13.7A	16.3A	51.5A	49.5A	29.3A	52.0A	34.3B	37.2A
	15-30	15.6AB	16.3A	20.3A	42.1AB	33.4B	39.2A	48.8A	48.8AB	42.5A
	30-45	12.5B	14.6A	22.3A	35.6AB	37.8B	40.9A	41.3A	66.1A	45.6A
	45-75	13.8B	14.5A	18.1A	37.6AB	36.2B	36.3A	44.1A	62.1A	46.4A
	75-100	13.1B	15.3A	14.7A	28.2B	27.6B	39.0A	39.0A	54.5AB	56.5A
Zn	0-15	1.6A	1.0B	0.8A	2.5AB	1.6A	2.2AB	3.7A	4.4A	3.1A
	15-30	0.4B	1.6A	0.7A	1.9B	1.2B	1.8B	2.0B	3.4B	2.1B
	30-45	0.3BC	1.5AB	0.8A	2.2AB	1.3B	2.0AB	2.0B	2.9B	2.0B
	45-75	0.3BC	1.3AB	0.9A	2.9A	1.3B	2.2AB	1.7B	2.5BC	1.8B
	75-100	0.1C	1.5AB	1.0A	2.8A	1.1B	2.4A	2.1B	2.0C	1.8B
Mn	0-15	12.7A	4.9A	7.4A	15.4A	11.7ABC	10.5B	10.4A	8.1A	8.9A
	15-30	10.7AB	3.0B	15.7A	11.2A	8.2C	10.2B	5.5B	4.8B	5.3B
	30-45	8.4BC	3.0B	17.3A	11.2A	10.3BC	12.1B	4.5B	6.6AB	6.5AB
	45-75	7.6C	3.7B	11.7A	12.3A	15.4A	15.4A	4.9B	8.7A	4.7B
	75-100	8.8BC	5.5A	8.6A	9.4A	12.9AB	17.1A	6.9AB	6.0AB	5.0B
Cu	0-15	0.2A	0.7B	0.7A	1.3B	0.8C	0.7B	1.2A	1.5B	1.6A
	15-30	0.2B	1.0A	0.9A	1.5AB	1.0AB	0.9A	1.5A	1.9AB	1.8A
	30-45	0.1B	0.9A	0.8A	1.4AB	1.0A	0.9A	1.6A	2.1A	1.8A
	45-75	0.1B	0.9A	0.8A	1.6A	1.0A	0.9A	1.3A	2.0A	1.8A
	75-100	0.1B	0.9A	0.8A	1.4AB	0.9BC	1.0A	1.2A	1.8AB	1.8A

Means in the same column and nutrient followed by the same letter are not significantly different at $P<0.05$. UM = unmined pasture. CP = crosspit spreader. MO = mixed overburden.

Extractable soil S in UM samples showed only slight differences with depth, with the highest concentrations in surface soil. Soil S levels in the UM site were generally much lower than for CP sites, especially in the subsoil (Table 9). Soil S concentrations increased to their overall highest levels in samples from CP5 before decreasing with time. No difference was observed in soil S concentrations between CP15 and CP20. All CP sites had increasing soil S concentrations with depth, but more differences were found between deeper intervals in younger sites (CP0, CP5, and CP10) compared to older sites (CP15, and CP20) (Table 10). Extractable soil S at 0-45 cm at MO sites was similar to CP15 and CP20, while soil S from 45-100 cm was similar to levels found with CP5 and CP10. All S concentrations in MO sites increased with depth beyond 15 cm, with the highest levels found at 75-100 cm. When comparing reclamation treatments, the extractable S levels from MO20 were numerically greater than from CP20, although statistically similar.

Sodium

Soil Na concentrations in UM samples ranged from 78 to 89 mg kg^{-1} and decreased with depth (Table 10). Despite increasing dramatically after reclamation began, Na decreased to near UM concentrations after 15 years for CP sites, followed by a plateauing for all reclamation sites older than 15 years (65 to 111 mg kg^{-1}) (Table 9). At all reclaimed sites, soil Na mostly increased with depth. Soil Na in MO samples was similar to that of UM, CP15 and CP20 samples.

Micronutrients: Iron, Manganese, Zinc, and Copper

Extractable soil Fe in UM soils (12.5 to 18.2 mg kg^{-1}) was slightly higher in surface soil compared to lower soil depth intervals (Table 10). A slight decrease in extractable Fe in surface soil occurred after reclamation began, but increased steadily in all soil depth intervals until CP10 (38.2 to 51.5 mg kg^{-1}) (Table 9). Soil Fe decreased in CP15 samples and again at CP20 in topsoil, but fluctuated at 15-100 cm. CP sites early in reclamation did not show soil Fe depth trends similar to the UM site until 10 years after reclamation began. Depth segregation of soil Fe in CP15 was similar to CP10, but no differences with depth were found in CP20 due to greater variability. MO samples from 0-30 cm were similar in extractable soil Fe to CP10 through CP20 (34.3 to 52.0 mg kg^{-1}). In soil below 30 cm, however, extractable soil Fe at MO sites exceeded that at CP sites. Among MO sites, higher soil Fe concentrations were observed in the MO20 topsoil, and soil Fe increased with depth in MO30 and MO40 sites. The highest soil Fe concentrations in MO sites increased with age and depth: MO20 at 0-30 cm, MO30 at 30-75 cm, and MO40 at 75-100 cm soil intervals. Comparing CP20 and MO20 sites, soil Fe for MO20 was numerically higher in most depth increments, but significantly greater only at 0-15 cm.

Extractable soil Mn concentrations in UM samples generally decreased with soil depth as did those of MO samples (Table 10). Soil Mn decreased after mining, but increased with age in CP sites up to 10 years (Table 9). Soil Mn in CP samples did not reach or exceed UM levels through the entire 1 m profile until CP10. In CP sites older than 10 years, soil Mn from 0-30 cm decreased with time, while soil Mn increased from 30-100 cm with time. Soil Mn in MO samples was similar and ranged from 4.5 to 10.4 mg kg^{-1}. Soil Mn concentrations in MO sites were mostly higher in topsoil, but did not show an observable trend with depth.

When comparing 20-yr old sites, soil Mn was similar at 0-15 cm, but higher with CP20 from 15-100 cm compared to MO20.

Extractable Zn in UM samples was in highest concentration in the surface and decreased with depth (Table 10). In CP sites, soil Zn (0.7 to 2.9 mg kg^{-1}) fluctuated, but mostly increased over time. The lowest concentrations were found in CP5 samples (0.7 to 1.0 mg kg^{-1}), while the highest were associated with CP10 (1.9 to 2.9 mg kg^{-1}) (Table 9). Soil Zn increased with depth in CP sites except for CP15. MO samples from 0-30 cm depth were higher in extractable Zn than all other sites at this depth. In MO sites from 30-100 cm, soil Zn ranged from 1.7 to 2.9 mg kg^{-1}, similar to soil in CP sites 10 years and older. Soil from MO30 had the highest extractable soil Zn concentrations of all MO sites (2.0 to 4.4 mg kg^{-1}). MO sites exhibited a decrease in soil Zn with depth. From 0-45 cm, MO20 had higher soil Zn concentrations than CP20. However, CP20 had higher Zn concentrations than MO20 from 45-100 cm.

Extractable soil Cu in UM samples (0.1 to 0.2 mg kg^{-1}) decreased with depth (Table 10). All reclamation sites, whether CP- or MO-reclaimed, had soil Cu concentrations greater than the UM site and showed soil Cu increasing with depth (Tables 9 and 10). In CP 0, 5, 15, and 20 sites, soil Cu was mostly similar (0.7 to 1.0 mg kg^{-1}), but there was an increase with CP10 (1.3 to 1.6 mg kg^{-1}). At MO sites, extractable soil Cu through 1 m of depth increased with age. MO20 had higher extractable soil Cu concentrations than CP20, but peaked at 30-45 cm instead of increasing with depth.

Correlations

Pairwise correlations between chemical and physical characteristics resulted in many significant correlations (Table 11). Soil EC and SAR had many significant correlations, while pH had few. Sand, silt, and clay correlated well with all chemical properties and nutrient concentrations, with the exception of pH and soil Na. Most extractable soil nutrients, except soil NO_3^--N, Na, and S, correlated with other extractable concentrations. Nonparametric correlations, conducted with Spearman's ρ between chemical and physical characteristics, were similar to those from pairwise correlations, but resulted in fewer significant correlations with pH, and more with extractable soil S (Table 12).

DISCUSSION

Surface mine reclamation law dictates that disturbed land must be returned to a premined or improved condition (SMCRA, 1977). The objective of this study was to determine when soil chemical properties through a 1-m depth would return to an unmined or improved state, which is inherently dependent on the reclaimed mine soil (RMS) materials that are first backfilled and regraded on the mined area (Ingram et al., 2005; Neel et al., 2003). In Texas, the Railroad Commission (RRC) defines backfilled surface soil suitability as: < 80% sand, < 40% clay, pH 5.0-8.4, > 0.0 acid/base (T $CaCO_3$/1000 T soil), < 4.0 EC (mmhos/cm), and < 13.0 SAR. While seemingly limited, these standards are designed to enhance revegetation, and control erosion and runoff (Cummins et al., 1965; McFee et al., 1981; Nieman and Shannon, 1976; RRC, 2011).

Table 11. Correlations (pairwise) of chemical and physical properties from a post-lignite mining chronosequence in eastern Texas.

	pH	EC	SAR	Sand	Silt	Clay	NO_3^--N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu	TC	TN
pH	-																		
EC	0.10	-																	
SAR	0.12	**0.26**	-																
Sand	0.01	**-0.41**	**0.48**	-															
Silt	-0.11	**0.33**	**-0.44**	**-0.95**	-														
Clay	0.14	**0.45**	**-0.45**	**-0.92**	**0.76**	-													
NO_3-N	0.02	**-0.32**	-0.06	**0.28**	**-0.23**	**-0.32**	-												
P	0.06	**-0.44**	-0.01	**0.50**	**-0.41**	**-0.56**	**0.50**	-											
K	0.07	0.09	**-0.62**	**-0.80**	**0.75**	**0.76**	-0.06	-0.11	-										
Ca	**0.54**	**0.51**	**-0.31**	**-0.72**	**0.58**	**0.81**	**-0.24**	**-0.35**	**0.64**	-									
Mg	**-0.17**	**0.56**	**-0.27**	**-0.82**	**0.74**	**0.82**	**-0.36**	**-0.66**	**0.60**	**0.56**	-								
S	**-0.22**	**0.75**	**0.27**	**-0.20**	**0.20**	**0.18**	**-0.20**	**-0.32**	-0.03	**0.18**	**0.43**	-							
Na	**0.20**	**0.66**	**0.71**	-0.12	0.06	**0.18**	**-0.28**	**-0.35**	**-0.20**	**-0.31**	**0.33**	**0.47**	-						
Fe	**-0.51**	-0.01	**-0.56**	**-0.52**	**0.53**	**0.42**	0.01	-0.06	**0.55**	0.12	**0.40**	0.13	**-0.37**	-					
Zn	-0.14	0.12	**-0.45**	**-0.66**	**0.65**	**0.56**	0.01	0.05	**0.73**	**0.44**	**0.43**	0.05	-0.45	**0.56**	-				
Mn	**-0.64**	-0.12	0.07	**0.29**	**-0.22**	**-0.34**	**0.21**	**0.29**	**-0.23**	**-0.47**	**-0.15**	0.12	-0.07	**0.20**	0.06	-			
Cu	**-0.19**	**0.25**	**-0.49**	**-0.83**	**0.77**	**0.79**	-0.14	**-0.42**	**0.79**	**0.49**	**0.76**	**0.19**	-0.06	**0.71**	**0.70**	-0.12	-		
TC	0.10	**0.17**	**-0.58**	**-0.59**	**0.54**	**0.55**	0.08	0.05	**0.69**	**0.58**	**0.31**	0.06	**-0.29**	**0.56**	**0.74**	-0.14	**0.54**	-	
TN	0.11	0.09	-0.14	**-0.31**	**0.34**	**0.23**	0.09	**0.30**	**0.48**	**0.29**	**0.15**	0.01	0.02	**0.15**	**0.56**	0.00	**0.26**	**0.49**	-

Values in bold indicate significant correlation ($p < 0.05$).

Table 12. Correlations (Spearman's ρ) of chemical and physical properties from a post-lignite mining chronosequence in eastern Texas.

	pH	EC	SAR	Sand	Silt	Clay	NO_3-N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu	TC	TN
pH	-																		
EC	0.06	-																	
SAR	0.00	0.13	-																
Sand	-0.07	**-0.51**	**0.50**	-															
Silt	-0.06	**0.46**	**-0.44**	**-0.94**	-														
Clay	**0.29**	**0.55**	**-0.43**	**-0.84**	**0.65**	-													
NO_3-N	0.01	**-0.29**	-0.13	0.12	-0.10	**-0.18**	-												
P	-0.11	**-0.51**	0.02	**0.43**	**-0.33**	**-0.53**	**0.33**	-											
K	0.10	**0.31**	**-0.67**	**-0.82**	**0.75**	**0.75**	0.05	**-0.18**	-										
Ca	**0.47**	**0.61**	**-0.42**	**-0.75**	**0.62**	**0.88**	**-0.15**	**-0.41**	**0.73**	-									
Mg	-0.12	**0.64**	-0.10	**-0.68**	**0.63**	**0.67**	**-0.23**	**-0.62**	**0.55**	**0.55**	-								
S	**-0.22**	**0.86**	**0.25**	**-0.34**	**0.35**	**0.35**	**-0.30**	**-0.44**	0.14	**0.39**	**0.60**	-							
Na	0.05	**0.65**	**0.68**	-0.07	0.09	**0.16**	**-0.29**	**-0.35**	-0.11	**0.22**	**0.45**	**0.72**	-						
Fe	**-0.54**	0.04	-0.57	**-0.53**	**0.54**	**0.35**	0.12	0.05	**0.61**	**0.20**	**0.36**	0.12	**-0.26**	-					
Zn	**-0.17**	**0.25**	-0.53	**-0.67**	**0.66**	**0.52**	0.09	0.04	**0.71**	**0.46**	**0.40**	**0.17**	-0.13	**0.67**	-				
Mn	**-0.68**	**-0.24**	0.12	**0.36**	**-0.25**	**-0.48**	**0.25**	**0.54**	**-0.27**	**-0.54**	**-0.24**	-0.01	-0.12	**0.31**	0.11	-			
Cu	**-0.19**	**0.38**	**-0.53**	**-0.81**	**0.75**	**0.71**	0.00	**-0.33**	**0.81**	**0.55**	**0.69**	**0.32**	-0.01	**0.74**	**0.75**	-0.08	-		
TC	0.11	**0.31**	**-0.70**	**-0.71**	**0.63**	**0.67**	0.05	-0.14	**0.72**	**0.70**	**0.35**	**0.15**	**-0.20**	**0.59**	**0.73**	**-0.16**	**0.59**	-	
TN	**0.22**	**0.37**	**-0.26**	**-0.41**	**0.38**	**0.36**	0.12	0.06	**0.47**	**0.47**	**0.20**	**0.20**	0.05	**0.19**	**0.51**	-0.07	**0.31**	**0.59**	-

Values in bold indicate significant correlation ($p < 0.05$).

As Table 1 showed, recently backfilled soil (CP0) met five of these criteria through 1-m depth. Yet, these criteria may create conflicts if returning disturbed land to a premined condition is a priority. Soil materials at the CP0 site were more suitable for backfilling than UM materials based on soil texture (i.e. sand vs. clay loam), which made the return of this mined site to its original premined state undesirable (Schroeder et al., 2010). The criteria of <80% sand in reclaimed surface soil was established because, although sandy-textured soils are not necessarily unproductive soils, they do have some secondary properties that can possibly hinder the recovery of soil quality. Soils with less than 80% sand and less than 40% clay are more conducive for aggregate formation, and will also enhance gas and water movement through the soil profile. Aggregate formation in reclaimed sites has been previously reported to occur over time (Lorenz and Lal, 2005; Ussiri et al., 2006). It has also been observed that the formation of macroaggregates (>2 mm) early in the reclamation process resulted in organic C sequestration, a soil quality indicator, (Elliott and Coleman, 1988; Ingram et al., 2005; Oades, 1984; Six et al., 2000a; 2000b), which in turn influenced the presence of plant available nutrients (Anderson et al., 2008; Ussiri et al., 2006). However, aggregates in RMSs at the Big Brown Mine were measured for different C fractions and total N only within 0-15 cm, leading us to examine when soil nutrients and chemical properties recovered to a premined state in reclamation sites. We hypothesized that a disturbed site would improve and then plateau to a certain capacity, a trend established in post-disturbance ecosystem succession theory (Odum, 1969). In addition, we hypothesized that this return of soil conditions would comprise a decrease of nutrient concentrations with depth, resembling an undisturbed soil profile (UM site), as biological activity in the topsoil increased with age, declined deeper into the soil profile, and as continual nutrient removal from increased root

penetration occurred over time (Akala and Lal, 2000; Jobbagy and Jackson, 2001; Lal et al., 1998; 2001).

While the CP0 site was suitable as backfilling material, it was not similar to the UM site and had poorer conditions (e.g. EC, NO_3^--N, P, Zn, Mn) than all reclamation sites (Table 13). The uniformity of soil conditions with depth for CP0 after backfilling was expected given mixing of soil horizons, storage and respreading that is included in the process (Abdul-Kareem and McRae, 1984; Ingram et al., 2005). After that initial reclamation stage, results varied for the time required for macronutrient concentrations in a reclaimed site to return to unmined conditions. In previous research at the Big Brown Mine, a recovery of soil total nitrogen to UM concentrations was observed after 10 years with a fluctuating trend over time; NO_3^--N being a majority of the inorganic N in a mineralization experiment (Fig. 6). In comparison with our NO_3^--N analysis, stratification (higher concentrations in shallower soil) was observed after 5 years (Sourkova et al., 2005), but no reclamation site matched UM levels through 0-30 cm regardless of age or reclamation practice. While there was some minimal stratification, no observable trend of soil NO_3^--N with age was observed in reclaimed soils (Fig. 7). Interestingly, a previous study conducted at the same site found immediate recovery of NO_3^--N with urea utilized as a fertilizer (Waggoner, 1993). If sufficient urea was used consistently on reclamation sites for our study, NO_3^--N might approach UM levels sooner. However, other studies have agreed with our findings. The higher NO_3^--N in the UM site was similar to the higher N levels that Ussiri et al. (2006) found in an undisturbed forest, while our lower concentrations in RMS were similar to data from Komnitsas et al. (2010). Ussiri et al.'s (2006) findings were attributed to differences in litter quality, but our sites had mostly the same vegetation with any differences (i.e. legumes)

possibly resulting in higher NO_3^--N. Conversely, it could be the minor changes in vegetation [ie. wheat (*Triticum L.*) and rye grass (*Lolium spp.*) instead of only coastal bermudagrass (*Cynodon dactylon L. pers.*)] that may have resulted in increased uptake of NO_3^--N. The use of different vegetation affecting nitrate recovery needs to be taken into account by reclamation management especially with such a dramatic textural change occurring. The finer texture and labile carbon of the reclamation sites may be responsible for microbially-induced lowering of NO_3^--N as anaerobic conditions could have enhanced nitrogen loss by denitrification (Chodak and Nilinska, 2010), or increased carbon causing immobilization. A study examining the presence of nitrification and denitrification genes coupled with oxidation potential may shed some light on the loss of this macronutrient when researching variation across reclamation sites. Finally, observed differences in NO_3^--N in UM soils compared to the reclamation sites could be due to the change in N fertilizer application (40-0-0 vs. 13-13-13). While a change in fertilizer application could return post-mined NO_3^--N concentrations to a UM level sooner, revegetation efforts appeared successful on the reclamation sites, indicating adequate N uptake for developing plant cover. Additionally, the recovery of N cycling in reclaimed soils should be done so that biological processes are not uncoupled and more N is not lost as time continues (Banning et al., 2008; Davidson et al., 1992; Schimel and Bennett, 2004).

Table 13. List of soil quality parameters: chemical characteristics, macro-, secondary, and micronutrients recovered to premined or improved conditions through 1 m.

	Soil Property												
Yr	EC	SAR	NO_3	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
0		X			X	X	X	X					X
5		X			X	X	X	X					X
10		X			X	X	X	X		X	X	X	X
15		X			X	X	X	X	X	X	X		X
20 (CP)		X			X	X	X	X	X	X	X		X
20 (MO)		X			X	X	X	X	X	X	X		X
30		X			X	X	X	X	X	X	X		X
40		X			X	X	X	X	X	X	X		X

X = the soil quality parameter recovered to premined conditions. EC = electrical conductivity. SAR = sodium adsorption ratio.

Other macronutrients displayed opposite trends of recovery to N as soil P increased with time but did not reach UM levels, while soil K exceeded UM levels immediately likely due to the increase in minerals containing potassium (Fig. 8, Tables 11 and 12). Soil P stratification was observed almost immediately and the difference became more noticeable with age, while soil K stratification was slower, requiring at least 10 years. The trend of higher soil K in RMS was also observed in an abandoned Chinese coal mine by Komnitsas et al. (2010), and most likely due to a change in mineralogy as seen by the significant correlation with clay (Tables 11 and 12). Although taken up by plants at much higher rates than N or P, the abundance of soil K in RMS at CP and MO sites was one less soil fertility concern of reclamation managers. The trend in soil P with depth was similar to observations by Sourkova et al. (2005) and Zipper et al. (2011). The reasons for lower soil P could be due to a change in the microbial community or production of phosphatase enzymes (Stroo and Jencks, 1982) as hypothesized by Fan and Liu (2005). While a change in enzyme production is worth investigating, we can likely eliminate other reasons for a lack of P recovery: the quality of substrate given the recovery of C over time (Ingram et al., 2005), little erosion given the shallow slope, and climatic conditions that were favorable for mineralization

during the warmest time of the year. The increase in P seemed to correlate with increased pH at reclamation sites, although the correlation was relatively poor (Tables 11 and 12). The use of Mehlich III in measuring soil P was also appropriate given the pH, as it may underestimate P concentrations in calcareous soils. Our soils were neutral to acidic, but the increase in soil Ca (Table 9) in RMS could skew our data as indicated by significant correlations with soil P (Tables 11 and 12). All things considered, after observing the trend of soil P increase in CP and MO sites, a minimum of 50 to 60 years of current management might be required for soil P in surface soils to return to UM levels.

Given the relationship between the recovery of soil P, pH and soil Ca, we investigated the potential relationships between other RRC-mandated soil standards and nutrient concentrations. Relationships between soil pH and plant-availability of different nutrients are well established, but relationships with other RRC-established soil criteria (e.g. EC, SAR) are less developed. During the course of reclamation, soil EC and SAR were initially higher, but well within the set limits, compared to UM, and decreased over time (Table 7). As expected, both EC and SAR correlated well with soil Na, Mg, and Ca (Tables 11 and 12). The improvement in EC had been previously documented in other post-mining sites, and related to finer textured soils (Anderson et al. 2008, Emerson et al., 2009; Thorne and Cardina, 2011), while several papers had observed increases in Ca and Mg, thus lowering SAR over time (Chambers et al., 1987; Thorne and Cardina, 2011). By CP20, EC had decreased to $\leq$ 0.267 dS m^{-1} throughout the soil profile, which was mostly lower than MO EC at 20 years (0.249 to 0.509 dS m^{-1}). In addition to slightly better stratification and lower heterogeneity, the improvement in soil EC is one of the few advantages that was observed in CP20 compared to MO20 (Neel et al., 2003). One criterion, pH, did stand out as problematic in

reclaimed sites. CP sites had a trend of more acidic conditions below 45 cm after 15 years, and below 15 cm after 20 years. While the decrease of pH is a naturally occurring phenomenon, and perhaps an indication of the return of intrinsic soil properties (e.g. ion leaching), it is concerning from a reclamation viewpoint. Byrnes and Miller (1973) reported that the pH and specific conductance of RMS overburden from southern Indiana were limiting to natural plant establishment, but observed widely variably results over the tested areas. Of the 6 RRC suitable soil parameters, only the acid to base ratio wasn't measured. While pH, total soil S and Ca can suggest the presence/absence of acid- or acid-buffering materials, more could have been done to ensure the absence of any acid-forming materials (AFM) (Cummins et al., 1965; Pugh et al., 1984). As Byrnes et al. (1980) showed in a mine site in Indiana, the cleanliness of the mining reclamation process also influences soil quality. They showed that overburden materials could contain potentially toxic levels of micronutrients (Barnhisel and Massey, 1969; Cummins et al., 1965). As the presence of lignite would indicate a lack of cleanliness at the Big Brown Mine, it was previously observed that CP sites were relatively clean of residual extracted materials (Chapter II). We did observe increased levels of soil S and Fe, which correlated with pH, suggesting pyritic materials (Tables 10, 11 and 12). The correlations between pH, increased micronutrient levels, and lower return of macronutrients (NO_3^--N and P) suggest more research into the biological influences on acidification in reclaimed sites maybe warranted.

A secondary objective of this study was to observe changes in chemical properties and nutrient concentrations with depth and determine when an unmined stratification was observed. The heterogeneity of early reclamation topsoil is well-documented in terms of texture and carbon stratification, and could also be assumed for early MO sites (Ingram et al.,

2005). Yet given the extended time of our chronosequence, none of our reclaimed sites showed the same nutrient stratification as the UM site (Ussiri et al., 2006). Chemical and nutrient concentration measured through 1 m changed mostly in the upper 30 cm, but it is known that changes have been observed between shallower soil in RMS (Ingram et al., 2005). When comparing CP to MO reclamation, we observed greater stratification in CP sites, where topsoil was stored separately before backfilling. Were this experiment to be repeated, I would suggest sampling the top 25 to 50 cm and dividing it into 5 intervals to see if the soil profile had returned in shallower soil. As soil microorganisms are the main decomposers and nutrient cyclers in the soil, as well as concentrated in the topsoil, analysis into identifying the dominant organisms and processes should be completed in order to better understand the effects of post-mining rehabilitation on soil properties. The dramatic changes in soil properties in the upper 30 cm over 40 years indicate a major influence of biologic activity in CP and MO sites.

CONCLUSION

Reclamation managers at Big Brown Mine have successfully restored vegetative productivity to post-mined lands by returning many plant essential nutrients and chemical properties to premined or improved levels. Our studies of reclaimed mine soils have shown the importance of measuring nutrient distribution in the soil profile, especially for freshly seeded and revegetated plots or grasslands with shallower root systems that may be more susceptible to sudden soil changes within the root zone. While nutrient concentration and stratification are not regulated in surface mine reclamation, it is important that both chemical properties and nutrient distribution by age and depth be managed toward premined or

improved stages for long-term productivity. These decisions are balanced with economic and environmental sustainability as observed with the diversity of post-mine reclamation practices in the US and with the replacement of the mixed overburden technique with the newer crosspit spreader at the Big Brown Mine. Further analysis on the microbiological and vegetative effects on chemical properties is needed to fully document the effects that surface mining and reclamation have on global nutrient cycling.

CHAPTER IV

RECOVERY OF SOIL MICROBIAL COMMUNITIES AND FUNCTIONALITY IN A POST-LIGNITE MINING REHABILITATION CHRONOSEQUENCE IN EAST TEXAS

INTRODUCTION

Surface mining for coal results in the destruction of the original soil profile characteristics, and therefore has altered physical, chemical, and biological conditions at sites across the U.S. and world (McSweetney and Jansen, 1984). The goal of surface mine reclamation is to return a site to a premined or improved state (SMCRA, 1977). Unsuccessful revegetation of reclaimed minesoils (RMS) is often associated with deteriorated soil conditions, arising from low soil organic matter content, high salinity, poor soil structure, and reduced soil fertility (Kleeberg, 2008; Sencindiver and Ammons, 2000; Ussiri et al., 2006). However, the improvement of RMS quality has been conducted through the enhancement of the availability and cycling of soil nutrients (Barnhisel and Hower, 1997; Bradshaw, 1997; Coyne et al., 1998; Hons, 1978; Ingram et al., 2005; Lorenz and Lal, 2007; Palmer et al., 2010; Whitford, 1988).

Soil microorganisms have many roles within the soil ecosystem (ex. the decomposition of organic matter, nutrient cycling, and improving soil structure), and have been recognized as indicators of soil health and maturity in RMS (Anderson and Domsch, 1990; Ingram et al., 2005; Insam and Domsch, 1988; Machulla et al., 2005). Ingram et al. (2005) showed that soil biological properties (e.g. microbial biomass, C and N mineralization) correlated strongly with previously defined soil quality indicators (e.g. soil

organic C) in RMS of 40 years in age (Franzluebbers et al., 2000). The recovery of soil microbial properties has been reported to be relatively fast in several studies. Machulla et al. (2005) observed increased C mineralization within a year, while Banning et al. (2008) observed recovery within 3 years. These parameters have been observed to plateau over time (An et al., 2009). While the recovery of some biological parameters has been well-studied (Banning et al., 2011; Gros, et al., 2006; Herrera et al., 2007; Mummey et al., 2002a; Zhan and Sun, 2011). Banning et al. (2011) observed a shift of bacterial communities affected by macronutrients towards an unmined forest using a *16S rRNA* microarray. Mummey et al. (2002a) showed a trend of both bacterial and fungal communities affected by organic matter that trended towards an undisturbed grassland by fatty acid methyl ester analysis. While much is known about the major shifts in the structure of soil microbial communities during land management changes from undisturbed lands to agricultural systems and prairies, little is known regarding changes in soil microbial community structure and function following mineland reclamation (Bissett et al., 2011; Jangid et al., 2010; Pastorelli, et al., 2011; Plassart et al., 2008).

The focus of the research was to observe how microbial communities and functionality change over time and the interrelationships that occur between the soil microbial community and physical and chemical properties following surface mine reclamation. The effects of mining on the soil microbial community have been documented at the Big Brown Mine. Studies found that *Rhizobium* and microbial populations can recover relatively quickly after disturbance (Mott, 1984; Peach, 2001). Swanson (1996) came to a similar conclusion when he found increasing levels of soil microbial biomass with age, which correlated well with the growth of coastal bermudagrass. Further analysis of microbial

populations and soil conditions were conducted with Harris (1985) observing *Rhizobium* and microbial biomass increasing with N and P, while Jackson (1979) observed a correlation between pH and the index of chemoautotrophic activity. Waggoner (1993) observed nitrification recovery, and correlated microbial numbers and growth with age. Additionally, preliminary research at Big Brown Mine has revealed that soil microbial biomass and mineralization rates recover after 15 years (Chapter II). My proposed research expanded upon this previous work by including a biodiversity analysis of microbial communities to track compositional and long-term successional changes using molecular techniques that enable unprecedented levels of soil microbial characterization on RMS. The objective of this microbial study will be to analyze the microbial community from an ecological (functional) and an identification (taxonomic) standpoint, while combining this data in the context of soil physical and chemical properties. Microbial taxonomic and functional diversity was measured using community-level physiological profiling (CLPP), a functional gene array (GeoChip), quantitative polymerase chain reaction of *Bacteria* and *Fungi* (qPCR), and *16S rRNA* gene sequencing (*Bacteria*). A better understanding of the recovery of community structure and function during the reclamation process is important to the improvement of post-mine land reclamation practices and understanding microbial community succession.

MATERIALS AND METHODS

Site Description and Soil Sampling

The Big Brown Mine is a lignite surface mine located east of Fairfield, TX (Table 1). Axtell, Lufkin, and Tabor are the predominant soil series in the area (Peach, 2001). These soils are moderately well drained, fine sandy loams that formed on clayey sediments. The

area is gently rolling to hilly (100-270 m above sea level) and is located within the Post Oak Savannah vegetation region of Texas (Gould, 1975). The regional mean annual temperature is 18.9 °C with peaks in July and August at 35.6 °C. Annual precipitation averages 970 mm, with the highest monthly rainfall levels in May (120 mm) and the lowest rainfall in July (50 mm). During the early reclamation era (1970-1980s) of this mine, the mixed overburden technique (MO) was utilized where RMS were backfilled and regraded with a combination of topsoil and overburden mixed together. The MO provided a material with better chemical and physical properties to enhance revegetation compared to native topsoil alone (Bearden, 1984; Dixon et al., 1980; Hons, 1978). About 20 years ago (1986), the crosspit spreader (CP) technique replaced MO with its improved speed and segregation of topsoil/topsoil substitute material and overburden.

Sampling sites were chosen with minimal slope and near the tops of hills to avoid the accumulation of runoff and sediment. Five sites were selected with the CP reclamation treatment aged 0, 5, 10, 15, and 20 years (CP0, CP5, CP10, CP15, and CP20). Three sites were selected with the older MO treatment and were 20, 30 and 40 years post-reclamation (MO20, MO30, and MO40) (Table 1). For a comparison of reclamation success, in which land is returned to a pre-mined or improved state, a control site that was unmined (UM) with an undetermined age was selected. A chronosequence comparison between reclamation methods was possible only at 20 years old since this was the only age in common between the two methods.

All sites were vegetated primarily with coastal bermudagrass (*Cynodon dactylon L. pers.*) and managed as grassland and pastureland. The UM site classified as the Padina series and was additionally vegetated with Bahia grass (*Paspalum notatum*). On this site, hay was

baled (~two 1200 kg bales/ha) twice a year, grazed (~1 cow/2 ha) every year and fertilized with 40-0-0 at 140 kg/ha. The CP0 and CP5 sites were initially revegetated with wheat (*Triticum L.*) and ryegrass (*Lolium spp.*) for temporary cover before coastal bermudagrass was sprigged. The CP0 site was classified as a Nahatche-Hatliff soil association and fertilized with 13-13-13 at 280 kg/ha during seeding. The CP5 received the same fertilizer treatment as CP0. The CP10, 15, and 20 sites were fertilized with 17-17-17 at 280 kg/ha during their initial revegetation with yearly applications. The CP5 and CP10 sites were originally classified as part of the Tabor series. Sites CP10 to 20 were hayed (~three 1100 kg bales/ha) and grazed yearly at a density of ~1 cow/ha. CP15 was initially classified as part of the Edge series, while CP20 was classified as part of the Silawa series. Sites CP15 and CP20 were also revegetated with two legume species, Yuchi Arrowleaf clover (*Trifolium vesiculosum*) and Crimson clover (*Trifolium incarnatum*). The MO20 site was grazed and hayed similar to the CP sites from ages 10 to 20, but was fertilized additionally with chicken litter at 4.5 t/ha during a one time application. The MO30 site was grazed (~1 cow/ha), and received fertilized with 44-0-0 at 140 kg/ha. The MO40 site differed slightly from the other reclamation sites in that Johnsongrass (*Sorghum halepense*) encroached upon the coastal bermudagrass at the site. The site was hayed (~three 1100 kg bales/ha) and fertilized annually with 17-17-17 at 280 kg/ha. All MO sites were classified as part of the Bigbrown soil series.

We sampled during the summer, on June 22, 2009 and June 26, 2010, when peak biomass levels were expected (Swanson, 1996). Except for mineralization and biomass, all tests were completed with 2009 sampled soil. At each site, a spot was randomly chosen as the center of the sampling plot. We measured 15 m in each cardinal direction (N to S, and E to W) as well as in bisecting directions (SE to NW, and SW to NE) forming four 30-m

transects. Ten 15cm cores (5 on each side of the center to avoid repeat samples at the center) were taken across the diameter of the circle with roughly 3 m between each sample core. The soil core (1.5 cm radius) was taken using a truck-mounted Giddings Probe. Soil cores from each transect were composited and placed in plastic and brown paper bags. Samples were then transported back to Texas A&M University campus (College Station, TX) within 12 hours. Soil for CLPP analysis was processed immediately upon arrival. The soil in plastic bags was placed in a -80 °C freezer for DNA analysis. The soil in brown paper bags were dried and processed for chemical and physical characteristics.

Soil Chemical and Physical Characteristics

Physical and chemical tests were handled similar to soil from Chapters 1 and 2. Texture was determined using 50.0 g of soil and the hydrometer procedure. This method was performed at room temperature using deionized (DI) water in a 1L graduated cylinder (Day, 1965). Soil pH was determined in a 1:2 soil:water extract of the soil using DI water. Samples were stirred and allowed to equilibrate for a minimum of 30 minutes after adding DI water before determination with a hydrogen selective combination electrode (Schofield and Taylor, 1955). Soil EC was determined in a 1:2 soil:water extract of the soil using DI water. Samples were stirred and allowed to equilibrate for a minimum of 30 minutes after adding the water. The actual determination was made using a conductivity probe fitted with a 10-mm dipping electrode and reported in µmol/cm (Rhoades, 1982). Cation exchange capacity (CEC) was determined with an aliquot of soil was placed in a syringe and repeatedly extracted with pH 8.2 sodium acetate (NaOAc), pH 7.0 ammonium acetate (NH_4OAc), and ethanol. Flame

emission on an atomic absorption spectrometer was used to determine the final concentration of Na.

The combustion procedure was used to determine OC, TC and TN at 650 °C and 900 °C, respectively, with a vario MAX C/N analyzer (Elementar, Germany) (McGeehan and Naylor, 1988; Schulte and Hopkins, 1996; Storer, 1984). A 2-5-g subsample of dried and sieved soil was pulverized through a ring pulverizer, and 250 mg of soil were weighed into aluminum tins (Alpha Resources, Inc., Stevensville, USA). Inorganic carbon (IC) was determined as the difference between TC and OC. To determine the amount of lignite C in the soil samples, the chemi-thermal method was used (Ussiri and Lal, 2008). Briefly, soil samples were extracted repeatedly with 1M HCl and 0.5M NaOH to remove soil inorganic carbon and recent OC. Highly recalcitrant OC was removed with 50% HNO_3, and 10% HF was used to release mineral bound organic matter (OM). After oven drying at 60 °C and use of a muffle furnace at 340 °C for 3 hr, TC was determined by combustion analysis using a vario MAX C/N analyzer (Elementar, Germany) at the SWFTL at Texas A&M University. Non-lignitic organic soil carbon (SC) and was determined as the difference between organic carbon and lignitic carbon.

Soil nitrate-nitrogen (NO_3^--N) was extracted using a 1 N KCl solution and determined by reduction to nitrite-nitrogen (NO_2^--N) using a cadmium column followed by spectrophotometric measurement (FIALab 2500) (Bellevue, WA, USA) (Keeney and Nelson, 1982). Soil P, K, Ca, Mg, Na, and S were extracted using the Mehlich III extractant and determined with a Genesis Radial Inductively Coupled Plasma (ICP) (Spectro, Germany). Mehlich III was a dilute acid-fluoride-EDTA solution of pH 2.5 that consisted of 0.2 N CH_3-COOH, 0.25 N NH_4NO_3, 0.015 N NH_4F, 0.013 N HNO_3, 0.001 M EDTA (Mehlich, 1984).

The sodium adsorption ratio (SAR) was calculated by dividing Na (meq/L) by taking the square root of Ca + Mg (meq/L) divided by 2. Micronutrients (Cu, Fe, Mn and Zn) were extracted using a 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.10 M triethanolamine solution (TEA) (Lindsay and Norvell, 1978). The analytes were determined by an Arcos Axial ICP (Spectro, Germany).

Soil Biological Properties

Ten grams of air-dry soil was wetted to 50% water holding capacity as calculated by the van Genuchten equation (van Genuchten, 1980) and incubated at 25 °C for 5 days in a glass jar in the dark. Soils were subsequently fumigated with ethanol-free $ClCH_3$ (Ricca Lab, Arlington, Texas) for 24 hr in the dark at room temperature alongside a non-fumigated set of soils. Soils were vented after the incubation and extracted with 40 mL of 0.5 M K_2SO_4 by shaking for 1 hr. The solutions were centrifuged (3250 x g) for 5 min, and gravity-filtered through 2.5-µm pore-size filter paper (Fisher Scientific). The filtrate was frozen prior to analysis on a Shimadzu DOC analyzer. Soil microbial biomass was calculated using the formula of Paul et al. (1999). A k_{EC} and k_{EN} of 0.45 and 0.54, respectively, were used in the calculations (Appuhn and Joergensen, 2006; Beck et al., 1997; Brookes et al., 1985; Pothoff et al., 2009; Wu et al., 1990).

Forty grams of air-dry soil was wetted to 50% water holding capacity (van Genuchten, 1980). Soils were placed in glass jars in the dark with 20 mL 1M KOH and some DI water to maintain soil moisture. Jars with no soil were used as controls. Soil and jars were incubated at 30°C. After 3, 7, 14, 21, 28, and 35 days, the KOH was titrated with 0.5 M HCl to the phenolphthalein endpoint following precipitation of carbonates with $BaCl_2$ (Zibilske,

1994). On those days, all jars were vented. CO_2-C trapped was calculated as ($HCl_{blank\ avg}$ − HCl_{sample})/2*22. After titration, soils were dried, pulverized, and analyzed for TC and TN as described previously.

Nitrogen mineralization was determined based on the concentrations of nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) in each soil sample after CO_2 titrations minus the concentrations in air-dry samples prior to incubation. Five grams of dried soil were shaken for 1 hr with 25 mL of 1 M KCl. Samples were then centrifuged (3250 x g) for 5 min and filtered through 2.5 µm cellulose filter paper. The filtrate was stored at 4 °C. Concentrations of NH_4^+-N and NO_3^--N were subsequently determined spectrophotometrically with a FIAlab-2500 instrument (FIAlab Instruments Inc., Bellevue, WA) equipped with a cadmium column for the reduction of NO_3^--N to nitrite (NO_2^--N).

Community-Level Physiological Profiles

For each site transect, 10 g of fresh unsieved soil was diluted to 10^{-3} (g/ml) in NaCl solution (0.87%). Field moist soil was added to 95ml NaCl and shaken for 30 s. A 150 µl aliquot of the 10^{-3} solution was added to each well so that each soil solution was added to each of the 31 C substrates and control well. The plates were incubated at 25 °C while being shaken lightly, and then read through an ELx808Automated Microplate Reader (Bio-Tek, Hayward, CA) every 24 hours, during the 72 hr incubation, at 590 nm. We calculated a normalized average well-color development (AWCD) as follows: normalized AWCD = {Σ ODi/[(Σ ODi)/31]}/31 where ODi is the optical density value of each well subtracted by the reading given off of the control well, containing water. We used an OD of 0.25 as threshold to determine which day of the data to analyze (Garland, 1997). The Shannon–Weaver index

was calculated as follows: $H = - \Sigma p_i (\ln p_i)$ where p_i is the ratio of the activity of each substrate (ODi) to the sum of activity from all wells (Σ ODi).

DNA Extraction, Purification

Soil community DNA was extracted from a composite sample (0.50 g) from each transect at each site using a PowerSoil DNA extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). Soil community DNA was purified with illustra MicroSpin™ S-400 HR columns (GE Healthcare, Piscataway, NJ) and concentrated with a vacuum centrifuge. Some transects required multiple DNA extractions in order to obtain the required amount of DNA for analysis. In these cases, DNA from the multiple extractions was combined for each sample.

qPCR

Community quantitative PCR (qPCR) methodology was performed identical to Ng et al. (2012) on four replicate soil samples from each plot targeting bacterial *16S rRNA* and fungal *ITS* genes (Fierer et al., 2005), with the *ITS* primer set described by Boyle et al. (2008). Plasmid standards for the *16S rRNA* and *ITS* reactions were constructed using *Escherichia coli* DH10B (pUC19) (obtained from Carlos Gonzales, Texas A&M University) and *Neurospora crassa* (obtained from Heather Wilkinson, Texas A&M University), respectively. Concentrations of DNA were converted to copy numbers of genes per DNA as described by Smits et al. (2004), and then converted to copy numbers of genes on a soil basis.

16S rRNA Pyrosequencing

16S rRNA pyrotag sequencing was performed on a Roche 454 FLX Genome Sequencer (454 Life Sciences, Branford, CT) system by the Research and Testing Laboratory (Lubbock, TX). Mothur v.1.23.1 software was used for all sequence analysis (Schloss et al., 2009). Quality control commands included trimming primers and barcodes, screening, creating unique sequences, aligning, filtering, and removing chimeras. Sequences were classified by using a template (nogap.bacteria) and referencing taxonomy from the SILVA bacterial references (silva.bacteria.silva.tax) (Wang et al., 2007). Templates and references were downloaded from the mothur website (http://www.mothur.org/wiki/ Silva_reference_files). The relative abundances of phyla were calculated as an average from four sequence libraries. Mothur was also used for OTU-based analyses: distance matrices, clustering, sub-sampling and calculating Shannon-Weaver and Simpson's inverse indices, Bray-Curtis dissimilarity indices, and trees. Bray-Curtis analyses were performed between the 9 sites (e.g. UM, CP10) and between the 36 transects (e.g. CP201 is the first transect from CP20).

GeoChip

Purified DNA from 3 transects from the UM, CP0, CP10, CP20, and MO40 sites was separated into 100 ng aliquots. The DNA was analyzed with GeoChip 3.0 at the Institute for Environmental Genomics (Norman, OK). Further details on hybridization parameters can be found in Xie et al. (2011). Microarray hybridization array data was processed and normalized by following the data analysis pipeline mentioned in He et al. (2010). For comparative analysis among the samples, we used relative signal intensity, which was calculated by

comparing genes from carbon cycling, metal resistance, nitrogen, organic remediation, phosphorus and sulfur to the total gene signal intensity of these categories.

Statistical Analysis

Analysis of variance (ANOVA), Student's t-test, and pairwise (r^2) comparisons were used to determine significant differences in measured characteristics between ages and correlations using JMP 9 (SAS Institute 2011). Correlations were made between soil chemical and biological parameters and relative phylum abundance. qPCR data for Bacteria and Fungi were log10 transformed before correlation. A p-value of 0.05 was used as a minimum for determining significant differences. PC-ORD was used to perform principal component analyses of normalized Biolog Ecoplate™ data (McCune and Mefford, 1999). PAST software was used to perform an ANOSIM with Biolog data (Hammer et al., 2001). R project was used to conduct a DCA on GeoChip data (R Development Core Team, 2011).

RESULTS

Soil Chemical and Physical Properties

Unmined pastures (UM) had a near neutral pH (Table 14). The CP sites began slightly alkaline, but acidified with age. Soil pH from 20 to 40 years was about equal to UM. Cation exchange capacity (CEC) in the UM site was 5-10 times lower than all reclamation sites, with CP0 highest of all CP sites, and MO30 highest of the MO sites. While CEC decreased with age in CP sites, all MO sites were similar. Electrical conductivity (EC) in the UM site was lowest of all sites. The EC was highest in CP0, but decreased two-fold with time (Table 14). The MO sites had the highest EC levels at age 30. Sodium adsorption ratio

(SAR) in the UM site was highest of all sites (Table 14). The SAR decreased two-fold with age in CP sites, and was similar across MO sites. All reclamation sites contained much finer textured soils than the sandy UM site, and were either clay loams or sandy clay loams (Table 14). Bulk density (BD) in the UM site was lower than most CP sites, except the oldest site which was similar (Table 14). Bulk density returned to premined conditions at sites 20 years and older.

Table 14. Chemical and physical characteristics of an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by site. Data are displayed as an average of data from 4 transects at each site.

	Soil Property								
	pH	CEC (cmol (+) kg^{-1})	EC (μmhos/cm)	SAR	BD (g/cm^3)	Sand	Silt	Clay	Texture
UM	6.70b	2.90f	45.3e	1.13a	1.37b	92.8a	2.5e	5.3c	s
CP0	7.78a	22.93c	438.8a	0.83b	1.66a	44.3c	24.7cd	31.0a	cl
CP5	6.63bc	12.45e	342.5ab	0.67	1.61a	57.0b	21.5d	21.5b	scl
CP10	5.30d	16.38d	210.3cd	0.50d	1.66a	35.0d	37.0ab	28.0a	cl
CP15	5.40d	14.68de	177.5d	0.46d	1.66a	60.5b	18.0d	21.5b	scl
CP20	6.15c	16.78d	171.0d	0.32e	1.34b	56.5b	23.0cd	20.5b	scl
MO20	6.38bc	26.15b	333.0ab	0.35e	1.27b	38.8cd	34.0b	27.2a	scl
MO30	6.73b	33.23a	339.3ab	0.28e	1.28b	25.0e	44.0a	31.0b	cl
MO40	6.88b	28.50b	294.0bc	0.31e	1.38b	40.0cd	30.5bc	29.5a	cl

Means in the same column followed by the same letter are not significantly different by site ($P<0.05$). UM = unmined pasture. CP = crosspit spreader. MO = mixed overburden.CEC = cation exchange capacity. BD = bulk density. s = sandy. c = clay. l = loam.

Soil Nutrients

Total (TC), organic (OC), and non-lignitic organic soil carbon (SC) pools followed similar trends over age (Table 15). The lowest concentrations of carbon were found in the UM and CP5 site. The CP0, CP10, and CP15 sites had about 1.5-2.0 times higher TC than the UM site. The TC increased from CP15 to CP20. The MO sites had more TC than any CP site, but decreased with age. Inorganic carbon (IC) had no pattern with age (Table 15), but UM was similar to CP sites, and MO sites had greater IC concentrations than CP sites. More than 90% of the TC pool was OC (Table 15). Lignitic carbon (LC) ranged from 4.5 to 10.0% of OC (Table 15). The LC was 1.6 to 9.0 times higher in reclamation sites than the UM site, with MO > CP. The CP0 site had increased SC compared to UM. After CP5, SC increased up to 20 years. The MO sites had significantly increased SC levels compared to the UM and CP sites, but decreased with age.

Table 15. Nutrient concentrations from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by site. Data are displayed as an average of data from 4 transects at each site.

	TC	IC	OC	LC	SC	TN	NO_3^--N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	----------g kg^{-1}----------						----------mg kg^{-1}----------										
UM	5.72e	0.32c	5.39f	0.31e	5.08f	1.17d	4.19a	39.42a	69.3c	387d	53e	11b	89d	18.2de	1.6e	12.7ab	0.2d
CP0	9.64d	0.94ab	8.71e	0.81c	7.90e	0.97d	1.09c	9.56e	196.7b	4331a	688b	38b	222a	13.7e	1.0f	4.9e	0.7c
CP5	5.75e	0.33b	5.41f	0.51de	4.90f	1.09d	2.80ab	14.24de	108.3c	1950bc	547cd	85a	129b	16.3de	0.8f	7.4de	0.7c
CP10	11.31d	0.52ab	10.79d	0.91c	9.88d	2.07ab	1.30c	26.22c	313.8a	2010bc	778a	38b	104c	51.5a	2.5d	15.4a	1.3b
CP15	10.92d	0.36b	10.56de	0.69cd	9.87d	1.24cd	2.53bc	28.24bc	218.6b	1596c	522cd	18b	84d	49.5ab	1.6e	11.7abc	0.8c
CP20	19.14c	0.61ab	18.54c	0.84c	17.70c	1.73bc	1.89bc	18.22d	180.9b	2275b	518cd	19b	65e	29.3cd	2.2d	10.5cd	0.7c
MO20	28.99a	1.47a	27.52a	1.40b	26.13a	2.45a	2.95ab	32.44b	358.5a	3977a	559c	39ab	89d	52.0a	3.7b	10.4bcd	1.2b
MO30	28.87a	1.1ab	27.78a	2.80a	24.97a	2.53a	2.03bc	27.02c	339.6a	4477a	488d	24b	75de	34.3c	4.4a	8.1cde	1.5a
MO40	24.34b	1.33a	23.01b	1.34b	21.68b	2.16ab	2.30bc	23.94c	352.5a	4072a	635b	23b	79d	37.2bc	3.1c	8.9bcde	1.6a

Means in the same column followed by the same letter are not significantly different by site ($P<0.05$). UM = unmined pasture. CP = crosspit spreader. MO = mixed overburden. TC = total carbon. IC = inorganic carbon. OC = organic carbon. LC = lignitic carbon. SC = non-lignitic organic carbon.

Total nitrogen (TN) in the UM site was similar to CP sites through 5 years (Table 15). The highest TN concentrations were found in CP10 before decreasing and then increasing at CP20. MO sites had TN concentrations that were significantly higher than UM and most CP sites. The NO_3-N in the UM site was highest of all sites, while the CP0 site was lowest (Table 15). Soil NO_3-N fluctuated with age in reclamation sites. Soil NO_3-N in MO sites was greater than most CP sites. Soil P in the UM site was highest of all reclaimed sites (Table 15). Soil P decreased significantly after mining, but increased up to 15 years. All MO sites were similar in soil P. As opposed to soil P, soil K was lowest of all sites (Table 15). Soil K fluctuated with time, with CP10 having the highest concentrations (313.8 mg/kg). Soil K in MO sites was 5 times greater than the UM site, but not significantly different by age. Soil Ca, Mg, and S were lowest in the UM site compared to other sites (Table 15). The CP0 site had soil Ca that was 1.9 to 2.7 times higher than all CP sites before stabilizing, while soil Ca in MO was greater than most CP sites. Soil Mg in the UM site was lowest of all sites (Table 15). Soil Mg in the CP sites was 9.8 to 14.6 times greater than the UM site. With the exception of CP10, soil Mg in CP sites had a trend of decreasing with age. Soil Mg levels in MO sites were 9.2 to 12.0 times higher than the UM site, but similar to CP sites. Soil S levels in the UM site were 1.6 to 7.7 times lower than CP sites (Table 15). Soil S increased to its highest levels in CP5 before decreasing. Soil S in MO sites decreased with age and was similar to most CP sites. Despite increasing after reclamation, Na decreased to near UM concentrations at 15 years followed by a plateauing (Table 15).

Soil Fe in the UM site was similar through CP sites of 5 years before increasing more than three-fold (Table 15). The MO sites were similar in soil Fe to CP sites from 10 to 20 years. Soil Zn decreased to half of UM concentrations after mining before increasing at 10

years (Table 15). In CP sites, soil Zn fluctuated, but mostly increased over time compared to the UM site. The MO sites were higher in soil Zn than all other sites. Soil Mn decreased more than half of UM concentrations after reclamation was initiated, but increased more than three-fold with age in CP sites up to 10 years. Soil Mn exceeded UM levels at CP10, but decreased in older sites. Soil Mn among the MO sites was similar. All reclamation sites had soil Cu concentrations at least three times greater than the UM site. In CP sites 0 to 20, soil Cu was mostly similar, but there was an increase at CP10. In MO sites, soil Cu increased with age.

Bacterial and Fungal Quantitative PCR

The UM site had higher copy numbers of *16S rRNA* and *ITS* genes than early reclamation sites up to 15 years, but was statistically similar to all sites (Fig. 9). The CP0 site had the lowest copy numbers of *16S rRNA* and *ITS* genes of all other reclamation sites. Both *16S rRNA* and *ITS* genes recovered and exceeded the UM site at CP20, as well as peaking at MO20. A decline was observed in both gene copy numbers after MO20. *Bacteria* to *Fungi* ratios were highest in the UM site (16.2) and lowest in the CP0 site (4.3). The ratios increased with time and peaked at MO30, but decreased at MO40. All reclamation sites except MO30 were statistically similar to each other. While sites aged 15 to 30 years were statistically similar to the UM site, reclamation sites did not have an equal ratio. *Bacteria* and *Fungi* numbers had a significant positive correlation with soil microbial biomass carbon and nitrogen data (data not shown). There was no correlation between soil microbial biomass data with *Bacteria* to *Fungi* ratios.

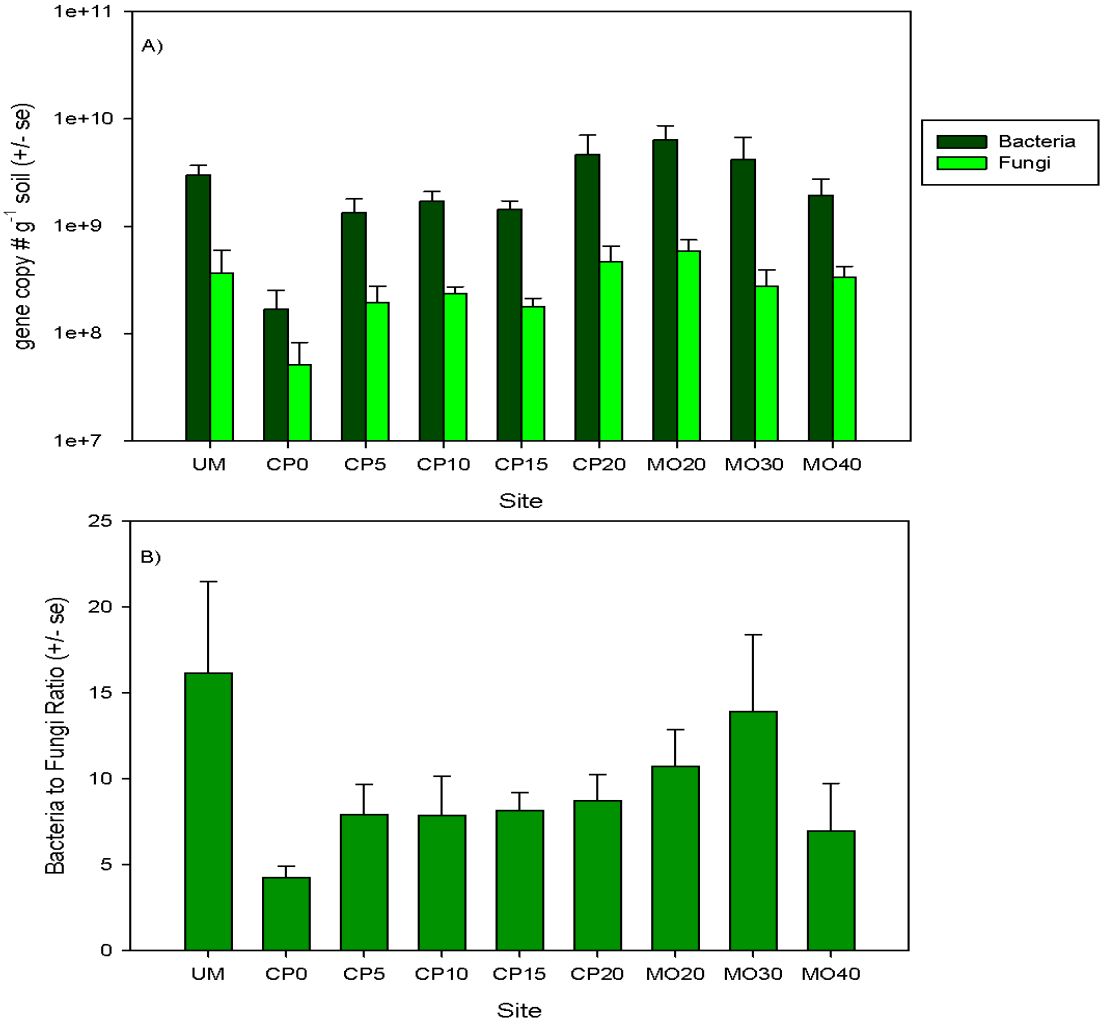

Fig. 9. qPCR measurements of A) *Bacteria* and *Fungi*, and B) *Bacteria* to *Fungi* ratios in a post-lignite mining rehabilitation chronosequence. Unmined soil (UM) and mined soils, crosspit spreader (CP) from 0 to 20 years and mixed overburden (MO) from 20 to 40 years, were sampled. Vertical bars indicate standard error of the mean.

16S rRNA Gene Sequence Analyses and Correlations

Of the >70,000 sequences analyzed from the 36 samples, representatives from 38 phyla were detected (Fig. 10). *Acidobacteria*, *Actinobacteria*, and *Proteobacteria* accounted for more than 70% of all sequences. The proportion of *Acidobacteria* decreased after mining, but returned to premined levels at 10 years. Sites aged 15 years and older all had statistically similar proportions of *Acidobacteria* (Table 16).

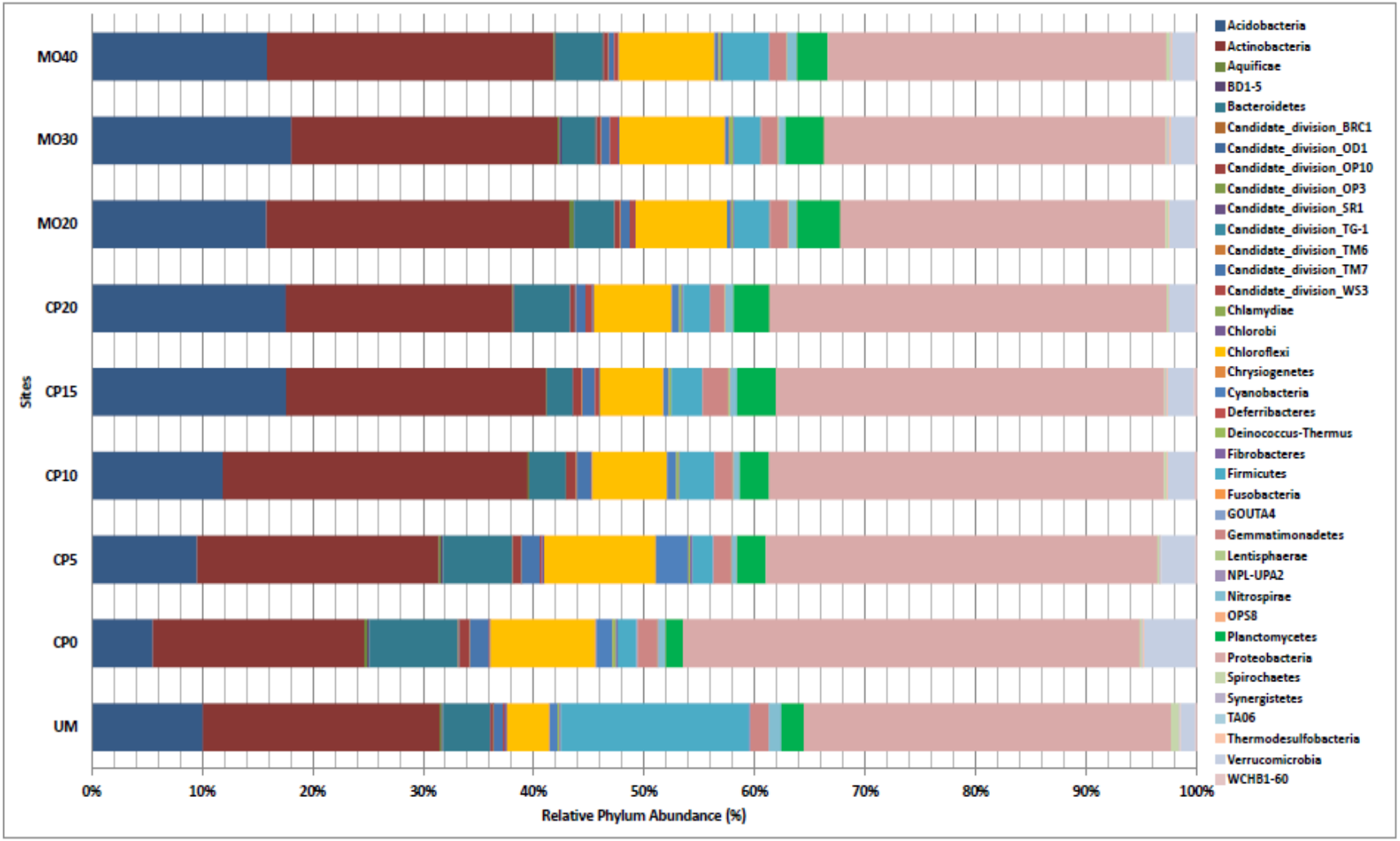

Fig. 10. Relative phyla abundances of bacteria in a post-lignite mining rehabilitation chronosequence. Unmined soil (UM) and mined soils, crosspit spreader (CP) from 0 to 20 years and mixed overburden (MO) from 20 to 40 years, were sampled.

Table 16. Relative phylum abundances (%) from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by site. Data are displayed as an average of data from 4 transects at each site.

	Site								
	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
Acidobacteria	10.07b	5.53c	9.53b	11.86b	17.62a	17.57a	15.81a	18.08a	15.87a
Actinobacteria	21.42cd	19.16d	21.87cd	27.60a	23.50bcd	20.53cd	27.43ab	24.06abc	25.93ab
Aquificae	0.20b	0.23b	0.20b	0.13b	0.08b	0.11b	0.38a	0.18b	0.16b
BD1-5	0.10abcd	0.18ab	0.18abc	0.04cd	0.03d	0.10bcd	0.04bcd	0.23a	0.08bcd
Bacteroidetes	4.25cd	8.06a	6.30b	3.30de	2.33e	4.93bc	3.63cde	3.05de	4.21cd
Cand_div_BRC1	0.01b	0.14a	0.04ab	0.04ab	0.01b	0.07ab	0.04ab	0.08ab	0.10ab
Cand_div_OD1	0.05ab	0.01ab	0.02ab	0.00b	0.01ab	0.07a	0.04ab	0.02ab	0.01b
Cand_div_OP10	0.21c	0.84a	0.72ab	0.82ab	0.73ab	0.36c	0.46bc	0.33c	0.37c
Cand_div_OP3	0.01a	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
Cand_div_SR1	0.03ab	0.04a	0.01bc	0.02abc	0.01bc	0.00c	0.00c	0.00c	0.00c
Cand_div_TG-1	0.03a	0.03a	0.03a	0.01a	0.00a	0.03a	0.00a	0.04a	0.00a
Cand_div_TM6	0.06a	0.07a	0.02a	0.12a	0.10a	0.08a	0.06a	0.05a	0.06a
Cand_div_TM7	0.78cd	1.69a	1.64ab	1.27abc	1.12bc	0.85cd	0.83cd	0.77cd	0.46d
Cand_div_WS3	0.16cd	0.03d	0.20cd	0.07d	0.44bc	0.56ab	0.48ab	0.74a	0.36bc
Chlamydiae	0.00a	0.01a	0.00a	0.00a	0.01a	0.01a	0.00a	0.00a	0.02a
Chlorobi	0.22a	0.08ab	0.19a	0.04b	0.06b	0.22a	0.04b	0.12ab	0.06b
Chloroflexi	3.83e	9.46a	10.10a	6.74cd	5.67de	6.99bcd	8.26abc	9.57a	8.64ab
Chrysiogenetes	0.02b	0.11a	0.02b	0.02b	0.04ab	0.04ab	0.02ab	0.02b	0.05ab
Cyanobacteria	0.69bc	1.43b	2.90a	0.82bc	0.47c	0.64bc	0.32c	0.30c	0.30c
Deferribacteres	0.02ab	0.01b	0.02ab	0.01b	0.01b	0.01b	0.06a	0.06ab	0.05ab
Deinococcus-Thermus	0.16bc	0.34a	0.14c	0.26ab	0.21bc	0.23abc	0.18bc	0.35a	0.20bc
Fibrobacteres	0.16ab	0.14abc	0.21a	0.08bc	0.01c	0.14abc	0.10abc	0.08bc	0.20ab
Firmicutes	17.01a	1.72d	1.81d	3.05bcd	2.80bcd	2.40cd	3.16bc	2.41cd	4.18b
Fusobacteria	0.00a	0.00a	0.01a	0.01a	0.00a	0.00a	0.02a	0.01a	0.00a
GOUTA4	0.10a	0.08abc	0.10ab	0.04abcd	0.00d	0.04abcd	0.06bcd	0.05abcd	0.02cd
Gemmatimonadetes	1.67ab	1.83ab	1.60b	1.67ab	2.34a	1.33b	1.63ab	1.51b	1.57b
Lentisphaerae	0.02b	0.07ab	0.04b	0.08ab	0.15a	0.05b	0.06ab	0.08ab	0.06b
NPL-UPA2	0.02ab	0.04ab	0.04ab	0.04ab	0.07a	0.00b	0.00b	0.02ab	0.02ab
Nitrospirae	1.09a	0.60bc	0.41c	0.49bc	0.58bc	0.71bc	0.66bc	0.56bc	0.83ab
OPS8	0.00a	0.01a	0.02a	0.00a	0.00a	0.00a	0.04a	0.00a	0.01a
Planctomycetes	2.05de	1.56e	2.57cd	2.67cd	3.50ab	3.26abc	3.93a	3.50ab	2.79bcd
Proteobacteria	33.23bc	41.33a	35.46b	35.74b	35.18b	35.93b	29.43d	30.92cd	30.64cd
Spirochaetes	0.73a	0.18b	0.19b	0.22b	0.15b	0.21b	0.28b	0.16b	0.35b
Synergistetes	0.01b	0.07ab	0.06ab	0.01b	0.04b	0.04b	0.02b	0.12a	0.01b
TA06	0.05a	0.01ab	0.02ab	0.00b	0.00b	0.00b	0.00b	0.04a	0.05a
Thermodesulfobacteria	0.07ab	0.12ab	0.07ab	0.10ab	0.10ab	0.04b	0.08ab	0.16a	0.12ab
Verrucomicrobia	1.37c	4.69a	3.10b	2.43bc	2.40bc	2.33bc	2.32bc	2.18bc	2.08bc
WCHB1-60	0.10a	0.08a	0.11a	0.19a	0.23a	0.11a	0.12a	0.15a	0.15a

Means in the same row followed by the same letter are not significantly different by site ($P<0.05$).

Among chemical properties, *Acidobacteria* showed significant, negative correlations with pH and SAR (Table 17). *Acidobacteria* positively correlated with all carbon pools, macronutrients, and most micronutrients. Among biological parameters, *Acidobacteria* correlated well with SMB-C and N, C and N mineralization, *Bacteria* and *Fungi* (Table 18). *Actinobacteria* proportions returned to premined levels after 5 years. The CP sites 15 years and older had higher *Actinobacteria* levels than younger sites. The MO sites were similar to CP15 and CP20 and had 1.4 to 3 times higher proportions of *Actinobacteria* than CP sites 10 years and younger. Similar to *Acidobacteria*, *Actinobacteria* negatively correlated with pH and SAR, while positively correlating with carbon, TN, K and micronutrients. *Actinobacteria* correlated with SMB-C and N, Cmin and *Fungi*. The relative abundance of *Proteobacteria* increased after mining, but decreased at 5 years. The MO sites had about 14% lower proportions of *Proteobacteria* than the CP sites. *Proteobacteria* seemed to take have opposite relationship to chemical properties from *Acidobacteria* and *Actinobacteria*. *Proteobacteria* had positive correlation with SAR and Na, but a negative correlation with carbon macronutrients, and micronutrients. *Proteobacteria* also had negative correlations with SMB-C and N, mineralization rates, and *Bacterial* copy numbers.

Table 17. Correlations (pairwise) of chemical properties and relative phylum abundance from a post-lignite mining chronosequence in eastern Texas.

	Soil Properties																		
	pH	EC	SAR	CEC	TC	OC	SC	TN	NO_3^-	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
Acidobacteria	**-0.39**	-0.21	**-0.73**	**0.35**	**0.65**	**0.66**	**0.66**	**0.63**	0.03	**0.37**	**0.49**	0.14	0.00	-0.28	**-0.78**	**0.50**	**0.62**	0.12	**0.44**
Actinobacteria	**-0.40**	-0.02	**-0.41**	0.28	**0.39**	**0.40**	**0.40**	**0.44**	-0.11	0.32	**0.49**	0.08	0.21	-0.03	**-0.39**	**0.52**	**0.44**	0.31	**0.50**
Aquificae	0.18	0.32	-0.00	0.22	0.24	0.25	0.26	0.18	0.13	0.07	0.11	0.24	-0.04	0.32	0.11	0.08	0.21	-0.12	0.10
BD1-5	**0.35**	**0.39**	0.11	0.20	0.07	0.08	0.05	-0.01	0.03	-0.18	-0.09	0.24	-0.06	0.32	0.26	-0.28	0.07	-0.26	0.00
Bacteroidetes	**0.57**	**0.43**	**0.35**	-0.07	-0.32	**-0.33**	**-0.33**	**-0.41**	-0.15	**-0.64**	**-0.38**	0.18	0.13	0.32	**0.70**	**-0.60**	**-0.46**	**-0.49**	-0.31
Cand_div_BRC1	**0.34**	0.23	-0.08	**0.40**	0.16	0.17	0.16	-0.05	**-0.39**	**-0.42**	0.13	**0.38**	0.27	-0.08	0.23	-0.21	0.12	-0.22	0.23
Cand_div_OD1	0.08	-0.11	0.12	-0.16	-0.01	0.00	0.01	-0.10	0.28	0.06	-0.26	-0.14	-0.27	-0.03	-0.09	-0.11	-0.00	0.10	-0.26
Cand_div_OP10	-0.14	**0.38**	0.08	-0.08	**-0.35**	**-0.36**	**-0.36**	-0.26	-0.28	**-0.46**	-0.02	0.03	**0.47**	**0.33**	**0.52**	0.14	**-0.37**	-0.12	-0.01
Cand_div_OP3	0.06	-0.27	**0.35**	-0.31	-0.20	-0.20	-0.20	-0.19	0.22	0.28	-0.27	-0.29	**-0.42**	-0.11	-0.09	-0.15	-0.15	0.13	-0.29
Cand_div_SR1	0.16	0.12	**0.50**	**-0.33**	**-0.45**	**-0.46**	**-0.46**	-0.31	-0.01	-0.03	**-0.35**	-0.17	-0.11	0.22	**0.43**	-0.28	**-0.41**	-0.04	**-0.35**
Cand_div_TG-1	0.31	-0.04	0.22	-0.12	-0.10	-0.10	-0.11	0.06	0.25	0.09	-0.19	-0.05	-0.22	0.06	0.07	-0.30	-0.07	-0.07	-0.24
Cand_div_TM6	-0.14	-0.01	-0.04	-0.00	-0.06	-0.05	-0.05	0.06	-0.22	-0.07	0.08	-0.02	0.21	-0.04	0.01	0.17	0.00	0.21	0.08
Cand_div_TM7	0.19	**0.35**	0.28	-0.18	**-0.48**	**-0.48**	**-0.49**	**-0.34**	-0.17	**-0.45**	-0.29	-0.01	0.28	**0.34**	**0.61**	**-0.35**	**-0.55**	-0.30	-0.30
Cand_div_WS3	-0.05	-0.06	**-0.60**	**0.45**	**0.66**	**0.68**	**0.67**	**0.48**	-0.03	0.23	**0.36**	0.29	-0.08	-0.28	**-0.54**	0.18	**0.57**	-0.08	0.32
Chlamydiae	0.13	0.09	-0.13	0.10	0.06	0.06	0.06	-0.04	-0.08	-0.13	0.07	0.13	0.07	-0.10	0.03	-0.03	-0.07	-0.09	0.04
Chlorobi	0.22	-0.31	0.27	**-0.36**	-0.25	-0.26	-0.26	-0.28	0.08	-0.01	**-0.55**	**-0.33**	**-0.42**	-0.12	-0.10	**-0.54**	**-0.33**	-0.15	**-0.51**

Table 17
continued

	Soil Properties																		
	pH	EC	SAR	CEC	TC	OC	SC	TN	NO_3^-	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
Chloroflexi	**0.38**	**0.71**	**-0.33**	**0.62**	0.32	0.31	0.30	0.13	-0.26	**-0.55**	0.33	**0.64**	**0.51**	**0.38**	0.33	-0.14	0.14	**-0.46**	**0.40**
Chrysiogenetes	0.26	0.33	0.13	0.05	-0.11	-0.11	-0.11	-0.19	-0.05	-0.28	0.04	0.30	0.14	0.16	**0.40**	-0.12	-0.10	-0.18	-0.04
Cyanobacteria	0.24	0.22	**0.34**	-0.30	**-0.50**	**-0.50**	**-0.51**	**-0.43**	0.09	**-0.42**	**-0.44**	-0.12	0.10	**0.35**	**0.47**	**-0.45**	**-0.55**	-0.20	**-0.35**
Deferribacteres	-0.03	0.08	-0.20	0.32	**0.42**	**0.43**	**0.43**	0.33	0.00	0.21	0.31	0.21	-0.07	0.08	-0.22	0..28	**0.40**	0.02	0.26
Deinococcus-Thermus	0.23	0.20	-0.09	**0.34**	0.13	0.12	0.10	0.18	**-0.35**	-0.19	0.19	**0.38**	0.26	-0.17	0.24	-0.14	0.21	-0.15	0.24
Fibrobacteres	0.29	0.05	0.22	-0.10	-0.07	-0.07	-0.07	-0.06	0.15	-0.11	-0.15	-0.01	-0.13	0.19	0.12	-0.27	-0.14	-0.18	-0.13
Firmicutes	0.06	**-0.61**	**0.68**	**-0.49**	-0.32	-0.32	-0.31	-0.21	**0.52**	**0.64**	**-0.46**	**-0.57**	**-0.84**	-0.26	-0.19	-0.21	-0.13	0.32	**-0.48**
Fusobacteria	-0.22	0.03	-0.12	0.15	0.26	0.26	0.26	0.22	0.04	0.12	0.16	0.00	0.03	0.10	-0.07	0.44	0.19	0.21	0.12
GOUTA4	**0.42**	0.06	**0.52**	-0.33	**-0.36**	**-0.35**	**-0.36**	-0.30	0.15	-0.14	**-0.34**	-0.09	-0.25	0.03	**0.34**	**-0.49**	-0.26	-0.19	**-0.35**
Gemmatimon-adetes	-0.19	0.01	0.13	-0.04	-0.17	-0.16	-0.16	-0.24	0.17	0.04	0.02	-0.09	0.05	0.03	0.14	0.30	-0.11	0.26	0.01
Lentisphaerae	-0.31	-0.04	-0.27	0.18	0.10	0.09	0.08	0.03	-0.06	0.04	0.25	0.03	0.23	-0.19	-0.11	0.27	-0.00	0.02	0.12
NPL-UPA2	-0.11	0.03	0.18	-0.11	-0.31	-0.32	**-0.34**	-0.18	-0.17	-0.19	-0.20	-0.17	0.10	0.10	0.18	-0.10	-0.28	-0.01	-0.07
Nitrospirae	0.17	**-0.41**	0.30	-0.21	-0.01	-0.01	0.00	-0.02	0.24	**0.40**	-0.12	-0.16	**-0.47**	-0.29	-0.18	-0.07	0.07	0.25	-0.17

Table 17
continued

	Soil Properties																		
	pH	EC	SAR	CEC	TC	OC	SC	TN	NO_3^-	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
OPS8	0.14	0.17	-0.09	0.12	0.10	0.12	0.12	0.12	0.09	-0.01	0.09	0.21	0.06	0.16	0.03	-0.01	0.10	-0.07	0.10
Planctomycetes	**-0.50**	0.02	**-0.63**	**0.34**	**0.59**	**0.60**	**0.61**	**0.52**	0.12	0.28	**0.40**	0.12	0.05	0.09	**-0.62**	**0.58**	**0.56**	0.18	**0.40**
Proteobacteria	0.13	0.11	**0.37**	-0.32	**-0.63**	**-0.64**	**-0.65**	**-0.61**	-0.28	**-0.59**	**-0.42**	-0.14	0.26	0.07	**0.66**	**-0.34**	**-0.64**	-0.13	**-0.40**
Spirochaetes	0.12	**-0.41**	**0.57**	**-0.44**	-0.21	-0.22	-0.21	-0.14	**0.41**	**0.47**	**-0.35**	**-0.39**	**-0.63**	-0.17	-0.10	-0.19	-0.10	0.22	**-0.37**
Synergistetes	0.27	0.28	-0.12	0.29	0.16	0.15	0.13	0.07	-0.08	-0.19	0.07	0.33	0.07	-0.04	0.16	-0.18	0.06	-0.31	0.07
TA06	**0.35**	-0.01	0.15	0.14	0.05	0.07	0.06	0.05	0.12	0.05	-0.08	0.08	-0.23	-0.07	-0.00	-0.25	0.20	-0.10	0.19
Thermodesulfo-bacteria	0.11	0.14	-0.03	0.29	0.08	0.08	0.06	0.07	-0.12	-0.07	0.21	0.23	0.11	-0.02	0.09	0.06	0.23	-0.13	0.31
Verrucomicrobia	**0.38**	**0.50**	0.10	0.14	-0.17	-0.17	-0.18	-0.28	-0.29	**-0.53**	-0.02	**0.36**	**0.39**	0.23	**0.68**	-0.24	-0.33	**-0.45**	-0.08
WCHB1-60	-0.22	-0.13	-0.16	0.04	-0.00	-0.01	-0.02	0.12	-0.04	0.10	0.09	-0.09	0.09	-0.16	-0.19	0.07	0.08	0.02	0.16

Values in bold indicate significant correlation ($p < 0.05$). EC = electrical conductivity. SAR = sodium adsorption ratio. CEC = cation exchange capacity. TC = total carbon. OC = organic carbon. SC = non-lignitic organic carbon. NO_3^- = NO_3^--N.

Table 18. Correlations (pairwise) of soil biological parameters and qPCR with relative phylum abundance.

	Soil Parameters									
	SMB-C	SMC-N	SMB-C/N	Cmin	Nmin	NO_3^-min	NH_4^+min	*Bacteria*	*Fungi*	*B:F*
Acidobacteria	**0.67**	**0.67**	-0.02	**0.56**	**0.60**	**0.59**	**0.45**	**0.37**	**0.40**	0.20
Actinobacteria	**0.46**	**0.34**	0.11	**0.43**	0.26	0.26	0.20	0.26	**0.40**	0.05
Aquificae	0.22	0.26	-0.01	0.26	0.21	0.20	0.20	0.04	0.03	0.05
BD1-5	-0.03	0.05	-0.15	-0.08	0.08	0.05	0.14	-0.11	**-0.41**	**0.35**
Bacteroidetes	**-0.40**	**-0.35**	-0.01	**-0.45**	**-0.39**	**-0.38**	-0.28	-0.30	-0.25	**-0.40**
Cand_div_BRC1	0.11	0.10	-0.11	0.08	0.10	0.06	0.24	-0.13	-0.32	0.03
Cand_div_OD1	0.06	-0.03	0.09	0.12	0.08	0.09	-0.01	-0.13	-0.16	0.03
Cand_div_OP10	**-0.42**	**-0.44**	-0.10	**-0.37**	**-0.36**	**-0.33**	**-0.37**	-0.17	-0.20	**-0.36**
Cand_div_OP3	-0.10	-0.10	-0.02	0.06	-0.12	-0.06	-0.29	0.07	-0.00	0.14
Cand_div_SR1	-0.48	-0.49	-0.02	-0.33	-0.37	-0.35	-0.33	-0.25	**-0.38**	0.00
Cand_div_TG-1	-0.16	-0.06	-0.12	-0.11	-0.10	-0.08	-0.13	-0.03	-0.20	0.31
Cand_div_TM6	-0.14	-0.11	0.05	0.08	0.02	0.03	-0.02	-0.27	-0.30	-0.14
Cand_div_TM7	**-0.66**	**-0.60**	-0.08	**-0.44**	**-0.41**	**-0.42**	-0.28	**-0.42**	**-0.59**	-0.18
Cand_div_WS3	**0.59**	**0.68**	-0.18	**0.53**	**0.54**	**0.60**	**0.57**	**0.36**	0.23	**0.33**
Chlamydiae	0.02	0.12	-0.08	-0.02	-0.04	-0.03	-0.08	0.05	0.06	-0.12
Chlorobi	-0.27	-0.10	-0.20	-0.18	-0.21	-0.16	-0.32	0.12	-0.03	0.18

Table 18 continued

	Soil Parameters									
	SMB-C	SMC-N	SMB-C/N	Cmin	Nmin	NO_3^-min	NH_4^+min	*Bacteria*	*Fungi*	*B:F*
Chloroflexi	0.19	0.14	0.00	0.19	0.12	0.05	**0.36**	-0.21	-0.32	-0.08
Chrysiogenetes	-0.11	-0.08	-0.12	-0.21	-0.16	-0.18	-0.01	-0.22	**-0.33**	-0.12
Cyanobacteria	-0.57	-0.50	-0.06	-0.41	-0.50	-0.48	-0.41	-0.25	-0.29	-0.20
Deferribacteres	**0.43**	**0.49**	0.00	**0.41**	**0.35**	**0.33**	0.30	0.13	0.12	0.14
Deinococcus-Thermus	-0.07	-0.10	0.07	0.12	0.15	0.13	0.19	-0.27	-0.24	-0.11
Fibrobacteres	-0.01	-0.07	0.30	-0.08	-0.19	-0.17	-0.23	-0.22	-0.12	0.10
Firmicutes	-0.18	-0.16	0.05	-0.16	-0.18	-0.13	-0.28	0.13	0.10	**0.41**
Fusobacteria	0.21	0.20	-0.05	0.06	0.11	0.13	-0.01	0.24	0.21	0.26
GOUTA4	**-0.35**	-0.29	-0.18	-0.13	-0.29	-0.28	-0.26	-0.12	**-0.36**	0.13
Gemmatimonadetes	-0.15	-0.18	0.01	-0.14	-0.05	-0.08	0.05	-0.17	-0.29	0.08
Lentisphaerae	0.08	0.09	-0.18	0.02	0.27	-0.01	0.16	0.06	-0.01	-0.15
NPL-UPA2	**-0.35**	-0.32	-0.09	-0.26	-0.30	-0.31	-0.17	-0.22	-0.28	-0.29
Nitrospirae	0.13	0.15	-0.07	0.09	0.04	0.05	0.01	0.22	-0.05	**0.46**
OPS8	0.13	0.25	-0.08	0.16	0.13	0.11	0.14	0.04	0.05	-0.10
Planctomycetes	**0.62**	**0.64**	-0.22	**0.59**	**0.57**	**0.56**	**0.41**	**0.52**	**0.43**	0.17

Table 18 continued

	Soil Parameters									
	SMB-C	SMC-N	SMB-C/N	Cmin	Nmin	NO_3^-min	NH_4^+min	*Bacteria*	*Fungi*	*B:F*
Proteobacteria	**-0.68**	**-0.64**	-0.06	**-0.62**	**-0.51**	**-0.50**	**-0.39**	**-0.35**	-0.32	**-0.47**
Spirochaetes	-0.12	-0.25	**0.37**	-0.09	-0.22	-0.18	-0.30	-0.17	-0.03	0.25
Synergistetes	0.03	0.04	-0.11	0.06	0.18	0.13	0.30	-0.13	-0.29	0.03
TA06	-0.04	-0.08	**0.34**	0.05	0.16	0.13	0.18	-0.33	-0.14	-0.03
Thermodesulfobacteria	0.12	0.03	0.11	0.18	0.21	0.17	0.28	-0.19	-0.16	-0.12
Verrucomicrobia	-0.31	-0.29	0.05	-0.27	-0.15	-0.16	-0.06	**-0.48**	**-0.58**	-0.19
WCHB1-60	0.01	-0.08	0.19	0.12	0.02	0.04	-0.05	-0.09	0.17	**-0.35**

Values in bold indicate significant correlation ($p < 0.05$). SMB-C = soil microbial biomass carbon. SMB-N = soil microbial biomass nitrogen. Cmin = carbon mineralization. Nmin = nitrogen mineralization. NO_3^- min = portion of nitrogen mineralization as nitrate. NH_4^+ min = portion of nitrogen mineralization as ammonium. *B:F* = ratio of Bacteria to Fungi.

Bacteroidetes, *Candidate division TM7*, *Chloroflexi*, *Firmicutes*, *Gemmatinmonadetes*, *Planctomycetes*, and *Verrucomicrobia* were minority phyla but made up between 1 to 8% of all sequences (Table 16). *Bacteroidetes* increased almost two-fold after mining but decreased back to premined levels and maintained after 10 years. *Bacteroidetes* were positively correlated with pH, but negatively correlated with macro- and micronutrients, as well as with SMB-C and N, mineralization rates, and *B:F* ratios. *Candidate division TM7* contributed more to total sequences out of the 9 candidate divisions. The relative abundance of *Candidate division TM7* increased more than two-fold after mining, but decreased to premined levels and maintained after 10 years. *Candidate division TM7* correlated positively with EC and Na, but negatively correlated with carbon pools, SMB-C and N, C and N mineralization rates, *Bacteria* and *Fungi*. *Chloroflexi* proportions in reclaimed sites were higher than premined levels. There was a two-fold increase of *Chloroflexi* after mining, followed by a decrease at 10 years. The MO sites had *Chloroflexi* proportions similar to CP0 and CP5. *Chloroflexi* correlated significantly with all chemical properties, and secondary nutrients. *Firmicutes* was highest in the UM site. Reclaimed proportions of *Firmicutes* were similar in CP and MO sites, which correlated positively with SAR, NO_3^--N and P, and negatively with EC, CEC, Ca, and Mg. *Firmicutes* correlated positively with the *B:F* ratio. *Gemmatinmonadetes* were similar through all sites, and peaked at CP15. *Planctomycetes* in the UM site was similar to early reclamation sites through 10 years. At 15 years, *Planctomycetes* increased and stayed statistically similar to 40 years. *Planctomycetes* correlated negatively with pH, SAR, and Na, but positively with carbon pools, K, and some micronutrients. *Planctomycetes* correlated positively with SMB-C and N, mineralization rates, *Bacteria* and *Fungi*. *Verrucomicrobia* increased after mining began, but

decreased shortly after and maintained. *Verrucomicrobia* correlated positively with pH and EC, Ca, Mg, and Na. *Verrucomicrobia* negatively correlated with *Bacteria* and *Fungi*.

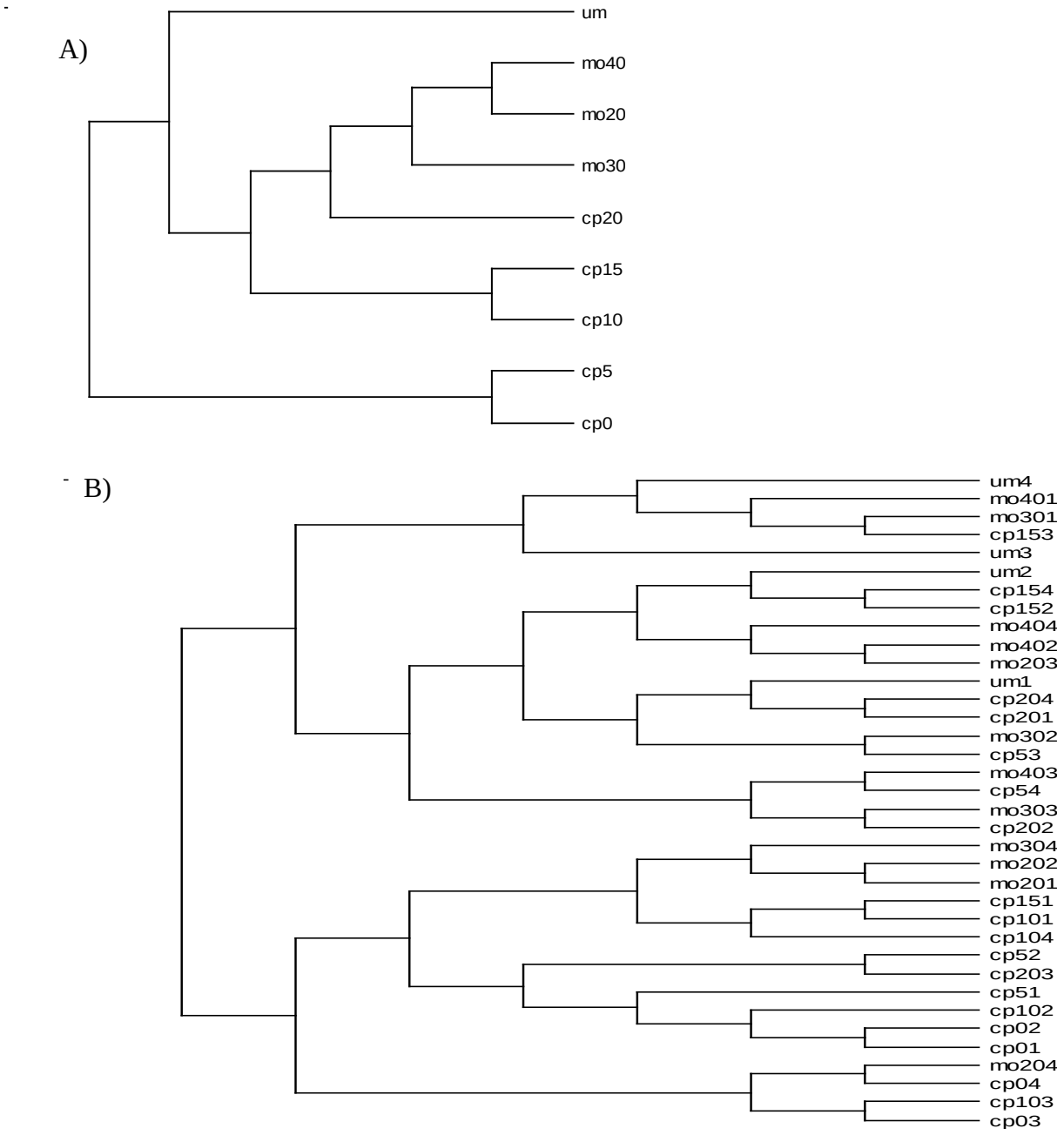

Fig. 11. Cladograms based upon Bray-Curtis dissimilarity between A) sites and B) transects from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by site.

A subsample of 4,964 sequences from each site was used for similarity and diversity analyses. We observed the reclamation site with highest similarity to the UM site was the CP20 site. However, reclamation sites 10 years and older had comparable Bray-Curtis values (Fig. 11A and Table 19). The CP0 was the most dissimilar to the UM site. Similarity between reclamation sites increased with age (CP0 vs. CP5, CP5 vs. CP10 etc.). Bray-Curtis values showed that the most similarity between UM transects was with older sites: CP153, CP201, MO302, and MO403 (Figure 11B). There were about 3,000 OTUs identified at each site during the richness and diversity analyses. Chao's richness estimator (*S*) decreased after mining and reclamation, but reclamation sites had mostly similar *S* to the UM site when improving after 5 years (Table 20). The highest *S* was found in CP5 and CP10. MO20 had higher S than CP20, but was statistically similar. In addition to richness, CP5 also had the highest diversity indices among all sites (Table 20). The Shannon's diversity index (*H*), decreased significantly after mining, but exceed premined levels after 5 years. The highest *H* was observed in the CP5 site, and lowest in the CP15 site. *H* was similar between CP20 and MO20 and was higher than older sites which were similar in diversity (i.e. MO30 and MO40). Simpson's Inverse Index (*D*) increased after mining through 5 years, but was lower than the UM site in CP sites 10 and 15. Sites 20 years and older were similar to the UM site.

Table 19. Bray-curtis values between sites of operational taxonomic units (OTUs) from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas.

	Site								
	UM	CP0	CP5	CP10	CP15	CP20	MO20	MO30	MO40
UM	-								
CP0	0.90	-							
CP5	0.87	0.79	-						
CP10	0.84	0.89	0.82	-					
CP15	0.86	0.94	0.88	0.75	-				
CP20	0.83	0.87	0.81	0.82	0.80	-			
MO20	0.83	0.88	0.83	0.81	0.78	0.73	-		
MO30	0.84	0.88	0.83	0.85	0.83	0.74	0.72	-	
MO40	0.84	0.87	0.84	0.84	0.79	0.75	0.71	0.73	-

Higher values mean more dissimilarity. UM = unmined pasture. CP = crosspit spreader. MO = mixed overburden.

Table 20. Richness and diversity indices from OTUs (operational taxonomic units) and a Biolog Ecoplate™ from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and mixed overburden reclamation treatments (MO) in eastern Texas compared by site. Data are displayed as an average of data from 4 transects at each site.

	OTU							Biolog
	OTU	Chao	Confidence Interval*	Shannon	Confidence Interval*	Invsimpson	Confidence Interval*	Shannon
UM	3220	13377	12116 to 14817	7.67	7.63 to 7.70	818.3	698.7 to 988.1	2.68ab
CP0	2924	10143	9232 to 11183	7.54	7.50 to 7.58	963.4	856.0 to 1101.1	2.75ab
CP5	3324	13502	12272 to 14900	7.74	7.70 to 7.77	1083.4	945.0 to 1270.2	2.73ab
CP10	3282	13400	12160 to 14813	7.70	7.67 to 7.74	660.5	545.5 to 836.6	2.85ab
CP15	3076	12412	11233 to 13761	7.51	7.47 to 7.55	396.4	334.8 to 485.8	2.98a
CP20	3254	13139	11931 to 14515	7.67	7.64 to 7.71	793.0	679.8 to 951.8	2.91ab
MO20	3234	13361	12101 to 14796	7.66	7.62 to 7.69	634.1	535.7 to 777.5	2.75ab
MO30	3130	13043	11793 to 14475	7.59	7.55 to 7.63	745.5	651.2 to 871.7	2.62b
MO40	3125	12399	11240 to 13722	7.60	7.56 to 7.64	707.7	609.0 to 844.6	2.82ab

Means in the same column followed by the same letter are not significantly different by site ($P<0.05$). UM = unmined pasture. CP = crosspit spreader. MO = mixed overburden. *95% Confidence Intervals calculated by mothur.

Community-level Physiological Profiling

Principal component analysis axes 1 and 2 explained 12.52 and 9.55% of the variance, respectively, between sites (Fig. 12). No clear separation was observed between sites or explained by the type of carbon substrate. Some clustering of CP15, CP20, and MO20 was observed. An ANOSIM revealed that significant differences at the $p < 0.05$ level were observed between CP15 and CP0 and CP15 and MO20 (data not shown). Some differences ($p < 0.1$) were observed between CP20 and younger sites. Metabolic diversity in the Biolog Ecoplate™ was statistically similar among all sites, but mostly had higher values in reclamation sites than the UM site. Shannon's diversity index peaked at CP15 and

bottomed at MO30, which were the only sites that were significantly different from each other (Table 20).

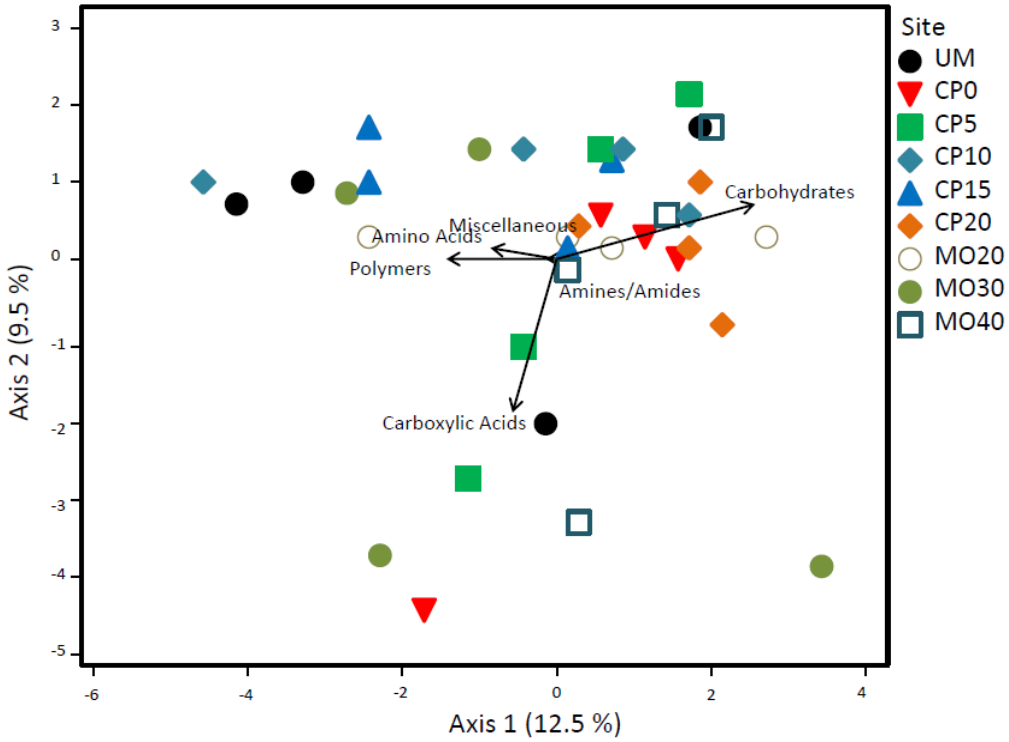

Fig. 12. Principal component analysis of carbon substrate use in a Biolog Ecoplate™ among an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) aged 0, 5, 10, 15, and 20 and mixed overburden (MO) reclamation treatments aged 20, 30, and 40 in eastern Texas compared by site. Data are displayed from 4 transects at each site.

GeoChip

A detrended correspondence analysis (DCA) showed good grouping among transects from each of the sites (Fig. 13). DCA 1 and DCA 2 accounted for 20.3 and 18.1% of variance, respectively. There was good separation between transects from the UM site and transects from the reclamation sites with MO40 being the most similar site to the UM site. Among the reclamation sites, there appeared to be separation with age as CP10 and CP20

grouped together and separated from CP0. Signal intensity percentages from gene categories showed that reclamation sites were similar or exceeded that of the UM site, differing by no more than 1.5% (Fig. 14). Organic remediation genes had the highest percentage of genes (41.6%), followed by metal resistance and carbon cycling (19.0%). Nitrogen, phosphorus, and sulfur genes represented 10.4%, 2.6%, and 6.8%, respectively. Genes that were associated with carbon cycling and sulfur were higher in reclaimed sites than the UM site. Genes associated with metal resistance, nitrogen, organic remediation, and phosphorus had at least one reclamation site with a lower percentage than the UM site.

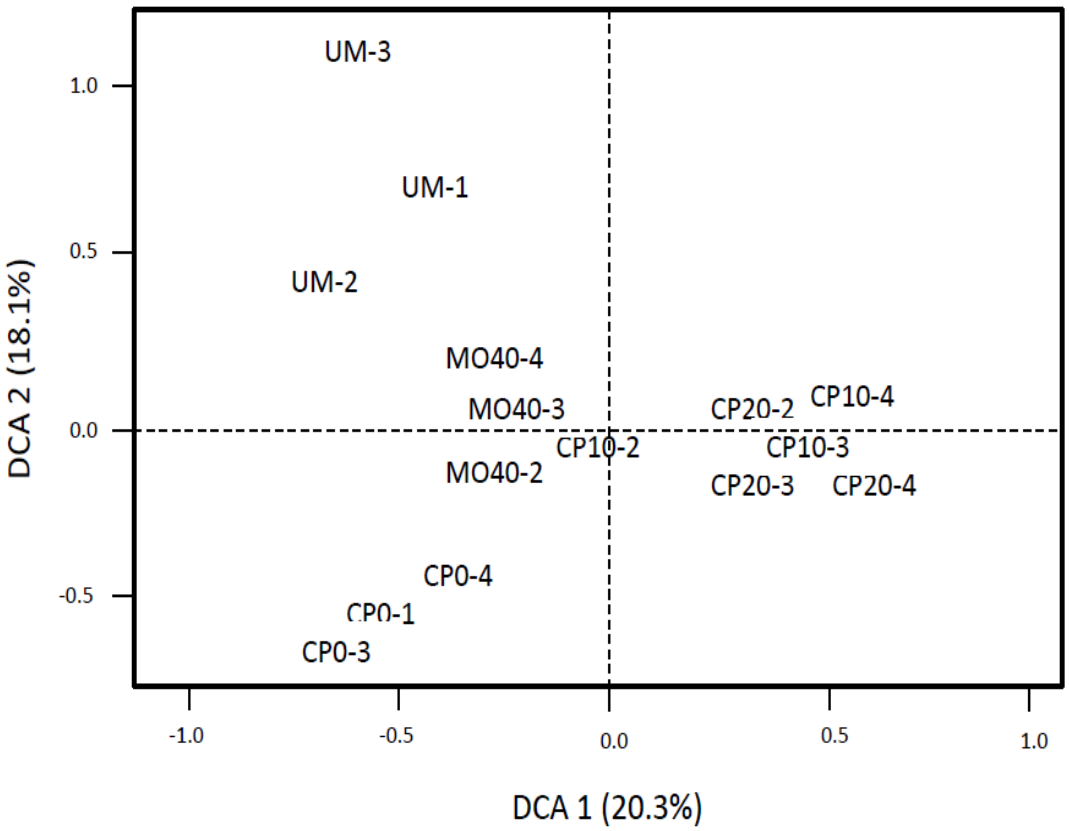

Fig. 13. Detrended correspondence analysis (DCA) of GeoChip data from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) aged 0, 10, and 20and mixed overburden (MO) reclamation treatments aged 40 in eastern Texas compared by site. Data are displayed 3 transects from each site.

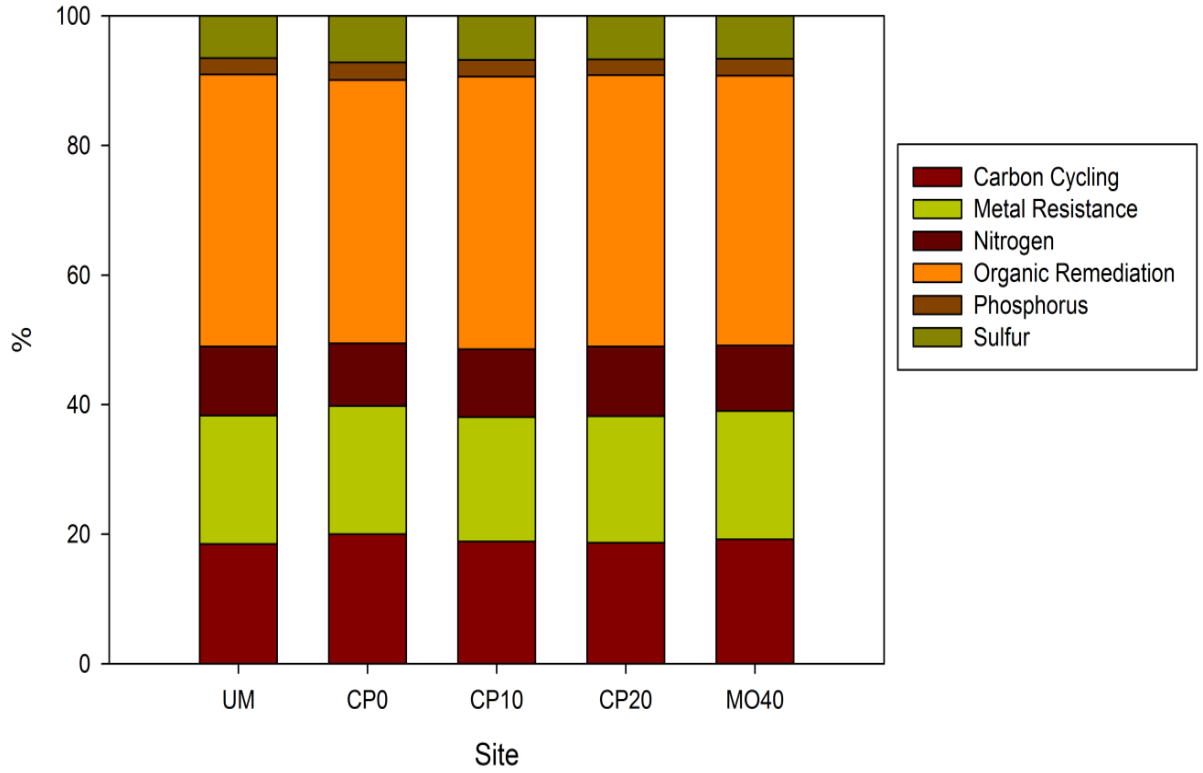

Fig 14. Contribution of GeoChip gene signal intensities (carbon cycling, metal resistance, nitrogen, organic remediation, phosphorus, and sulfur) from an unmined site (UM) and reclaimed minesoils from crosspit spreader (CP) and a mixed overburden reclamation treatment (MO) in eastern Texas compared by site. Data are displayed as an average of data from 3 transects at each site.

DISCUSSION

As post-mined sites are rehabilitated, reclamation land managers aim to return sites to a premined or improved condition as mandated by SMCRA (1977). Surface mine reclamation standards for soil in Texas are intended to promote revegetation, and minimize erosion and runoff: < 80% sand, < 40% clay, pH 5.0-8.4, > 0.0 acid/base, < 4.0 EC (mmhos/cm), and < 13.0 SAR. In addition to these standards, we found that many soil physical and chemical parameters in the chronosequence improved from or returned to a premined state (Tables 9 and 10). These soil properties in turn correlated with many phyla of

the soil bacterial community, major drivers of soil functionality: decomposition and nutrient cycling (Tables 11 and 12). While we have observed a broad return of soil functionality through mineralization rates and soil microbial biomass, the objective of this study was to determine if the soil microbial community returned to a premined or improved state.

No one soil microbial community from any RMS had an identical composition to the unmined pasture (Table 19). However, we found that the sites 10 years and older were most similar to the UM site (Fig. 11A, Table 19). While CP20 had the highest overall similarity, we found that individual transects from CP15, CP20, MO30, and MO40 were similar to transects from the UM site. Odum's (1969) theory of ecological succession states that after disturbance, an ecosystem's soil quality indicators will decrease after time, and increase before reaching a maximum capacity. The composition of any microbial community is dependent on the environment around it (Harris, 2003). Since soil physical and chemical properties were not returned to the exact premined state (instead actually being improved in many cases), we would not expect identical soil microbial communities to be found in the unmined and a successfully reclaimed sites (Tables 9, 10 and 12) (Banning et al., 2011). Given that sites 15 years and older had transects that were most similar to the UM site, we examined the soil parameters at these sites to determine which, if any, parameters influenced the soil bacterial phyla. Soil parameters: EC, SAR, BD, clay, pH, SC, TN, NO_3^--N, P, K, S, Na, Fe, Mn and Cu either stabilized from 15 to 40 years or were similar through the older MO sites, suggesting that importance of these properties in returning the microbial community to a premined state. It is important to note that returning the soil properties in a reclaimed site to near similar levels found in an unmined site may not guarantee an exact microbial population because of additional environmental factors (ex. temperature, aeration)

and soil-forming factors (ex. time) that influence the soil microbial populations. If a soil microbial community is unlikely to return to an unmined state, the use of richness and diversity indices as soil microbial quality indicators may be an option as these parameters exceeded premined conditions early in reclamation (5 years), similar to other predefined soil quality indicators, soil carbon and mineralization (Franzluebbers et al., 2000; Ingram et al., 2005) (Table 20). The diversity of the soil microbial community in reclamation sites could be equated with functionality, resilience to disturbance, and their importance to total ecosystem health, and therefore should be considered as a soil health indicator in future research (Torsvik and Ovreas, 2002).

The dominant phyla in our chronosequence were *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and accounted for more than 70% of all sequences (Fig. 10 and Table 16). *Acidobacteria* and *Actinobacteria* increased with age (Chronakova et al., 2010) and correlated with pH, and have been identified as important in the soil stabilization of RMS (Carrasco et al., 2009; Kuramae et al., 2010). As the pH of CP sites showed a trend of declining with age, land managers at the Big Brown Mine should ensure stable pH to maintain the abundance of these phyla (Table 9). These phyla also correlated with multiple C pools, macronutrients, and mineralization (Tables 12 and 13), which have been previously documented (Banning et al., 2011; Nielsen et al., 2011). Not only is the abundance of these nutrients and fluxes indicative of healthy microbial community, but also supportive of a plant community that is continually inputting C into that ground that can then be mineralized and stabilized into different aggregate fractions (Chapter II).

The correlation of bacterial populations with metal concentrations is a well-documented field, as acid mine drainage and metal solubilization are problems commonly

associated with poorly managed mines (Aceves, et al. 1999; Piotrowska-Seget et al. 2005). Concentrations of some metals were slightly higher in the reclaimed sites, but were not an observable inhibitor to plant growth. Metals had negative correlations with several dominant phyla: *Bacteroidetes*, *Candidate division TM7*, *Chlorobi*, and *Cyanobacteria*. The negative correlation with *Proteobacteria*, a very diverse phylum of soil bacteria, differed from a study in which mine tailings were dominated by members of this group (Chen et al., 2008). At the same time, a more general scope of the microbial population analysis could mask the effects of metals on specific genera. Further investigation into the more specific taxa within the *Proteobacteria* phylum may reveal data supported by Chen et al. (2008). We also observed several positive correlations of metals with other abundant phyla: *Acidobacteria*, *Actinobacteria*, and *Planctomycetes*. As previously discussed, these phyla correlated with pH, and other soil quality indicators, suggesting that the increased metal concentrations at the mine may be more important in influencing the proportion of other beneficial phyla of RMS.

Our observation of the minor representatives of phyla had been sparsely documented in other mine sites: *Candidate division TM7* (Banning et al., 2011), *Firmicutes* (Valverde et al., 2011), and *Verrucomicrobia* (Jangid et al., 2010). *Firmicutes*, which include *Bacillus spp.*, were found in the highest abundance in the UM site, and never recovered to similar levels in the RMS. As it correlated well with *Bacteria* to *Fungi* ratios and macronutrients, this phylum could be a potential soil health indicator (Table 13). *Candidate division TM7* and *Verrucomicrobia* had weak or negative correlations with soil C pools and mineralization, but showed a relationship with EC and Na. While not prioritized as high as soil C, EC is a regulated soil parameter in RMS, and may indicate some significance of these less abundant phyla.

On a broad scale, qPCR showed that *Bacteria* and *Fungi* returned to premined levels, agreeing with SMB-C and SMB-N data (Figure 9). While our SMB data improved to premined levels after 16 years, qPCR data showed a return after 20 years. While similar, the discrepancy of results could originate from the differences of the techniques used (carbon vs. gene copy numbers), or absence of other unaccounted soil microorganisms (ex. algae, protozoa, *Archaea*). The range of copy numbers of soil bacteria are supported by past data (Rosello-Mora and Amann, 2001) and indicate that the parent material used for reclamation at the Big Brown Mine was suitable for reclamation, soil functionality and revegetation. The poor quality and recovery (ie. low SMB and activity) of mine spoils mismanaged as parent material further emphasize the importance of soil health management in disturbed ecosystems (Akala and Lal, 2001; Mummey et al., 2002b; Stroo and Jenks, 1982). The ratio of *Bacteria*-to-*Fungi* also showed a predictable trend of decreasing and then increasing with reclamation age through 30 years. While statistically similar, the MO30 ratio only reached 81% of UM levels. This trend suggests that this soil biological parameter could be a potential soil quality indicator in mines as it has been used in comparing tillage sites and other prairie ecosystems (Allison et al., 2005; Helgalson et al., 2009). The ratio did not correlate positively with any of our dominant phyla (ie. *Acidobacteria*, *Actinobacteria*, and *Proteobacteria*), but showed minor correlations with some lesser representatives: *Bacteroidetes* and *Firmicutes*. We are unsure of the cause of this lack of correlation. At the very least, more in-depth analysis is needed to better understand how management of RMS affects populations of soil microorganisms.

From a management viewpoint, the composition of the soil microbial community may not be important unless it correlates with soil functionality, which was measured with a

Biolog Ecoplate™. The C substrate utilization patterns did not differ much between sites, indicating that the microbial community had recovered functionally much faster than by taxonomic composition (Figure 12). This finding was similar to what had found in a study comparing functionality and taxonomy with the Biolog Ecoplate™ and FAME analysis (Peach, 2001). The diversity of substrates utilized increased over time and peaked in CP15 and 20, indicating that the soil microbial functionality in reclaimed sites exceeded the functional diversity of premined sites, which agreed with previous work from Chodak et al. (2009). The increase in diversity may have been influenced by the vegetation change, and additional plant-derived carbon inputs than age (Garcia-Palacios et al., 2011). If all sites had been vegetated with the same species, we might expect a decrease or slower increase in metabolic diversity as microorganisms would be selected to compete for the same carbon sources over an extended period of time.

Alternatively, GeoChip, a functional gene microarray that has been used in soil microbial analysis in grasslands, was used to analyze soil microbial functionality on our RMS chronosequence (He et al., 2007). A DCA showed differences among reclamation sites with older reclamation sites being more similar to the UM site than younger reclamation sites (Fig. 13). While we observed a difference between sites, the analysis of signal intensities from gene categories showed that all reclaimed sites had mostly similar percentages to the UM site agreeing with CLPP results (Fig. 14). However, further analyses could reveal the differences between subcategories, individual genes, their correlations with soil properties, and then the soil microorganisms to which they belong to (i.e. another analysis of community composition (He et al., 2010; Pastorelli et al., 2011; Reeve et al., 2010). Additionally, as

sequencing of the fungal community improves, identifying this important group of decomposers will be valuable in RMS management (Fontaine et al., 2011).

CONCLUSION

Several parameters of the soil microbial community of a reclaimed surface mine were able to meet and exceed the premined levels, indicating potential use as a soil quality indicator. Sites 10 years and older were more closely related to the unmined site than younger sites and, but CP20 had was most similar to the UM site. Dominant phyla (*Acidobacteria*, *Actinobacteria*, and *Proteobacteria*) comprised 70% of the bacterial community, and levels of these phyla returned to premined levels after 10 years. Soil parameters: EC, SAR, BD, clay, pH, SC, TN, NO_3^--N, P, K, S, Na, Fe, Mn and Cu either stabilized from 15 to 40 years or were similar through the older MO sites, suggesting that importance of these properties in returning the microbial community to a premined state. Several phyla also correlated well with previously defined soil quality indicators: soil microbial biomass and mineralization. The return of community diversity and richness may be an adequate parameter in measuring soil health recovery due to its importance in functionality and resilience to disturbance. The diversity of soil functionality measured by a Biolog Ecoplate^{TM} also was similar between reclaimed sites and the premined site, but few differences between substrate utilization patterns were observed between sites. While this suggests that the functionality of microbial community had recovered much faster than by taxonomic composition, GeoChip showed that differences persist between sites even after 40 years, but that older sites were more similar to the UM site. Further analysis of functional

genes and fungal sequencing will expand upon this reclamation research so that a better understanding of the role that soil microbial communities play is made.

CHAPTER V

CONCLUSION

SUMMARY

The Big Brown Mine's surface mine reclamation practices have been in operation for 40 years. While vegetation and surface hydrology have been maintained, we showed through the use of a chronosequence that soil physical and chemical parameters through 1 m of soil, and soil biological characteristics to 15 cm of soil improved over time compared to a premined condition. Changes in soil texture (sand to clay loam) increased C sequestration up to ~250 Mg C ha^{-1} at the MO20 site. Most soil C was non-lignitic organic C and associated with macroaggregates and small macroaggregates (> 2 mm, 2 mm – 250 μm), with a small amount in lignitic C. Soil organic C and N reached or exceeded premined concentrations after 0 and 10 years, respectively. Levels of soil C and N throughout the profile equaled or exceeded premined levels after 15 years. Nitrate fluctuated during reclamation and never returned to premined levels, but developed profile stratification after 5 years of reclamation. Soil P also did not reach premined conditions but increased steadily over time. Soil K and Ca exceeded premined conditions and exhibited stratification after 10-15 years. Contrastingly, soil Mg and S exceeded unmined conditions and displayed increases in depth. Post-mining concentrations of Fe, Zn, and Cu exceeded premined concentrations, while Mn returned to unmined concentrations after 10 years. Soil microbial biomass and C and N mineralization required 15 years before exceeding unmined levels. *Bacteria* and *Fungi* recovered to premined levels after 20 years. Many of these soil parameters correlated with the relative

abundance of soil microbial populations, and older sites (10 to 40 years) had more communities more similar to the UM site than younger sites (0 and 5 years). Three phyla (*Acidobacteria*, *Actinobacteria*, and *Proteobacteria*) represented 70% of the soil microbial community, while four more phyla (*Bacteroidetes*, *Chloroflexi*, *Firmicutes*, and *Planctomycetes*) represented up to 20% of all sequences of the 37 phyla represented. Diversity and richness estimates also recovered to premined levels. Soil microbial functionality appeared to recover faster than taxonomic composition of the soil microbial community. Soil functionality measured by community-level physiological profiling did not differ much between sites indicating their speedier recovery compared to taxonomic measurements. A functional gene array (GeoChip) showed separation between the UM sites and reclamation sites, although older sites were more similar to the UM site than younger sites. Further analysis of functional genes and *Fungi* sequencing will expand upon this research so that we may better quantify the soil quality.

RECOMMENDATIONS

Establishing and maintaining vegetation, minimizing erosion, and restoring hydrologic function in addition to future productivity are concerns for land managers and owners of reclaimed mine sites. Surface mine reclamation managers must follow suitable surface soil criteria when rehabilitating post-mined lands so that a premined or improved condition is established. We found that all reclaimed sites in our chronosequence initially met these criteria and supported vegetation. Over time, the reclaimed sites exceeded the conditions found in our unmined site. The use of the crosspit spreader replacing mixed overburden improved soil quality in terms of returning a disturbed site to a premined state in

terms of soil nutrient distribution, and the composition of the soil microbial community. However, the mixed overburden sites achieved higher carbon and nutrient levels. While we cannot observe younger mixed overburden sites, it is suggested that the older methods of overburden removal, backfilling and regrading be revisited to enhance future mining and reclamation techniques. Additionally, routine maintenance of liming and P fertilizer application is suggested to maintain pH consistency and provide adequate nutrient concentrations as the sites age. Other surface mining companies could use the Big Brown Mine as a model for land reclamation, or at least consider using their reclamation techniques to improve the quality of the surface soil that is initially backfilled on post-mined land and ensure revegetation. I feel that further analysis of functional genes and fungal sequencing will expand upon this reclamation research so that a better understanding of the role that soil microbial communities play is made. Additionally, time will tell if soil microbial communities in the CP and MO sites continue to become more similar to the UM site.

REFERENCES

Abdul-Kareem, A.W., and S.G. McRae. 1984. The effects on topsoil of long-term storage in stockpiles. Plant Soil 76:357-363.

Aceves, M.B., C. Grace, J. Ansorena, L. Dendooven, P.C. Brookes. 1999. Soil microbial biomass and organic C in a gradient of zinc concentrations in soils around a mine spoil tip. Soil Biol. Biochem. 31:867-876.

Adu, J.K., and J.M. Oades. 1978. Physical factors influencing decomposition of organic materials in soil aggregates. Soil Biol. Biochem. 10:109-115.

Aguilar, J., C. Dorronsoro, E. Fernandez, J. Fernandez, I. Garcia, F. Martin, and M. Simon. 2004. Soil pollution by a pyrite mine spill in Spain: Evolution in time. Environ. Pollut. 132:395-401.

Akala, V.A., and R. Lal. 2000. Potential of mineland reclamation for soil carbon sequestration in Ohio. Land Degrad. Develop. 11:289-297.

Akala, V.A., and R. Lal. 2001. Soil organic carbon pools and sequestration rates in reclaimed minesoils in Ohio. J. Environ. Qual. 30:2098-2104.

Allison, V.J., R.M. Miller, J.D. Jastrow, R. Matamala, and D.R. Zak. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Sci. Soc. Am. J. 69:1412-1421.

An, S.-S., Y.-M. Huang, and F.-L. Zheng. 2009. Evaluation of soil microbial indices along a revegetation chronosequence in grassland sols on the Loess Plateau, Northwest China. Appl. Soil Ecol. 41:286-292.

Anderson, J.D., L.J. Ingram, and P.D. Stahl. 2008. Influence of reclamation management practices on microbial biomass carbon and soil organic carbon accumulation in semiarid mined lands of Wyoming. Appl. Soil Ecol. 40:387-397.

Anderson, T.-H., and K.H. Domsch. 1990. Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biol. Biochem. 22:251-255.

Angel, P.N. 1973. A soil analysis of the strip mine spoil bank at Fairfield, Texas. M.S. Thesis. Stephen F. Austin State University, Nacogdoches, TX.

Appuhn, A., and R.G. Joergensen. 2006. Microbial colonisation of roots as a function of plant species. Soil Biol. Biochem. 38:1040-1051.

Askenasy, P.E. 1977. Soil factors influencing row crop production and phosphate adsorption on leveled lignite mine soil banks. Ph.D. Dissertation. Texas A&M University, College Station, TX.

Banning, N.C., C.D. Grant, D.L. Jones, and D.V. Murphy. 2008. Recovery of soil organic matter, organic matter turnover and nitrogen cycling in a post-mining forest rehabilitation chronosequence. Soil Biol. Biochem. 40:2021-2031.

Banning, N.C., D.B. Gleeson, A.H. Grigg, C.D. Grant, G.L. Anderson, E.L. Brodie, and D.V. Murphy. 2011. Soil microbial community successional patterns during forest ecosystem restoration. Appl. Environ. Microbiol. 77:6158-6164.

Barnhisel, R.I., and J.M. Hower. 1997. Coal surface mine reclamation in the eastern United States: The revegetation of disturbed lands to hay/pasture or cropland. Adv. Agron. 61:233-275.

Barnhisel, R.I., and H.F. Massey. 1969. Chemical, mineralogical, and physical properties of eastern Ky acid-forming coal spoil materials. Soil Sci. 108:367-372.

Barth, A.K. 2002. Land capability classification of minesoils in east Texas. M.S. Thesis, Texas A&M University, College Station, TX.

Bearden, E.D. 1984. A comparison of variability of undisturbed and surface-mined soils in Freestone county, Texas. M.S. Thesis, Texas A&M University, College Station, TX.

Beck, T., R.G. Joergensen, E. Kandeler, F. Makeschin, E. Nuss, H.R. Oberholzer, and S. Scheu. 1997. An Inter-laboratory comparison of ten different ways if measuring soil microbial biomass C. Soil Biol. Biochem. 29:1023-1032.

Bissett, A., A.E. Richardson, G. Baker, and P.H. Thrall. 2011. Long-term land use effects on soil microbial community structure and function. Appl. Soil Ecol. 51:66-78.

Boyle, S.A., R.R. Yarwood, P.J. Bottomley, and D.D. Myrold. 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biol. Biochem. 40:443-451.

Bradshaw, A. 1997. Restoration of mine lands-using natural processes. Ecol. Eng. 8:255-269.

Bradshaw, A.D. 1987. The reclamation of derelict land and the ecology of ecosystems. In: W.R. Jordan, III et al., editors, Restoration ecology: A synthesis approach to ecological research. Cambridge Univ. Press, Cambridge, U.K. p. 53-74.

Brookes, P.C., A. Landman, G. Pruden, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17:837-842.

Byrnes, W.R. W.W. McFee, and J.G. Stockton. 1980. Properties and plant growth potential of mineland overburden. EPA-600/7-80-054, SEA/CR-10, U.S.D.A./E.P.A. Intraagency Environmental Research and Development Series, Washington, DC.

Byrnes, W.R. and J.H. Miller. 1973. Natural revegetation and case overburden properties of surface-mine coal lands in southern Indiana. In: R.W. Hutnik and B. Davis, editors, Ecology and Reclamation of Devastated Land, Gordon and Breach, New York. p. 285-306.

Cambardella, C.A., and E.T. Elliot. 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grasslands soils. Soil Sci. Soc. Am. J. 57:1071-1077.

Carrasco, L. F. Caravaca, R. Azcon, and A. Roldan. 2009. Soil acidity determines the effectiveness of an organic amendment and a native bacterium for increasing soil stabilization in semiarid mine tailings. Chemosphere 74:239-244.

Chambers, J.C., R.W. Brown, and R.S. Johnston. 1987. A comparison of soil and vegetation properties of seeded and naturally revegetated pyritic alpine mine spoil and reference sites. Landscape and Urban Planning 14:507-519.

Chen, Y, -Q., G.-J, Ren, S.-Q. An, Q.-Y. Sun, C.-H. Liu, and J.-L. Shuang. 2008. Changes of bacterial community structure in copper mine tailing after colonization of reed (*Phragmites communis*). Pedosphere. 18:731-740.

Cheng, H.H., and J.M. Kimble. 2001. Characterization of soil organic carbon pools. In: R. Lal, J.M. Kimble, R.F. Follett, and B.A. Stewart, editors, Assessment methods for soil carbon. Lewis Publishers, Boca Raton, FL.

Chodak, M., and M. Niklinska. 2010. Effect of texture and tree species on microbial properties of mine soils. Appl. Soil Ecol. 46:268-275.

Chronakova, A. V. Kristufek, M. Tichy, and D. Elhottova. 2010. Biodiversity of streptomycetes isolated from a succession sequence at a post-mining site and their evidence in Miocene lacustrine sediment. Microbiol. Res. 165:594-608.

Coyne, M.S., Q. Zhai, C.T. Mackown, and R.I. Barnhisel. 1998. Gross nitrogen transformation rates in soil at a surface coal mine site reclaimed for prime farmland use. Soil Biol. Biochem. 30:1099-1106.

Cummins, D.G., W.T. Plass, and C.E. Gentry. 1965. Chemical and physical properties of spoil banks in eastern Kentucky coal fields. U.S. Department of Agriculture, Forest Serv. Res. Paper CS-17.

Davidson, E.A., S.C. Hart, and M.K. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148-1156.

Day, P.R. 1965. Particle fractionation and particle-size analysis. p. 545-567. In: C.A. Black, et al., editors, Methods of Soil Analysis: Part 1. Agronomy Monogr. 9. ASA and SSSA, Madison, WI.

Dixon, J.B., H.S. Arora, F.M. Hons, P.E. Askenay, and L.R. Hossner. 1980. Chemical, physical, and mineralogical properties of soils, mine soil, and overburden associated with lignite mining. In: L.R. Hossner, editor, Reclamation of Surface-Mined Lignite Spoil in Texas, The Texas Agricultural Experiment Station and The Center for Energy and Mineral Resources. p. 12-21.

Elliott, E.T., and D.C. Coleman. 1988. Let the soil work for us. Ecol. Bull. 39:23-32.

Emerson, P. J. Skousen, and P. Ziemkiewicz. 2009. Survival and growth of hardwoods in brown versus gray sandstone on a surface mine in West Virginia. J. Environ. Qual. 38:1821-1829.

Fan, J.F., and Z.B. Liu. 2005. Experimental study of soil physical and chemistry property and improve measures of dump in Haizhou open-pit coal mine. Opencast Mining Technology. 5:7-77.

Fierer, N., J.A. Jackson, R. Vilgalys, and R.B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71:4117-4120.

Fontaine, S., C. Henault, A. Aamor, N. Bdioui, J.M.G. Bloor, V. Maire, B. Mary, S. Revaillot, and P.A. Maron. 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol. Biochem. 43:86-96.

Franzluebbers, A.J., R.L. Haney, C.W. Honeycutt, H.H. Schomberg, and F.M. Hons. 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64:613-623.

Garcia-Palacios, P., M.A. Bowker, S.J. Chapman, F.T. Maestre, S. Soliveres, A. Gallardo, F. Valladares, C. Guerrero, and A. Escudero. 2011. Early-successional vegetation changes after roadside prairie modify processes related with soil functioning by changing microbial functional diversity. Soil Biol. Biochem. 43:1245-1253.

Garland, J.L. 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol. Ecol. 24:289-300.

Golchin. A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994. Soil structure and carbon cycling. Aust. J. Soil. Res. 32:1043-1068.

Gould, F.W. 1975. Texas plants: A checklist and ecological summary. Miscellaneous publication (MP-585) Texas Agricultural Experiment Station, College Station, TX.

Gregorich, E.G., R.P. Voroney, and R.G. Kachanoski. 1991. Turnover of carbon through microbial biomass in soils with different textures. Soil Biol. Biochem. 23:799-805.

Gros, R., L.J. Monrozier, and P. Faivre. 2006. Does disturbance and restoration of alpine grassland soils affect the genetic structure and diversity of bacterial and N_2-fixing populations? Environ. Microbiol. 8:1889-1901.

Haering, K.C., W.L. Daniels, and J.A. Roberts. 1993. Changes in minesoil properties resulting from overburden weathering. J. Environ. Qual. 22:194-200.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: Paleontological Statistics software package for education and data analysis. Palaeontologica Electronica. 4:9.

Harris, J.A. 2003. Measurements of the soil microbial community for estimating the success of restoration. Eur. J. Soil Sci. 54:801-808.

Harris, P.A. 1985. Evaluation of the *Rhizobium*-subterranean clover symbiosis and ecology of *Rhizobium* in lignite mine spoil. Ph.D. Dissertation. Texas A&M University, College Station, TX. (Diss. Abstr. 91-06936).

He, Z., Y. Deng, J.D. Van Nostrand, Q. Tu, M. Xu, C.L. Hemme, X. Li, L. Wu, T.J. Gentry, Y. Yin, J. Liebich, T.C. Hazen, and J. Zhou. 2010 GeoChip 3.0 as high-throughput tool for analyzing microbial community composition, structure and functional activity. ISME J. 4:1167-1179.

He, Z., T.J. Gentry, C.W. Schadt, L. Wu, J. Liebich, A.C. Chong, Z. Huang, W. Wu, B. Gu, P. Jardin, C. Criddle, and J. Zhou. 2007. GeoChip: A comprehensive microarray for investigating biogeochemical, ecological and environmental processes. ISME J. 1:67-77.

Helgalson, B.L., F.L. Walley, and J.J. Germida. 2009. Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. Soil Sci. Soc. Am. J. 73:120-127.

Herrera, A., M. Hery, J.E.M. Stach, T. Jaffre, P. Normand, E. Navarro. 2007. Species richness and phylogenetic diversity comparisons and soil microbial communities affected by nickel-mining and revegtation efforts in New Caledonia. Euro. J. Soil Biol. 43:130-139.

Holmgren, G.S., R.L. Juve, and R.C. Geschwender. 1977. A mechanically convariable rate leaching devices. Soil Sci. Soc. Am. J. 41:1207-1208.

Hons, F.M. 1978. Chemical and physical properties of lignite spoil material and their influence upon successful reclamation. Ph.D. Dissertation. Texas A&M University, College Station, TX. (Diss. Abstr. 78-17183)

Ingram, L.J., G.E. Schuman, P.D. Stahl, and L.K. Spackman. 2005. Microbial respiration and organic C indicate nutrient cycling recovery in reclaimed soils. Soil Sci. Soc. Am. J. 69:1737-1745.

Insam, H., and K.H. Domsch. 1988. Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. Microb. Ecol. 15:177-188.

Jacinthe, P-.A., and R. Lal. 2007. Carbon storage and minesoil properties in relation to topsoil application techniques. Soil Sci. Soc. Am. J. 71:1788-1795.

Jackson, J.A. 1979. Chemoautotrophic metabolism and acid production in reclaimed soil from a lignite surface mine. M.S. Thesis. East Texas State University, Commerce, TX.

Jangid, K., M.A. Williams, A.J. Franzuebbers, J.M. Blair, D.C. Coleman, and W.B. Whitman. 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biol. Biochem. 42:302-312.

Jobbagy, E.G., and R.B. Jackson. 2001. The distribution of soil nutrients with depth: Global patterns and the imprints of plants. Biogeochemistry. 53:51-77.

Keeney, D.R., and D.W. Nelson. 1982. Nitrogen - inorganic forms. In: A.L. Page, et al., editors, Methods of Soil Analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI. p. 643-687.

KET. Kentucky Educational Television. COAL: Ancient Gift Serving Modern Man. Types of Coal. http://www.ket.org/Trips/coal/agsmm/agsmmtypes.html. Accessed on January 31, 2012.

Kleeberg, A., A. Schapp, and D. Biemelt. 2008. Phosphorus and iron erosion from non-vegetated sites in a post-mining landscape, Lusatia, Germany: Impact on aborning mining lakes. Catena 72:315-324.

Komnitsas, K., X. Guo, and D. Li. 2010. Mapping of soil nutrients in an abandoned Chinese coal mine and waste disposal site. Minerals Engineering 23:627-635.

Kuramae, E.E., H.A. Gamper, E. Yergeau, Y.M. Piceno, E.L. Brodie, T.Z. DeSantis, G.L. Andersen, J.A. van Veen, and G.A. Kowalchuk. 2010. Microbial secondary succession in a chronosequence of chalk grasslands. ISME J. 1-5.

Lal, R., J.M. Kimble, R.F. Follet, and C.V. Cole. 1998. The potential of U.S. cropland to sequester carbon and mitigate the greenhouse effect. Ann Arbour Press, Chelsea. p. 128.

Lindsay, W.L. and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer. J. 42:421-428.

Lorenz, K., and R. Lal. 2005. The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. Adv. Agron. 88:35-66.

Lorenz, K., and R. Lal. 2007. Stabilization of organic carbon in chemically separated pools in reclaimed coal mine soils in Ohio. Geoderma. 141:294-301.

Machulla, G., M.A. Bruns, and K.M. Scow. 2005. Microbial properties in mine spoil materials in the initial stages of soil development. Soil Sci. Soc. Am. J. 69:1069-1077.

McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate Analysis of Ecological Data. Version 5.0, MjM Software, Gleneden Beach, Oregon, U.S.A.

McFee, W.W., W.R. Byrnes, and J.G. Stockton. 1981. Characteristics of coal mine overburden important to plant growth. J. Environ. Qual. 10:300-308.

McGeehan, S.L., and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Commun. Soil Sci. Plant Anal. 19:493-505.

McSweetney, K., and I.J. Jansen. 1984. Soil structure and associated rooting behavior in minesoil. Soil Sci. Soc. Am. J. 48:607-612.

Mehlich, A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. Commun. Soil Sci. Plant Anal. 15:1409-1416.

Merrill, S.D., J.F. Power, and R.E. Ries. 1998. Subsoil characteristics and landscape position affect productivity of reconstructed minesoils. Soil Sci. Soc. Am. J. 62:263-271.

Mott, J.B. 1984. Evaluation of microbial populations, *Rhizobium trifolii* and endomycorrhizal associations in reclamation of surface mine spoils in Texas. Ph.D. Dissertation. Texas A&M University, College Station, TX. (Diss Abstr. 84-19857).

Mummey, D.L., P.D. Stahl, and J.S. Buyer. 2002a. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Appl. Soil Ecol. 21:251-259

Mummey, D.L., P.D. Stahl, and J.S. Buyer. 2002b. Soil microbiological properties 20 years after surface mine reclamation: spatial analysis of reclaimed and undisturbed sites. Soil Biol. Biochem. 34:1717-1725.

Neel, C., H. Bril, A. Coutrin-Nomade, and J-.P. Dutreuil. 2003. Factors affecting natural development of soil soil on 35-year-old sulphide-rich mine tailings. Geoderma. 111: 1-20.

Ng., J.P., E.B. Hollister, M. del C.A. Gonzalez-Chavez, F.M. Hons, D.A. Zuberer, J.A. Aitkenhead-Peterson, R. Loeppert, and T.J. Gentry. Impacts of cropping systems and

long-term tillage on soil microbial population levels and community composition in a dryland agricultural setting. ISRN Ecology. 12. Article ID 487370.

Nielsen, U.N., E. Ayres, D.H. Wall, and R.D. Bardgett. 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity-function relationships. Eur. J. Soil. Sci. 62:105-116.

Nieman, R.H., and M.C. Shannon. 1976. Screening plants for salinity tolerance. P. 359-367. In M.T. Wright and S.A. Ferrari (ed.) Plant adaptation to mineral stress in problem soils. Spec. Pub. Cornell Univ., Agric. Exp. Stn., Ithaca, N.Y.Oades, J.M. 1984. Soil organic matter and structural stability: Mechanisms and implications for management. Plant Soil 76:319-337.

Oades, J.M. 1984. Soil organic matter and structural stability: Mechanisms and implications for management. Plant Soil. 76:319-337.

Odum, E.P. 1969. The strategy of ecosystem development. Science 164:262-270.

Palmer, M.A., E.S. Bernhardt, W.H. Schlesinger, K.N. Eshleman, E. Foufoula-Georgiou, M.S. Hendryx, A.D. Lemly, G.E. Likens, O.L. Loucks, M.E. Power, P.S. White, and P.R. Wilcock. 2010. Mountaintop Mining Consequences. Science 327:148-149.

Pastorelli, R., S. Landi, D. Trabelsi, R. Piccolo, A. Mengoni, M. Bazzicalupo, and M. Pagliai. 2011. Effects of soil management on structure and activity of denitrifying bacterial communities. Appl. Soil Ecol. 49:46-58.

Paul, E.A., D. Harris, M.J. Klug, and R.W. Ruess. 1999. The Determination of Microbial Biomass. In: G.P. Roberston, D.C. Coleman, C.S. Bledsoe, and P. Sollins, editors, Standard Soil Methods for Long-term Ecological Research, Oxford University Press, New York, NY.

Peach, A.E. 2001. Functional and taxonomic diversity of microbial communities in reclaimed East Texas lignite mine sites. M.S. Thesis. Texas A&M University, College Station, TX.

Pearson, T.R.H., S.L. Brown, and R.A. Birdsey. 2007. Measurement Guidelines for the Sequestration of Forest Carbon. USDA Forest Service. NRS – 18.

Piotrowska-Seget, Z., M. Cycon, and J. Kozdroj. 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. Appl. Soil Ecol. 28:237-246.

Plassart, P., M. A. Vinceslas, C. Gangneux, A. Mercier, S. Barray, and K. Laval. 2008. Molecular and functional responses of soil microbial communities under grassland restoration. Agric, Ecosyst. Environ. 127:286-293.

Pothoff, M., L.E. Jackson, S. Sokolow, and R.G. Joergensen. 2009. Below and aboveground responses to lupines and litter mulch in a California grassland restored with native bunchgrasses. Appl. Soil Ecol. 42:124-133.

Pugh, C.E., L.R. Hossner, and J.B. Dixon. 1984. Oxidation rate of iron sulfides as affected by surface area, morphology, oxygen concentration, and autotrophic bacteria. Soil Sci. 137:309-314.

Reeve, J.R., C.W. Schadt, L. Carpenter-Boggs, S. Kang, J. Zhou, and J.P. Reganold. 2010. Effects of soil type and farm management on soil ecological functional genes and microbial activities. ISME J. 1-9.

Rhoades, J.D. 1982. Soluble salts. In: A.L. Page, et al., editors, Methods of Soil Analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI. p. 167-178.

Robertson, G.P., D.C. Coleman, C.S. Bledsoe, and P. Sollins. 1999. Standard methods for long-term ecological research. Oxford, U.K: Oxford University Press.

Rosello-Mora, R., and R. Amann. 2001. The species concept for prokaryotes. FEMS Microbiol. Rev. 25:39-67.

RRC. Railroad Commission of Texas. 1993. State of Texas Energy Policy Partnership. 2:282-283.

RRC. Railroad Commission. 2011. Texas Natural Resource Code Annuals Section 134.001. http://www.statutes.legis.state.tx.us/Docs/NR/htm/NR.134.htm

Schimel, J.P., and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591-602.

Schlesinger, W.H. 1997. Biogeochemistry. An analysis of global change. 2nd ed. Academic Press, San Diego, CA.

Schloss P.D. et al. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75:7537-41.

Schobert, H.H. 1990. The Chemistry of Hydrocarbon Fuels. Butterworth-Heinemann. Oxford, U.K.

Schofield, R.K., and A.W. Taylor. 1955. The measurement of soil pH. Soil Sci. Soc. Am. Proc. 19:164-167.

Schroeder, P.D., W.L. Daniels, and M.M. Alley. 2010. Chemical and physical properties of reconstructed mineral san mine soils in Southeastern Virginia. Soil Sci. 175:2-9.

Schulte, E.E., and B.G. Hopkins. 1996. Estimation of soil organic matter by weight by weight Loss-On-Ignition. In: F.R. Magdoff, M.A. Tabatabai and E.A. Hanlon, Jr., editors, Soil Organic matter: Analysis and Interpretation, publication No. 46. Soil Sci. Soc. Amer. Madison, WI. p. 21-32

Schutter, M.E., and R.P. Dick. 2002. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil. Soil Sci. Soc. Am. J. 66:142-153.

Sencindiver, J.C., and J.T. Ammons. 2000. Minesoil genesis and classification. Ch. 23. In: R.I. Barnhisel, W.L. Daniels, and R.G. Darmody, editors, Reclamation of Drastically Disturbed Lands. Agronomy Series No. 41. American Soc. Agronomy. Madison, WI. p. 595-613.

Shresta, R.K., and R. Lal. 2008. Land use impacts on physical properties of 28 years old reclaimed mine soil in Ohio. Plant Soil 306:249-260.

Six, J., E.T. Elliot, and K. Paustian. 2000a. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32:2099-2103.

Six, J., K. Paustian, E.T. Elliott, and C. Combrink. 2000b. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64:681-689.

SMCRA. Surface mining control and reclamation act of 1977. U.S. Congress 1977. Public Law 95-87.

Smits, T.H.M., C. Devenoges, K. Szynalski, J. Maillard, and C. Holliger. 2004. Development of a real-time PCR method for quantification of the three genera *Dehalobacter*, *Dehalococcoides*, and *Desulfitobacterium* in microbial communities. J. Microbiol. Methods 57:369-378.

Soil Survey Staff. 1996. Soil survey laboratory methods and procedures for collecting soil samples. Soil Survey Investigations Report No. 42, USDA, SCS. U.S. Government Printing Office, Washington, D.C.

Sourkova, M., J. Frouz, and H. Santruckova. 2005. Accumulation of carbon, nitrogen and phosphorus during soil formation on alder spoil heaps after brown-coal mining, near Sokolov (Czech Republic). Geoderma. 124:203-214.

Stapleton, R.E. 2004. Development of organic carbon pools in wetlands created on reclaimed lignite mine soil. M.S. Thesis. Stephen F. Austin State University. Nacogdoches, TX.

Stewart, W.M. 1996. Factors affecting the ratio of cation exchange capacity to clay content in east Texas lignite overburden. Ph.D. Dissertation. Texas A&M University, College Station, TX.

Stewart, W.M, and L.R. Hossner. 2001. Factors affecting the ratio of cation exchange capacity to clay content in lignite overburden. J. Environ. Qual. 30:1143-1149.

Storer. D.A. 1984. A simple high volume ashing procedure for determining soil organic matter. Commun. Soil Sci. Plant Anal. 15:759-772.

Stroo, H.F., and E.M. Jenks. 1982. Enzyme activity and respiration in mine soils. Soil Sci. Soc. Am. J. 46:548-553.

Swanson, E.S. 1996. Soil Microbial Biomass: An estimator of soil quality on reclaimed mine soils. M.S. Thesis. Texas A&M University, College Station, TX.

Thorne. M., and J. Cardina. 2011. Prairie grass establishment on calcareous reclaimed mine soil. J. Environ. Qual. 40: 1824-1834.

Torsvik, V., and L. Ovreas. 2002. Microbial diversity and function in soil: From genes to ecosystems. Curr. Opin. Microbiol. 5:240-245.

Toups, B.G. 1986. Comparison of site quality for loblolly pine on selected mined and non-mined soils in Panola County, Texas. M.S. Thesis. Stephen F. Austin State University, Nacogdoches, TX.

Ussiri, D.A.N., R. Lal, and P.A. Jacinthe. 2006. Soil properties and carbon sequestration of afforested pastures in reclaimed minesoils of Ohio. Soil Sci. Soc. Am. J. 70:1797-1806.

Ussiri, D.A.N., and R. Lal. 2008. Methods for determining coal carbon in the reclaimed minesoils contaminated with coal. Soil Sci. Soc. Am. J. 72:231-237.

Valverde, A., M. Gonzalez-Tirante, M. Medina-Sierra, I. Santa-Regina, A. Garcia-Sanchez, and J.M. Igual. 2011. Diversity and community structure of culturable arsenic-resistant bacteria across a soil arsenic gradient at an abandoned tungsten-tin mining area. Chemosphere 85:129-134.

van Genuchten, M.Th. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci. Soc. Am. J. 44:892-898.

van Veen, J.A., and P.J. Kuikman. 1990. Soil structure aspects of decomposition of organic matter by microorganisms. Biogeochemistry 11:213-223.

Waggoner, P.J. 1993. Microbial hydrolysis of urea and its subsequent nitrification in East Texas lignite mine spoil. M.S. Thesis. Texas A&M University, College Station, TX.

Wang, Q, G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl. Environ. Microbiol. 73:5261-7.

Whitford, W.G. 1988. Decomposition and nutrient cycling in disturbed arid ecosystems. In: E.B. Allen, editor, The reconstruction of disturbed arid lands: An ecological approach., Westview Press, Boulder. p. 136-161.

Wick, A.F., P.D. Stahl, and L.J. Ingram. 2009. Aggregate-associated carbon and nitrogen in reclaimed sandy loam soils. Soil Sci. Soc. Am. J 73:1852-1860.

Willet, R.L. 1978. Soil properties relating to height growth of loblolly pine on soils of the Bowie, Fuquay, Sacul, and Troup series. Ph.D. Dissertation. Stephen F. Austin State University, Nacogdoches, TX.

Wu, J., R.G. Joergensen, B. Pommerening, R. Chaussod, and P.C. Brookes. 1990. Measurement of soil microbial biomass C by fumigation-extraction – an automated procedure. Soil Biol. Biochem. 22:1167-1169.

Xie, J.P., Z.L. He, X.X. Liu, X.D. Liu, J.D. Van Nostrand, Y. Deng, L.Y. Wu, J.Z. Zhou, and G.Z. Qiu. 2011. GeoChip-Based Analysis of the Functional Gene Diversity and Metabolic Potential of Microbial Communities in Acid Mine Drainage. Appl. Environ. Microbiol. 77: 991-999.

Zhan, J., and Q. Sun. 2011. Diversity of free-living nitrogen-fixing microorganisms in wastelands of copper mine tailing during the process of natural ecological restoration. J. Envr. Sci. 23:476-487.

Zibilske, L. 1994. Carbon mineralization. In: R.W. Weaver, et al., editors, Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties. Soil Science Society of America. Madison, WI. P. 835-863.

Zipper, C.E. J.A. Burger, J.M. McGrath, J.A. Rodrigue, and G.I. Holtzman. 2011. Forest restoration potentials of coal-mined lands in the Eastern United States. J. Environ. Qual. 40:1567-1577.

VITA

Name: Justin Park Ho Ng

Address: Heep Center, Rm. 550C
2474 TAMU
College Station, TX 77843-2474

Email Address: Justin.Ng@tamu.edu

Education: B.S., Biology, Trinity University, 2005
M.S., Environmental Science,
The University of Texas at San Antonio, 2007
Ph.D., Soil Science, Texas A&M University, 2012