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RECLAMATION OF ABANDONED MINE LAND WITH POULTRY LITTER BIOCHAR

by

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A THESIS

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Abstract

Biochar, a recalcitrant, porous black carbon product from pyrolysis of organic residues, is a promising material in agricultural and environmental applications for improving soil fertility, enhancing carbon sequestration, reducing greenhouse gas emissions, and decreasing contaminant mobility. This study aimed at investigating the feasibility of poultry litter (PL)-biochar as a soil amendment for reclaiming abandoned mine land (AML). Greenhouse potting trials and laboratory analyses were conducted to examine soil quality improvement and plant growth enhancement by PL-biochar when applied to AML soils at varied rates. An AML soil collected from Lower Emigh Mine, PA was amended with PL-biochar at 0, 10, 20, and 30 g kg^{-1}, respectively, and packed into 18.5 cm i.d. × 19.0 cm growing pots. Poverty oatgrass (*Danthonia spicata*), a native plant at the AML site, was seeded in the pots at the recommended rate. Artificial irrigation simulating the natural rainfall was implemented to maintain the moisture content of the potting soils at 40-60% of their field water holding capacities. Growth of poverty oatgrass in the pots was monitored for plant height, leaf color, and tillering. Six months after germination the plants were harvested and measured for aboveground biomass yield. Before PL-biochar amendment and after plant growth, the AML soils were intensively characterized for various fertility parameters including pH, electrical conductivity, cation exchange capacity, organic matter content, total nutrient content, plant-available nutrient concentration, and microbial community composition. The results showed that PL-biochar amendment at 20 and 30 g kg^{-1} significantly promoted the growth of poverty oat grass in AML soils and improved its biomass yield. These

treatments reduced soil acidity by 90% and improved soil organic carbon content and cation exchange capacity. The total and water-extractable contents of the N, P, K, Ca, Mg, and S nutrients in the soils were also increased by PL-biochar amendment. No significant enhancement in microbial activity and microbial community structure was detected, largely due to the short time length of plant growth and the limitation of the analytical methods used. The results suggest that PL-biochar is a desirable soil quality enhancer and plant growth promoter in abandon mine land reclamation if applied at 20-30 g kg^{-1} soil amendment rates.

Keywords: Abandoned mine land; poultry litter; biochar; soil fertility; microbial community; ribosomal RNA

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AML: Abandoned Mine Land

CEC: Cation Exchange Capacity

DOC: Dissolved Organic Carbon

EC: Electrical conductivity

FDA: Fluorescein Diacetate

OM: Organic Matter

PL: Poultry litter

TDN: Total Dissolved Nitrogen

TDP: Total Dissolved Phosphorus

TN: Total Nitrogen

TP: Total Phosphorus

WHC: Water Holding Capacity

1. Introduction

Abandoned mine land (AML) is land disturbed by mining activities through which natural vegetation has been removed and soil has been mechanically disarranged (Shrestha and Lal, 2006). During mining, the landscape is severely disrupted as topsoil is moved. The landform is generally altered in appearance and/or structure by mechanical disturbance, leaving a scarred land surface by the extraction and processing of coal and ores (Kundu and Ghose, 1997; MonAme, 2009). A typical AML is characterized by a barren, rocky area with mine openings, waste rock dumps, mill tailing piles, and mine structures (CODNR, 2002).

Mining causes land disturbance and results in land degradation. During mining operations large quantities of soil and rocks are pulverized, relocated, and generally piled at the site. Changes in landform by mining activities alter the drainage pattern, soil structure, and vegetation coverage of the land, spurring surface instability, soil erosion, and soil quality degradation (MonAme, 2009). The AML soil is generally acidic, sandy/gravely, low in porosity, available nutrients, organic matter, and microbial activity, and high in toxic heavy metals (Reed, 2007). In Mongolia, for example, Erdenet copper industry produces 10-12 million tons of solid waste per year, while Baganuur coal mine dumped 162 million m^3 of solid waste during the years from 1978 to 2001. Due to gold mining, over 200 thousand tons of polluted tailings and soil were dumped in the territories of seven Aimags (Altantsetseg, 2011). Natural colonization of AML by plants

is challenging and it may take decades to hundreds of years to re-establish fair vegetation coverage if artificial reclamation efforts are not implemented.

Unreclaimed AMLs present hazards to the environment and life. In addition to wildlife habitat loss, surface instability, soil erosion, landslides, pitfalls, subsidence, surface and groundwater pollution from acid mine drainage, stream clogging by sediments, and dangers posed by portals, gob piles, and impoundments are other potential threats (DOI, 2012; NAAMLP, 2012). Effective reclamation of AML to restore the damaged environment and natural ecosystem provides tremendous environmental benefits such as water and air quality improvement, wildlife habitat rehabilitation, scenery enhancement, sustainable resource development, and valuable land for future economic investment (MonAme, 2009). However, there are many challenges existing in AML reclamation. Installation of barriers surrounding large openings is necessary when construction access is restricted. To divert runoff water away from stockpiled mine waste, diversion ditches must be dug using a backhoe or by hand. Frequently scattered mine waste dumps in an area have to be relocated, consolidated, capped, and revegetated; capping materials need a particular formula and can be costly. If mine waste piles cannot be relocated to avoid direct contact with flowing water, stream diversion may be necessary (CODNR, 2002). To reduce soil erosion, grading and leveling are commonly practiced in AML reclamation to make the land surface more uniform and gently sloping. This action requires different heavy construction machines (Vogel, 1981). The most challenging step in AML reclamation is revegetation, which is commonly employed in combination with other best reclamation approaches, to stabilize land and to restore the natural ecosystem. The disturbed area needs to be prepared for vegetation by grading,

smoothing, and shaping to reduce water erosion risks (Vogel, 1981). The disturbed mine soil is generally acidic, toxic, stony, and poor in plant nutrients, therefore unable to sufficiently support plant growth (Harris, 2001). Covering the surface to be vegetated with fertile soil or a similar rooting medium is usually imperative (Sydnor and Redente, 2002; Riley et al., 2003). Importing quality topsoil or mixing original shattered rock material with compost, however, can be cost-prohibitive (Troeh et al., 2004). Often the soil cap needs to be roughened to allow moisture retention. Nevertheless, heavy vehicle operation on topsoil spreading and leveling can cause soil compaction (Sharma et al., 1996), which decreases water and root permeability (Munshower, 1994). Therefore, care must be taken not to over tighten the surface. The topsoil needs to be tested for physical and chemical properties to determine essential fertility enhancement practices such as liming and application of fertilizer, manure, or sewage sludge (Sopper, 1992; Haering et al., 2000; Sheoran et al., 2010). Mine spoils are ideal sites for heavy applications of manure and sewage sludge. However, pyrite (FeS_2) in the mining spoil should be excavated and buried below plant root depth. Due to its shallow rooting depth, reclaimed land could be easily affected by drought (Marashi and Scullion, 2004). Therefore, artificial irrigation may be necessary.

Establishment of perennial vegetation or cultivated crops on the prepared surface requires scientific selection of plant species that fit the climate, soil conditions, and intended use of the area. As a rule of thumb, the artificial plant community should resemble that of the surrounding area. Generally, forage legume will not grow well if the soil pH is below 4.5. Deertongue, Korean lovegrass, weeping lovegrass, and switchgrass are recommended where the spoil pH is <4.0, while reed canarygrass, sand lovegrass, tall

fescue, and the legume plants birdsfoot trefoil, crownvetch, flatpea, and sericea lespedeza are suited to a pH between 4.0 to 5.5. Chinese silvergrass, costal panicgrass, orchardgrass, and perennial ryegrass grow well on spoils with pH above 5.5. Certain trees, including black locust, European black alder, American sycamore, eastern cottonwood, green ash, loblolly pine, northern red oak, Norway spruce, red maple, red pine, Scotch pine, silver maple, sweetgum, Virginia pine, and white pine may also be considered in revegetation due to their ability to grow on a poor soil. Mulches are usually applied immediately after seeding or planting. Follow up application of fertilizers and organic residues once every several years is necessary to ameliorate soil physical properties and provide plant nutrients (Troeh et al., 2004).

Many studies and projects have been conducted to effectively reclaim AML through revegetation. All the efforts recognized the importance of topsoil quality and plant species selection in AML reclamation. In a ten-year field study, Redente et al. (1997) found that a phosphorus-fertilized 15-cm topsoil layer over non-toxic surface coal mine spoils in Colorado supported rangeland vegetation. On reclaimed uranium lands in Wyoming, the biomass yield of wheatgrass and green needle grass and the water infiltration were significantly improved with 40-60 cm of soil topping (Schuman et al., 1985). Indeed, the required topsoil depth varies with the types of mine spoils. In a 6-year field study, Barth and Martin (1984) observed that healthy growth of perennial cool-season grasses on surface mining land required minimally 50 cm, 71 cm, and 0 cm depths of topsoil over generic, sodic, and soil-like spoils, respectively; plant root penetration in soil-unlike spoils was limited to <10 cm.

In addition to depth, quality of topsoil in AML reclamation is also critical. In an effort to revegetate a Colorado acidic mine site, it was found that appropriately limed stockpiled topsoils provided sufficient plant growth support and decreased trace element phyto-accumulation as compared with imported topsoils (Sydnor and Redente, 2002). Organic amendment plays a critical role in AML reclamation. Plant cover and biomass production in acidic mine waste amended with mushroom compost at 90 dry ton ha^{-1} by incorporation and limed with limestone at 102 dry ton ha^{-1} were comparable to those with soil-topping treatments (Sydnor and Redente, 2002). Dere et al. (2011) found that AML soil amendment with composted PL (27.1 g N kg^{-1} and 13.5 g P kg^{-1} soil) at 78 ton ha^{-1} or 1:2 wt/wt poultry litter and paper mill sludge mixture at 153 ton ha^{-1} (2117 kg N ha^{-1} and 1052 kg P ha^{-1}) promoted vegetation growth as compared with lime (13.4 ton ha^{-1}) and chemical fertilizer (112 kg N ha^{-1} and 196 kg P ha^{-1}) application. However, N leaching from the poultry litter and sludge mixture treatment at such a high application rate was significant. Chambers et al. (1994) noticed that initially high fertilization of topsoil facilitated establishment of seeded perennial legume and forage grasses on reclaimed phosphate mining land in Idaho and maintained their predominance after 14 years of revegetation. Artificial sowing of native plant species in well fertilized topsoils ensured rapid revegetation of a limestone quarry site (Riley et al., 2003). A revisit of a 28-year-old reclaimed coal mine site in North Dakota revealed that the nonnative seeded plant species crested wheatgrass (*Agropyron cristatum*) and smooth brome (*Bromus inermus*) predominantly remained after 28 years, while the less aggressive species Russian wildrye (*Psathyrostachys juncea*) and alfalfa (*Medicago sativa*) were largely replaced by other invaded plants (Wick et al., 2011). The results suggest that a diverse, sustainable plant

community should be established at the initial stage of revegetation to prevent undesirable species invasion. Nevertheless, survival and growth of desirable forest trees may be restricted in highly fertilized and heavily sown mine soils (Berger and Torbert, 1990). If herbaceous and woody species are used together in land reclamation, planting trees and sowing grasses in alternate strips is recommended (Vogel, 1980).

Poultry litter (PL)-biochar may be a desirable soil amendment in AML reclamation to provide long-term soil fertility enhancement. The material is a charcoal-like solid produced through pyrolysis by heating PL in the absence of air at a moderate temperature of 300-600°C (Guo et al., 2012). Poultry litter is rich in plant nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and sulfur (S) (Guo et al., 2009). Raw PL has been used as an organic soil conditioner in land reclamation to furnish nutrients and improve soil physiochemical properties (Lamey and Angers, 2012). In soil, however, raw PL decomposes rapidly and the inherent nutrients are released at high rates. Research has shown that PL nutrients are predominantly released within six months following application (Guo et al., 2009). The rapid decomposition and nutrient release is not desirable for AML reclamation, which requires years of soil amelioration in nutrient supply, acidity buffering, water retention, and microbial harboring. Biochar, however, is recalcitrant in the soil environment and may exist in soil for thousands of years (Lehmann et al., 2003). The stability of biochar against field weathering originates chiefly from the low O:C molar ratio (i.e., <0.2) developed during pyrolysis (Spokas, 2010). Chemical analyses revealed that PL-biochar generated at 300°C pyrolysis temperature inherited the majority (>80%) of the N and all the P, K, Ca, Mg, and S in the raw feedstock (Song and Guo, 2012). Compared with general soils, PL-biochar was much more capable in

buffering acidity, holding nutrients, and retaining water (Song and Guo, 2012). To date, PL-biochar has not been tested as a long-term soil quality enhancer in AML reclamation. Further research is clearly warranted.

2. Hypotheses

It is hypothesized that PL-biochar amendment will improve the physical, chemical and biological properties of AML soils and promote plant growth. The improvement in soil quality and promotion in plant growth are related to the biochar application rate.

3. Objectives

The overall objective of this study was to evaluate the potential of PL-biochar as a soil amendment in AML reclamation. Specifically, the study aimed to:

1) Quantify the quality enhancement of abandoned mine soils by PL-biochar amendment in physical, chemical, and biological characteristics
2) Evaluate vegetation establishment and growth in abandoned mine soils as enhanced by PL-biochar amendment
3) Determine optimal PL-biochar application rates for AML reclamation

4. Materials and Methods

4.1 Soil, biochar, and plant seeds

An AML soil was collected from the recently-terminated Lower Emigh Mine (~40°55'24.15" N, 78°13'18.60" W) in Philipsburg, Clearfield Country, Pennsylvania. The average summer temperature of the mining site is 17.0°C (May-August), with the warmest month in July at approximately 20.0°C and the coolest month in January at approximately 4.8°C (PSU State Climatologist, 2012). The average annual precipitation is 1167 mm (US Climate, 2012). The soil was passed through a 25.4-mm sieve on site to remove large rock fragments. In the laboratory, the <25.4 mm bulk soil was air-dried and determined for gravel percentage by physical sieving. The bulk soil contained by weight 46.67% <2 mm particles, 6.38% 2-4 mm gravels, 12.86% 4-6.3 mm fragments, and 34.09% 6.3-25.4 mm rocks. The <2 mm portion was further analyzed for pH, electrical conductivity (EC), cation exchange capacity (CEC), organic matter (OM) content, total and plant-available N, P, and K contents, and water holding capacity following the standard methods (Sparks, 1996; Gee et al., 2002).

Pelletized poultry litter was obtained from Perdue AgriRecycle Inc. (Seaford, DE) and converted to biochar by slow pyrolysis at 300°C using a bench-top pyrolyzer. Details of the poultry litter composition and the biochar production methods can be found in Song and Guo (2012).

Poverty oatgrass (*Danthonia spicata*) was selected as the test plant. The species was identified as the predominant plant in the soil sampling site. It is a native plant for

North America, usually growing in poor, dry, acidic and rocky soil (Navarrete-Tindall et al., 2010). Poverty oatgrass seeds were procured from the commercial supplier Silver Falls Seed Company, Silverton, OR.

4.2. Greenhouse potting trials

The bulk AML soil (<25.4 mm) was amended with <2 mm PL-biochar at 0, 10, 20, and 30 g kg^{-1}, respectively, and thoroughly mixed. The soils were transferred to 18.5 cm i.d. × 19.0 cm height plastic growing pots to a depth of 16.0 cm. Each treatment was conducted in triplicate. Each pot contained 6.36 kg soil by dry mass. Tap water was added to the pots to bring the moisture content to 75% of the soil water holding capacity (27.4% by weight). The pots were placed in the greenhouse for two weeks to stabilize the soils. Poverty oat grass seeds were then planted in the pots by mixing the seeds with the top 5-cm of soil at the recommended rate.

Biweekly artificial irrigation with tap water was conducted to ensure adequate water supply for the plants to grow. The amount of water applied in each irrigation event was adjusted according to the plant growth stage and greenhouse temperature. Leachates from the pots were collected in the underlying buckets. The total amount of the irrigation water was close to the annual precipitation of the soil sampling site in Philipsburg, PA.

Growth of poverty oatgrass in the greenhouse was maintained for 180 days. During the 180 days of greenhouse potting trials, the growing pots were monitored for seed germination and plant growth (e.g., leaf color, plant height, tillering, and flowering). At the end of the trials, oatgrasses were carefully harvested by hand cutting and water rinsing. Each pot was measured for fresh and dry aboveground biomass yields. Soils from

each of the pots were passed a 2-mm sieve and stored at -80°C for microbial activity and microbial community structure analyses. Aliquots of the soils were air-dried and analyzed for various fertility characteristics (e.g., pH, EC, CEC, OM content, and total and water extractable nutrient contents).

4.3. Analytical methods

4.3.1. Soil moisture content

Soil moisture content was determined by measuring the amount of water in moist soils per unit of dry soil mass. Briefly, a soil sample was transferred into a weight-known (m_1) crucible to a total weight of m_2. The soil was dried in an oven at 105°C for eight hours until the soil reached a constant weight. After bringing to room temperature in a desiccator, the soil-containing crucible was weighed again (m_3). The soil moisture content was calculated following the following equation:

$$\theta = \frac{\text{Mass of moist soil (g)} - \text{mass of dry soil(g)}}{\text{Mass of dry soil(g)}} \times 100\% = \frac{(m_2 - m_3)}{(m_3 - m_1)} \times 100\%$$

4.3.2. Soil pH and electrical conductivity

Soil (<2 mm) was soaked with deionized water at a 1:1 solid/water ratio for 24 h with occasional agitation. The slurry was then measured for pH using an Accumet AB15 pH meter with an Accumet 3-in-1 pH/ATC combination electrode (Fisher Scientific, Suwanee, GA) and for EC using an Oakton CON510 conductivity/TDS meter (Oakton Instruments, Vernon Hills, IL) with a CON 510 conductance cell (cell constant = 1.0 cm^{-1}) and a built-in ATC probe to normalize the reading to 25°C.

solution was determined and adjusted to the previous level taken before the third centrifugation by adding 0.05 M H_2SO_4. The sample EC was further adjusted to 1.5 mM $MgSO_4$ solution's conductivity by adding deionized water and also alternatively adjusted pH of the solution. The soil CEC was calculated based on the amount of $MgSO_4$ consumed.

4.3.5. Soil particle size distribution

To measure soil texture and particle size distribution, 50 g (<2 mm) of air-dry soil were weighed into a 1000-mL glass beaker, followed by addition of 250 mL deionized water and 100 mL hexametaphosphate (50 g L^{-1}) solution. The mixture was stirred for 5 minutes, settled at room temperature overnight, stirred again for 15 minutes, transferred into a 1000-mL graduate cylinder, and brought to the mark (1000 mL) by adding deionized water. The cylinder was then sealed with a piece of parafilm and shaken end-over-end for 1 minute. Once the mixing was completed, a hydrometer (ASTM no. 152H, with Bouyoucos scale in g L^{-1}) was gently placed into the mixture and recorded the density readings in the following time intervals: 30 seconds, 1, 3, 10, 30, 60, 90, 120 and 1440 minutes. A blank solution was prepared by diluting 100 mL of the hexametaphosphate (50 g L^{-1}) solution in a separate 1000-mL cylinder with 900 mL deionized water. Hydrometer readings were taken for the blank solution following the same time intervals as for the soil sample. Once the hydrometer reading was complete, the soil suspension was poured into a 53 μm sieve to obtain the sand fraction. The sand fraction retained on the sieved was washed by water and dried at 105°C. The dried sand was then transferred to a nest of sieves arranged from top to bottom in the following order: 1000, 500, 250, and 106 μm. After vigorous shaking for 3 minutes, the sand

fractions retained on each of the sieves were measured. The weight percentages of the differently-sized particle fractions in the soil were then computed.

4.3.6. Soil nutrient contents

Precisely 1.0 g of soil (<2 mm) was weighed into a 45-mL Teflon cylinder, followed by addition of 5.0 mL concentrated HNO_3 and 5.0 mL deionized water. The cylinder was installed into a Parr acid digestion bomb (Parr Instrument Company, Moline, IL) and heated in a 1.2 KW microwave oven (General Electric, Inc., Louisville, KY) for 2.5 min at a 50% power level. After cooling to room temperature, the digest was transferred into a 100 mL volumetric flask, brought to volume with deionized water, and filtered through a 0.45-μm glass fiber membrane. The total phosphorus (TP) concentration of the digest was measured following the phosphomolybdate blue method of Murphy and Riley (1962). The total concentrations of K, Ca, Mg, and S were determined using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) techniques. Another batches of soil were digested with a mixture of 2.0 mL concentrated H_2SO_4, 2.0 mL H_2O_2, and 8.0 mL deionized water. The filtered digests were analyzed for total nitrogen (TN) concentrations using the Shimadzu 5000A TC/TN analyzer.

Water extractable nutrients in the soils were also determined. Aliquots (25.0 g) of soil (<2 mm) were weighed into 50-mL HDPE centrifuge tubes, followed by addition of 25 mL of deionized water. The tubes were shaken for 24 h at room temperature. After centrifugation at 10,000 rpm for 20 min and filtration through 0.2-μm syringe filters, the extracts were analyzed for extractable nutrient concentrations. Extractable OC and extractable N contents were determined with a Shimadzu 5000A TC/TN analyzer. Extractable P contents were analyzed using the phosphomolybdate blue method after

digesting the extracts with H_2SO_4 and potassium persulfate in an autoclave. Extractable Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, Na^+, NH_4^+, K^+, Ca^{2+}, and Mg^{2+} were measured using a Metrohm 790 ion chromatography system (Metrohm Ltd., Herisau, Switzerland).

4.4. Soil biological analyses

4.4.1. Microbial activity analysis

Briefly, 2.0 g of soil were added to a 50-mL falcon tube, followed by addition of 20 mL 60 mM phosphate buffer. The tubes were shaken by vortex for 1 minute. Afterwards 100 μL of 2000 mg L^{-1} fluorescein diacetate (FDA) solution were added to the tube. The tube was placed in a 30°C shaking incubator (200 rpm) for 60 minutes. Later 20 mL of 2:1 chloroform/methanol solution were added to the tube to stop the enzymatic reaction. The tube was centrifuged at 5000×g for 5 minutes. Approximately 10 mL of the supernatant was filtered through a 0.20-μm membrane. The filtered solution was then measured for absorbance at 490 nm using spectrophotometer (GENESYS 10, Rochester, NY, USA). Control tubes without soil addition and without FDA addition were included.

4.4.2. Bacterial community analysis

Soil microbial community was analyzed by DNA extraction and sequencing. The DNA in soil was extracted using a Power Max soil DNA kit (MoBio Laboratories) following the manufacturer's instructions. Briefly, 10 g of moist soil were added to a tube containing 15 mL of bead suspension and vigorously mixed. 16S rRNA gene amplification was performed using the archaeal universal primers A751F (5'-CCGACGGTGAGRGRYGAA-3') and UA1406R (ACGGGCGGTGWGTRCAA-3')

(Baker et al., 2003). A PCR mixture (25 μL) consisting of 1 U *Taq* DNA polymerase, 5 pmol primer, 200 μM deoxynucleoside triphosphate (dNTP), 3.4-6.9 ng/uL extracted soil DNA, and PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl). The thermocycler program was as follows: 1 cycle at 94°C for 2 min, followed by 30 sequential cycles of 94°C for 1 min, 53.7°C for 1 min, and 72°C for 1 min and a final step at 72°C for 10 min. The gradient PCR was done for optimization of the PCR and investigating the right melting temperature for archael universal primer A751A and UA1406R. The melting temperature range for gradient PCR was set from 50 to 60°C. In the PCR machine, it was set to 60, 59.2, 58, 56.1, 53.7, 51.9, 50.7, 50°C automatically. Each different melting temperature had a negative control.

The PCR products (≈ 655bp) were cloned using TOPO-TA cloning kit for sequencing (Invitrogen) according to the manufacturer's instructions. Once the PCR products were ligated to the vector, the ligation was transformed into the TOPO TA one shot *Escherichia Coli,* chemically competent cells (50uL). Colony PCR was performed to check the insert.

The Plasmid DNA was purified by the alkaline lysis method (Sambrook et al., 1989). A total of 65 (5 colonies from each sample plate) plasmid mini-prepped samples were screened for the right insert by using *EcoR1* restriction enzyme. Of these, 39 plasmids were selected for further analysis. The cloned 16S rRNA genes were sequenced with the forward primer M13 (5'-GTAAAACGACGGCCAG-3') and the reverse primer M13 (5'-CAGGAAACAGCTATGAC-3') on a Sanger sequencer at Delaware Biotechnoly Institute (Newark, Delaware). The sequences were compared in BLAST

(http://blast.ncbi.nlm.nih.gov) program to determine the matching microorganisms from the database.

4.5. Statistical analysis

Analysis of variance (ANOVA) was conducted to test the effect of biochar amendment on soil quality enhancement and plant growth. One-way ANOVA test was conducted to test whether or not the fertility and plant growth of a specifically-treated soil was significantly different from the others. The null hypothesis H_0 was $\mu_1 = \mu_2 = \mu_3 = \mu_4$, indicating there were no significant differences between any treated groups. The alternative hypothesis H_A was not all μ_is (i =1, 2, 3, 4) were identical, suggesting that at least one of the PL-biochar amendments resulted in significant differences in soil fertility and plant growth from the others. The Least Significance Difference (LSD) analysis was conducted to compare the soil groups with different PL-biochar amendment rates at 95% confidence level. All the statistical analysis was performed using SPSS software (Version 20.0, IBM Corp., Armonk, NY)

5. Results and Discussion

5.1. Basic characteristics of abandoned mine land soils

The fundamental characteristics of the AML soil collected from Pennsylvania are given in Table 1. The soil (<2 mm fraction) was a silt loam, consisting of 34.5% sand, 51.5% silt and 14% clay. It was strongly acidic, with a pH value at 4.4, suggesting a zero base saturation and nearly no exchangeable K^+, Na^+, Ca^{2+}, and Mg^{2+} in the soil (Brady and Weil, 2010). Correspondingly, it was nutrient-poor, as indicated by its low EC of 0.069 dS m^{-1}. The EC of a soil is a comprehensive expression of the inherent water soluble inorganic salt ions including K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^- (Brady and Weil, 2010). In comparison, a strong acid (pH 5.3) agricultural soil in Delaware had an EC of 0.20 dS m^{-1} (Guo et al., 2012a). A pH 6.2 grassland soil in Pennsylvania showed an EC of 0.52 dS m^{-1} (Guo et al., 2001).

The cation exchange capacity (CEC) of the soil was measured at 3.35 meq/100 g (Table 1). Soil CEC indicates its ability to adsorb and retain cationic nutrients. General agricultural soils have a CEC ranging from 10 to 15 meq/100 g (Brady and Weil, 2010). The low CEC of the AML soil was consistent with its low pH and EC values.

The soil contained 48.9 mg kg^{-1} total nitrogen (TN) and 440.9 mg kg^{-1} total phosphorus (TP). Its Mehlich-3 P was low at 14.4 mg kg^{-1} (Table 1). A strong acid agricultural soil in Delaware showed a TN level of 88.5 mg kg^{-1} and Mehlich-3 P 55.4 mg kg^{-1} (Guo et al., 2012a). Water extractable nutrients of the AML soil were also

significantly lower (Table 1). Evidently, the soil was poor and would not be able support general plant growth.

Table 1. Basic physical and chemical properties of the abandoned mine land (AML) soil

Parameter	Value
pH[1]	4.4 ± 0.01
Electric conductivity[1] (dS m^{-1})	0.069 ± 0.0007
CEC[2] (meq 100g $^{-1}$)	3.35 ± 0.05
WHC[3] (%)	27.43 ± 0.23
Soil texture	Silt loam
Sand %	34.5
Silt %	51.5
Clay %	14.0
Total N (mg kg^{-1})	48.89 ± 0.10
Total P (mg kg^{-1})	440.9 ± 40.5
Mehlich-3 extractable soil P (mg kg^{-1})	14.37 ± 0.5
Water extractable components[4] (mg kg^{-1})	
Dissolved OC	48.08
Water soluble N	6.3
Water soluble P	3.89± 0.036
NH_4-N	ND[5]
NO_3-N	0.44 ± 0.007
NO_2-N	ND
Cl^-	63.18
SO_4^{2-}	192.24 ± 3.9
PO_4^--P	0.068 ± 0.02
K^+	5.87
Na^+	12.09 ± 2.43
Ca^{2+}	3.72 ± 0.32
Mg^{2+}	0.45 ± 0.007

[1]Measured in 1:1 soil/water phase
[2]CEC = cation exchange capacity
[3]WHC = water holding capacity
[4]Water extractable nutrients at 1:1 soil/water ratio for 24 h.
[5]ND = Nondetectable

5.2. Characteristics of poultry litter biochar

Nutrient contents of the PL-biochar generated from slow pyrolysis of raw poultry litter at 300°C are listed in Table 2. The biochar yield was 60.1% of the feedstock dry mass; nearly 40% of the feedstock mass was lost in vapors during pyrolysis (Song and Guo, 2012). Of the feedstock nutrients, 81.8% of N and 100% of P were retained in the biochar product (Song an Guo, 2012). The PL-biochar contained 41.5 g N kg^{-1}, 22.7 g P kg^{-1}, and 69.3 g K kg^{-1} (Table 2). The water extractable nutrients were also high at 4.91 g N kg^{-1}, 0.34 g P kg^{-1}, and 32.01 g K kg^{-1} (Table 2). It is expected that the nutrient-rich PL-biochar would furnish more nutrients to promote growth of plants in poor AML soils.

Table 2. Nutrient contents of PL biochar generated by 300°C slow pyrolysis (Song and Guo, 2012)

Parameter	Value
pH[1]	9.5
Electric conductivity[1] (dS m^{-1})	22.8
CEC (meq 100g $^{-1}$)	51.1
Ash content (%)	47.87 ± 0.12
OC content (%)	37.99 ± 0.50
WHC (%)	88
Total nutrients (g kg^{-1})	
N	41.71
P	22.73
K	69.28
Ca	71.75
Mg	18.61
S	26.95
Water extractable nutrients[2] (g kg^{-1})	
N	4.91
P	0.343
K	32.01
Ca	0.238
Mg	0.278
S	12.3

[1]Measured with poultry litter-water mixture at 1:5 dry mass:water ratio
[2]Water extractable nutrients at 1:1 soil/water ratio for 72h.

5.3. Soil fertility improvement by PL-biochar amendments

5.3.1. Improvement in total nitrogen content

The residual contents of total nitrogen (TN) in biochar-amended soils after one season of plant growth were illustrated in Figure 1. The residual TN content was elevated from 96.5 mg kg^{-1} in control soil to 203.3, 235.6 and 336.2 mg kg^{-1} in 1%, 2% and 3% PL-biochar amended soils, respectively. Evidently, PL-biochar amendment increased soil TN levels. In one-way ANOVA test, the null hypothesis (H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4$) was rejected at a confidence level of 95%. Further LSD tests showed that the residual TN levels were not significantly different between 1% and 2% biochar-amended soils.

5.3.2. Improvement in total phosphorus content

Phosphorus is the important macronutrient for plant growth and microorganisms. It is the important component of Adenosine Triphosphate (ATP), Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). The residual TP content was 440.9 mg kg^{-1} in the control soil (0% biochar amendment) and the level increased to 1135.1 mg kg^{-1} in 1% PL-biochar amended soils and to 1580.6 mg kg^{-1} in 3% PL-biochar amended soils (Fig. 1). The increase was linear with the PL-biochar amendment rate in the range of 1 -3%. The ANOVA and LSD tests indicated significant increase in soil TP by PL-biochar at an amendment rate greater than 1%.

5.3.3. Improvement in Mehlich-3 phosphorus content

Mehlich-3 extractable P is a common parameter to index plant available P in soils (Brady an Weil, 2010). The residual content of Mehlich-3 P was 14.0 mg kg^{-1} in the unamended soil. The level was elevated to 85.5, 137.6 and 169.8 mg kg^{-1}, respectively,

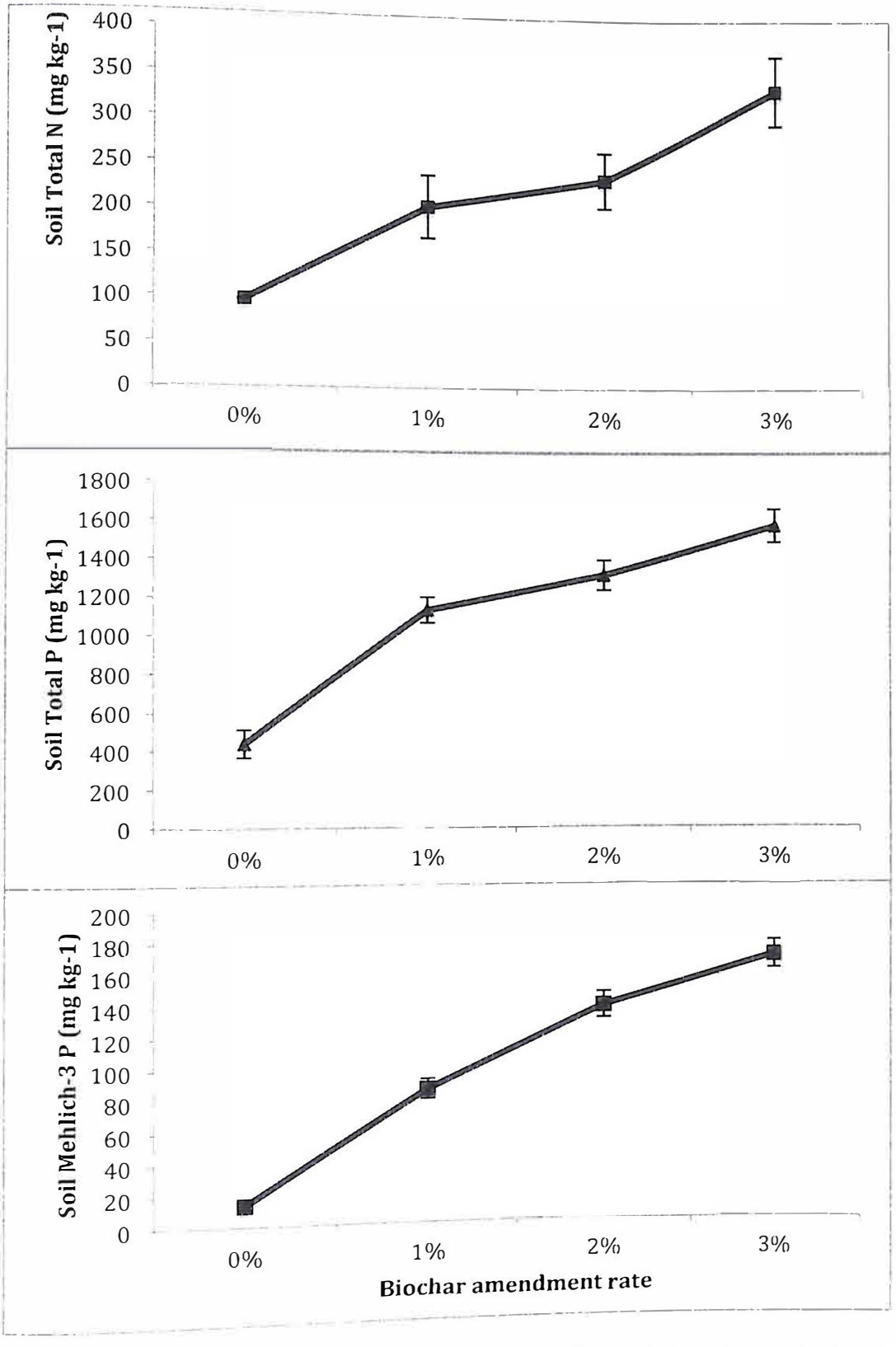

Figure 1: Residual contents of total nitrogen (TN), total phosphorus (TP), and Mehlich-3 P in PL-biochar-amended soils after one season of plant growth. Error bars represent standard deviations of the triplicate measurements. ($p \leq 0.05$ by ANOVA and LSD tests, indicating at least one treatment was significantly different from the others)

by 1%, 2% and 3% PL-biochar amendments (Fig. 1). Agricultural soils in Clearfield County, PA typically possess a Mehlich-3 P content of 59 mg kg^{-1} (Penn State Agricultural Analytical Service Lab, 2013). Both ANOVA and LSD analyses suggest that PL-biochar amendment significantly ($P < 0.05$) increased the Mehlich-3 P content of AML soils.

5.3.4. Decrease in soil acidity

The PL-biochar amendments reduced the acidity of the strongly acidic AML soil. After 6 months of incubation and plant growth, soil pH was increased from 5.6 in the control treatment to 6.3 and 6.9, respectively, in 2% and 3% PL-biochar amended soils; however, the pH showed a slight decrease to 5.5 in 1% PL-biochar amended soils (Fig. 2). Likely, PL-biochar amendment stimulated plant growth: at 1% amendment rate the basic cation nutrients released from PL-biochar did not compensate for the amounts absorbed by plants. The LSD tests indicated that the pH elevation and acidity reduction by PL-biochar at 2-3% amendment rates were significant at 95% confidence level. Indeed, PL-biochar amendment would reduce soil acidity. Schomberg et al. (2012) reported that 1% PL-biochar increased the pH of an agricultural soil from 5.6 to 8.4 after 127 days of incubation.

5.3.5. Increases in soil electric conductivity

The EC of the test soils increased linearly with the PL-biochar amendment rate (Fig. 2). After one season of plant growth, the unamended soils increased its EC from 0.07 dS m^{-1} (Table 1) to 0.19 dS m^{-1} (Fig. 2). With PL-biochar amendment, the soil EC was further increased to 1.03, 1.91, and 2.04 dS m^{-1}, respectively, at 1%, 2%, and 3% amendment rates. As the measurement of EC is directly related to the nutrient and

minerals contents of the soil, Hass et al. (2012) also found that chicken manure amendment increased the soil EC level. According to the soil EC standard characteristics (UD extension, 2013), soils with EC <1.3 dS m^{-1} in 1:1 soil/water slurry measurement are "not saline". Amendment of the AML soil with 3% PL-biochar resulted in a "slightly saline" soil. To avoid potential salinity toxicity to plants, PL-biochar amendment should be controlled at rates less than 3% of the root-zone soils (Guo et al., 2012).

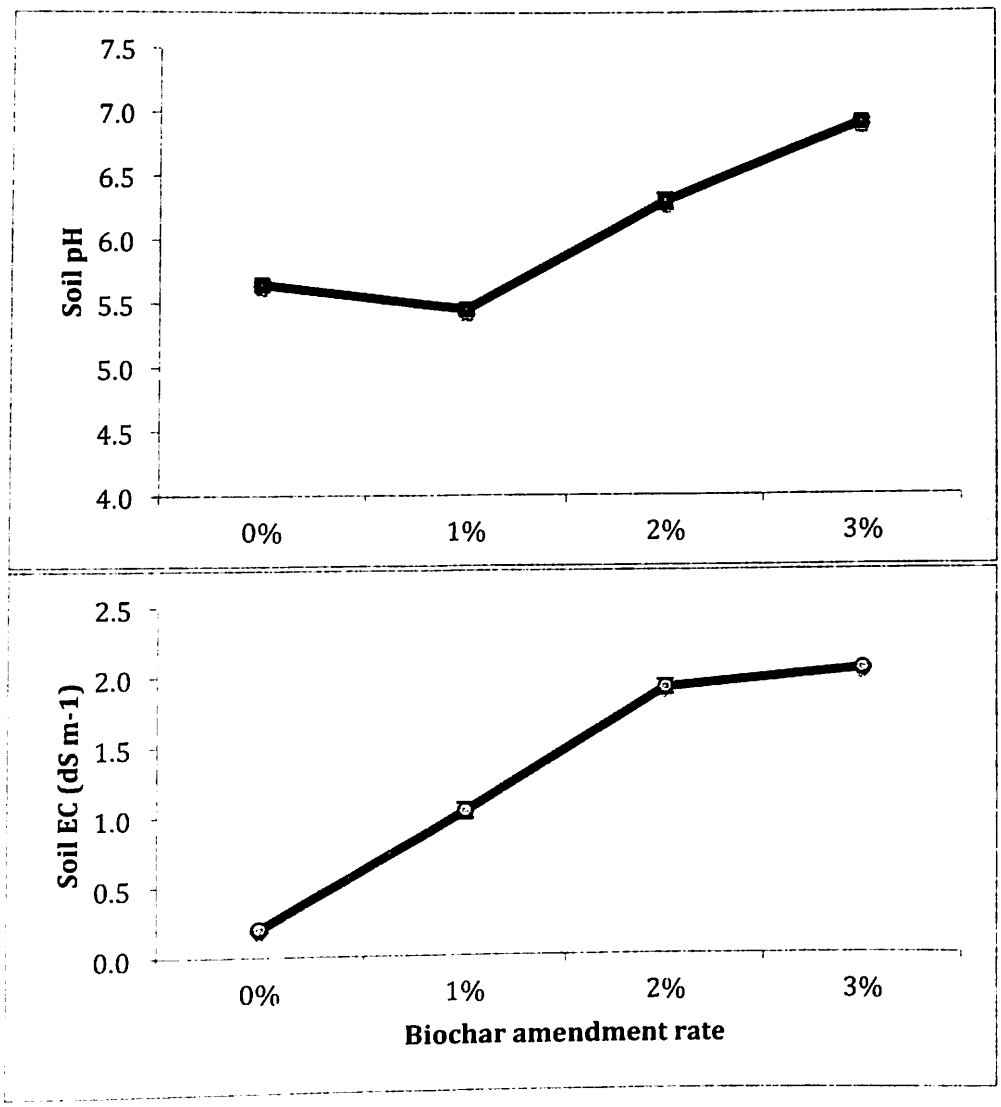

Figure 2: pH and electrical conductivity (EC) of PL-biochar amended soils after 6 months of plant growth. Error bars represent standard deviation of triplicate measurements ($p \leq 0.05$ by ANOVA and LSD tests).

5.3.6. Increases in soil cation exchange capacity

The cation exchange capacity (CEC) of the treated soils increased with increasing the PL-biochar amendment rate (Fig. 3). The unamended soil showed a CEC of 3.88 meq $(100\ g)^{-1}$. The level was elevated to 4.36 meq $(100\ g)^{-1}$ by 1% PL-biochar amendment and further to 6.37 meq $(100\ g)^{-1}$ by 3% PL-biochar amendment. The increases in CEC were significant at 95% confidence level. A typical agricultural soil in Clearfield County, PA has CEC at 12.5 meq $(100\ g)^{-1}$ (Penn State Agricultural Analytical Service Lab, 2013). Angst et al. (2013) found that 2% sycamore biochar amendment increased the soil CEC from 20.9 to 26.9 meq $(100\ g)^{-1}$ after 55 days of incubation.

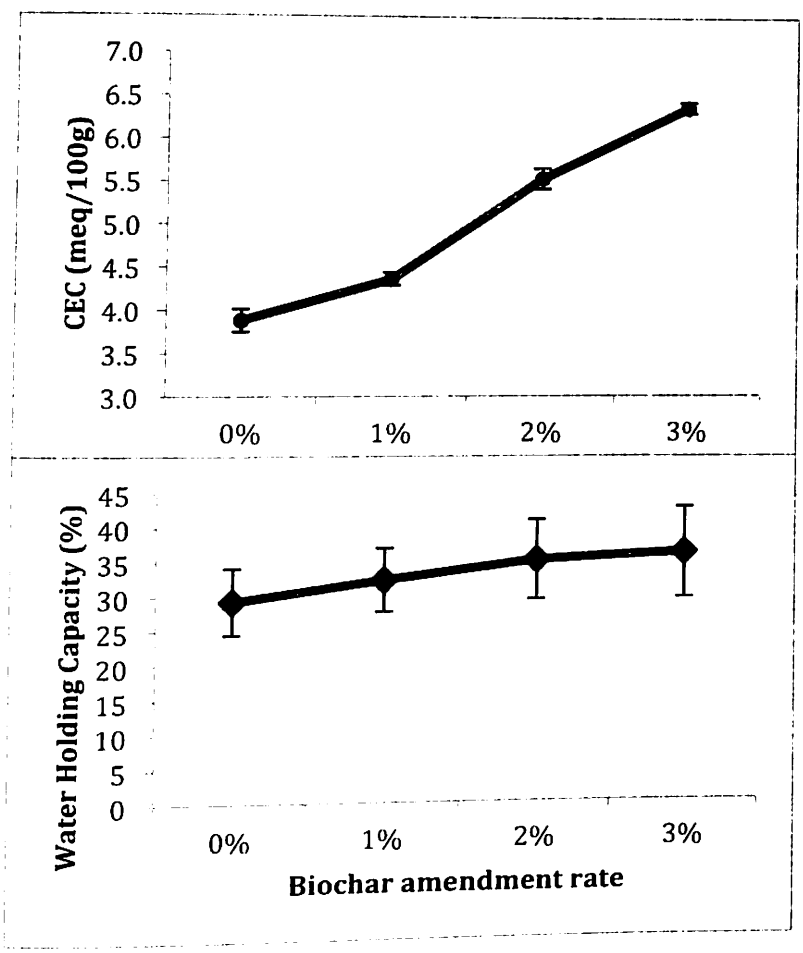

Figure 3. Increases in soil cation exchange capacity (CEC) and water holding capacity (WHC) by PL-biochar amendments. Error bars represent standard deviation of triplicate measurements. ($p \leq 0.05$ by ANOVA and LSD tests).

5.3.7. Increases in soil water holding capacity

At 1%, 2%, and 3% amendment rates, PL-biochar increased WHC of the AML soil from 27.2% to 30.1%, 30.2%, and 30.5%, respectively (Fig. 3). The increases were not significant, however, based on ANOVA analyses ($\alpha = 0.05$). Streubel et al. (2011) reported that amendment of mineral soils with switchgrass and softwood biochars at 39.0 ton ha^{-1} increased soil WHC by 1%.

5.3.8. Elevations in water-extractable nutrients

Water extractable nutrients are the nutrients in soil readily available to plants. Through PL-biochar amendment, the total dissolved nitrogen (TDN, or water-extractable N) of the AML soil was significantly increased ($P< 0.05$). The control soil had TDN at 5.87 mg kg^{-1}. In soils amended by PL-biochar at 1, 2, and 3%, the TDN contents were 8.94, 12.13, and 16.02 mg kg^{-1}, respectively (Fig. 4). Similarly, PL-biochar amendment significantly increased the total dissolved phosphorus (TDP), dissolved organic carbon (DOC), and water-extractable Ca^{2+}, Mg^{2+}, K^{+}, and SO_4^{2-} levels of the AML soil (Fig. 4; Table 3). Overall, the AML soil fertility was substantially improved by PL-biochar amendment.

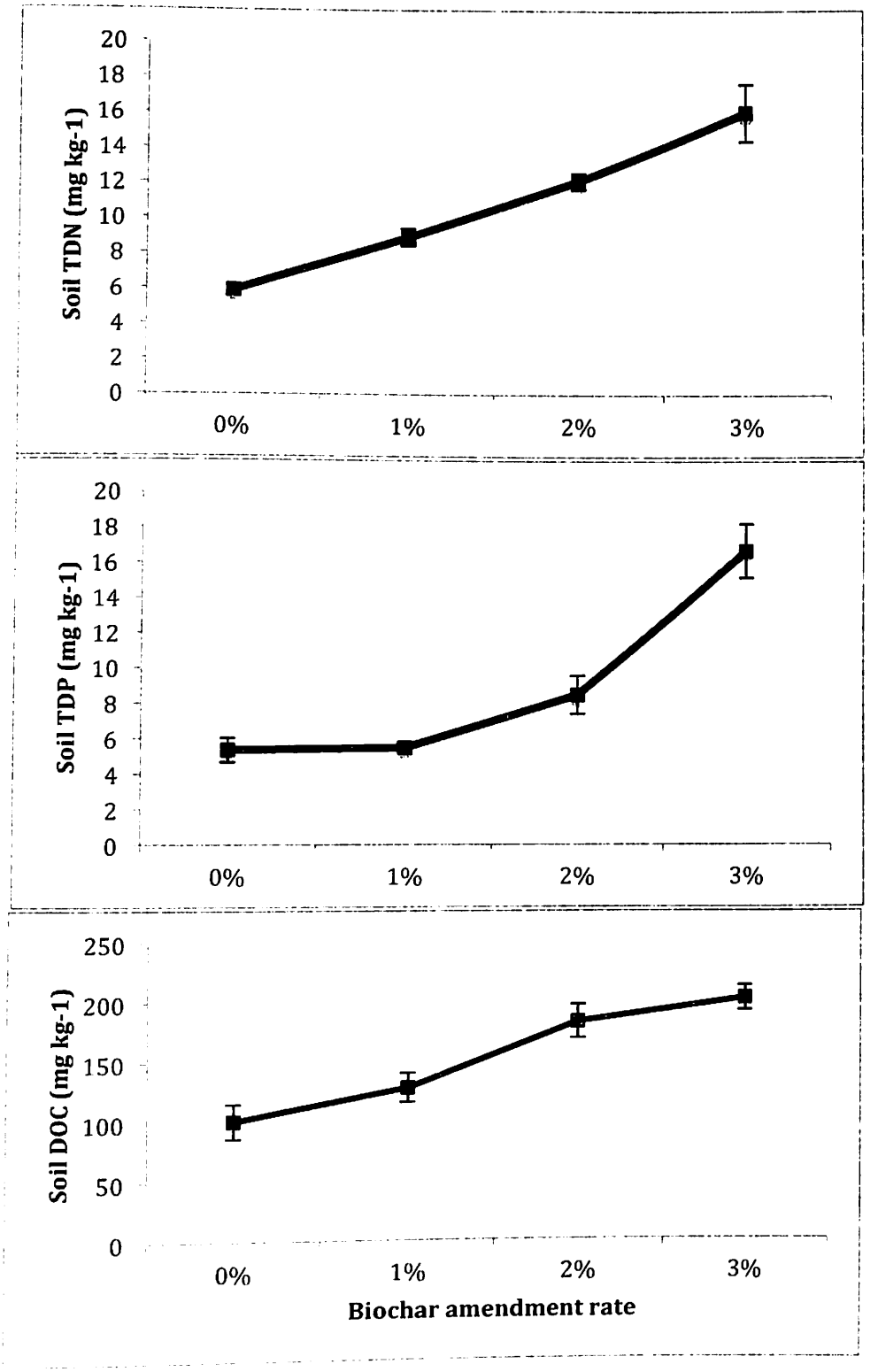

Figure 4. Residual contents of total dissolved nitrogen (TDN), total dissolved phosphorus (TDP) and dissolved organic carbon (DOC) in PL-biochar amended soils after 6 months of plant growth. Error bars represent standard deviations of triplicate measurements ($p \leq 0.05$ by ANOVA and LSD tests).

Table 3. Residual contents of water extractable salt nutrients in PL-biochar amended soils after 6 months of plant growth.

Nutrients	PL biochar amendment rate			
	0%	1%	2%	3%
K^+ (mg kg^{-1})	0.67 ± 0.1	12.2 ±0.6	33 ± 2.7	68.7 ± 1.6
Ca^{2+} (mg kg^{-1})	0.78	5.6 ± 0.7	30.6 ± 2.6	42.3 ± 1.4
Mg^{2+} (mg kg^{-1})	0.0	1.7 ± 0.3	11.6 ± 1.7	19.2 ± 1.0
SO_4^{2-} (g kg^{-1})	0.83 ± 0.09	5.4 ± 0.47	9.9 ± 0.4	11.5 ± 0.8
		Least Significance Difference test P value		
K^+		**	**	**
Ca^{2+}		**	**	**
Mg^{2+}		**	**	**
SO_4^{2-}		**	**	**

** Significantly different to compare with control soil at $\alpha = 0.05$
* Not significantly different to compare with control soil at $\alpha = 0.05$

5.4. Improvement of soil microbial community by PL-biochar amendment

5.4.1. Soil microbial activity improvement

No significant differences in microbial activity were detected among the differently treated soils. In the initial AML soil, the FDA enzymatic activity was measured at 52.05 μg g^{-1}. It remained nearly unchanged after 6 months of plant growth. In the soils amended with 1%, 2%, and 3% PL-biochar, the microbial activity levels were 52.2, 52.16 and 52.42 μg g^{-1}, respectively, not significantly different from the unamended control soil (Fig. 5). The PL-biochar itself was sterile and did not contain any microorganisms. It may require longer time (e.g., several years) of plant growth for soils to develop discernible microbial activity stimulation effects from PL-biochar amendment by providing additional habitats and nutrients to soil microorganisms.

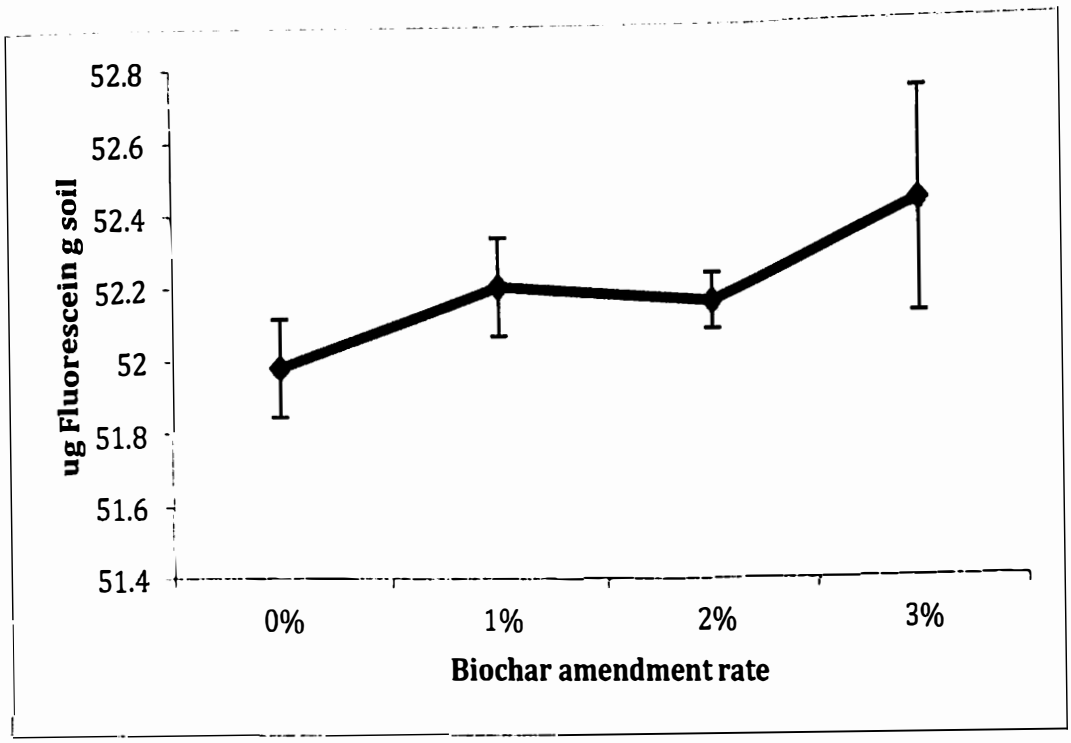

Figure 5. Microbial activity with Fluorescein Diacetate Assay of PL-biochar amended soils after 6 months of plant growth. Error bars represent standard deviations of triplicate measurements. ($p > 0.05$ by ANOVA an LSD tests).

5.4.2. Changes in soil microbial community structure

The soil samples analyzed by 16S rRNA sequencing are listed in Table 4.

Table 4. PL biochar amendment rate relating to their sample number used further in this section.

0% PL biochar amended soil	1% PL biochar amended soil	2% PL biochar amended soil	3% PL biochar amended soil
Sample 1	Sample 4	Sample 7	Sample 10
Sample 2	Sample 5	Sample 8	Sample 11
Sample 3	Sample 6	Sample 9	Sample 12

Effective extraction of DNA from the PL-biochar amended soils was rather difficult. It is probably due to the highly acidic, heavy metals-rich nature of the AML soil. After numerous attempts, Power Max soil DNA kit (MoBio Laboratories, Carlsbad, CA) was identified the most efficient for extracting DNA in 260/280 nm and 260/230 nm ratios and at nanodrop concentrations . Eventually, DNA was successfully extracted from all the soil samples except for Sample 3 and Sample 12 (Table 4) by using the Power Max soil DNA kits (Fig. 6). The microbial DNA in the initial, unamended AML soil was also extracted on lane 17 (Fig. 6). Soils collected from Delaware State University (DSU) campus gardens were used to verify the technique.

Overall, all the DNA extraction trials showed satisfactory results except for Sample 3 and Sample 12 in 260/280 ratio (Table 5). Nevertheless, the concentration of extracted DNA was low in all samples, ranging from 4.3-6.9 ng μL^{-1}. In soils from DSU gardens, the range was 34.1 and 81.1 ng μL^{-1} (Table 5).

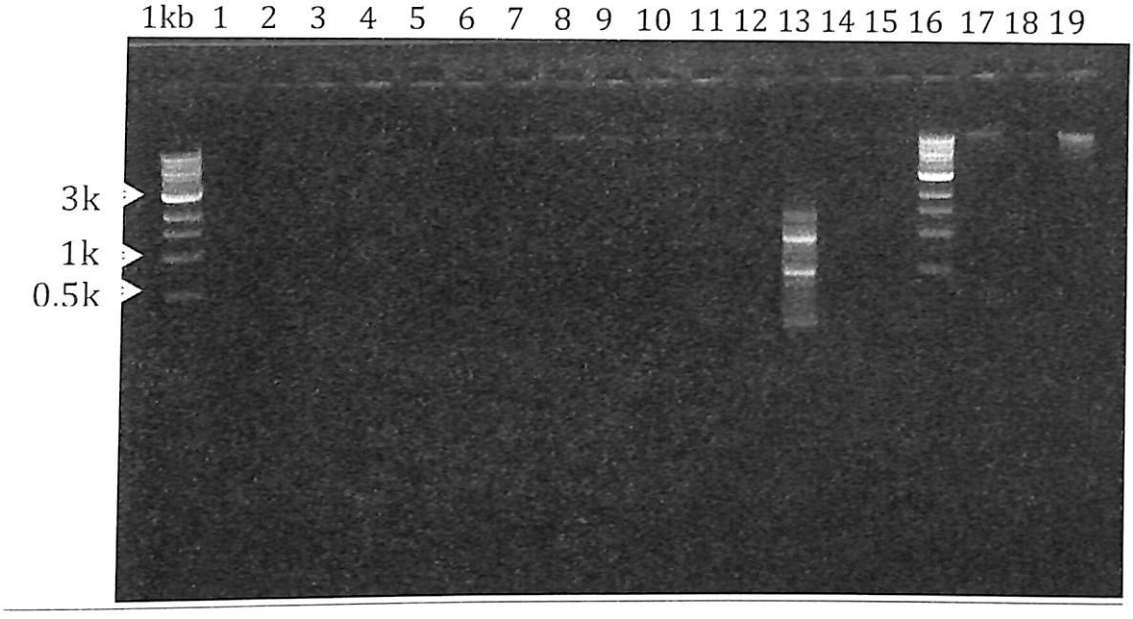

Figure 6. DNA extraction. First lane is the 1kb ladder. Lane number 1-12, sample 1-12 respectively. Lane 13-100bp ladder, 14-sample 9, 15-sample 10, 16-1kb ladder, 17-initial soil sample, 18-sample 1, 19-DNA from DSU garden.

Table 5. DNA concentrations from PL biochar amended soil samples after 6 months growth of Poverty oatgrass in greenhouse potting experiment. Positive control DNA was also isolated from DSU garden and lawn soils.

Sample ID	Nucleic acid conc. ng μl^{-1}	A260	A280	260/280	260/230
Initial soil	4.6	0.092	0.055	1.69	0.25
Sample 1 (0%)	4.5	0.091	0.035	2.61	0.92
Sample 2 (0%)	6.2	0.122	0.076	1.62	1.16
Sample 3 (0%)	4.6	0.092	0.035	2.64	0.52
Sample 4 (1%)	6.7	0.139	0.07	1.93	0.88
Sample 5 (1%)	6.2	0.125	0.039	3.23	0.56
Sample 6 (1%)	5.9	0.118	0.067	1.75	1.39
Sample 7 (2%)	5.8	0.115	0.072	1.59	1.57
Sample 8 (2%)	6.9	0.139	0.068	2.03	1.28
Sample 9 (2%)	5.0	0.101	0.058	1.75	1.14
Sample 10(3%)	4.3	0.086	0.052	1.65	1.28
Sample 11(3%)	6.3	0.126	0.055	2.29	0.96
Sample 12 (3%)	6.9	0.137	0.043	3.17	0.55
DSU garden soil	34.1	0.681	0.488	1.40	0.47
DSU lawn soil	81.1	1.623	1.042	1.56	1.21

Gradient PCR was also conducted for identifying correct melting temperature for archeal universal primer A751F and UA 1406R. The melting temperature range was 50 to 60°C. Results on 50 to 56.1°C showed the brighter bands. All the bands showed the expected result (~655 bp). Consequently, 53.7°C was selected as the melting temperature in further PCR analysis. In negative controls, no bands were observed (Fig. 7).

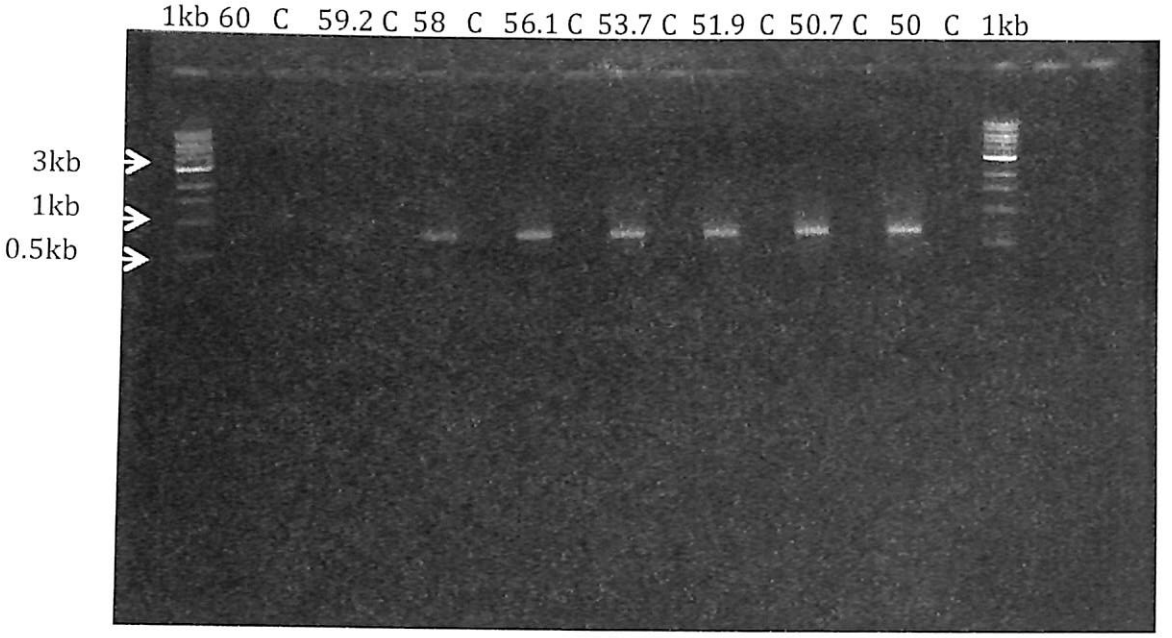

Figure 7. Gradient PCR using A751F/ UA1406R archeal universal primer. The melting temperature between 50-60°C. C-represent the negative control in each melting point.

The PCR amplification was successfully conducted using the archeal 16S rRNA universal primer A751A and UA1406R. All the samples, except for Sample 3 and Sample 12, showed the correct size result on 1% gel electrophoresis. The expected result was around 655 bp. There was no amplification on Sample 12. Double bands occurred on Sample 3. One of the band on Sample 3 was correct band of ~655 bp but the other band was a primer dimer (Fig. 8).

The fresh DNA products were successfully cloned on TOPO TA PCR-4 vector (Fig. 9). The only kanamycin resistant colonies could grow on the LB-kanamycin plates. TOPO TA PCR-4 vector has the kanamycin resistant gene thus the 16S rRNA PCR products which ligated into the vector has that ability to resist kanamycin.

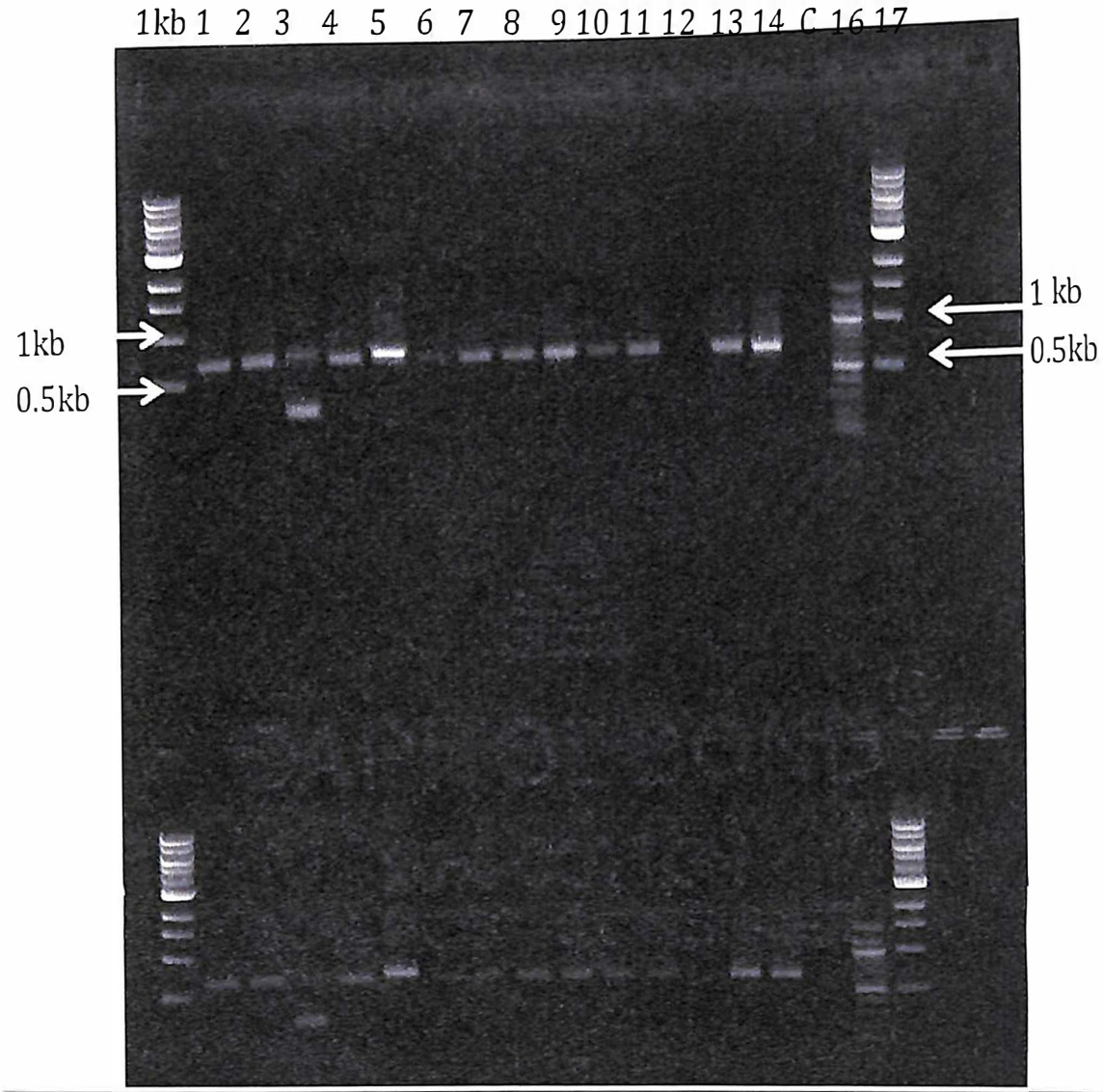

Figure 8. Archeal universal primer A751F and UA1406R PCR amplification (1% agarose gel). The lane numbers 1-12 represent the sample number. Lane number 13- initial soil DNA, 14- DSU lawn soil sample, 15- negative control, 16- 100bp, 17-1kb.

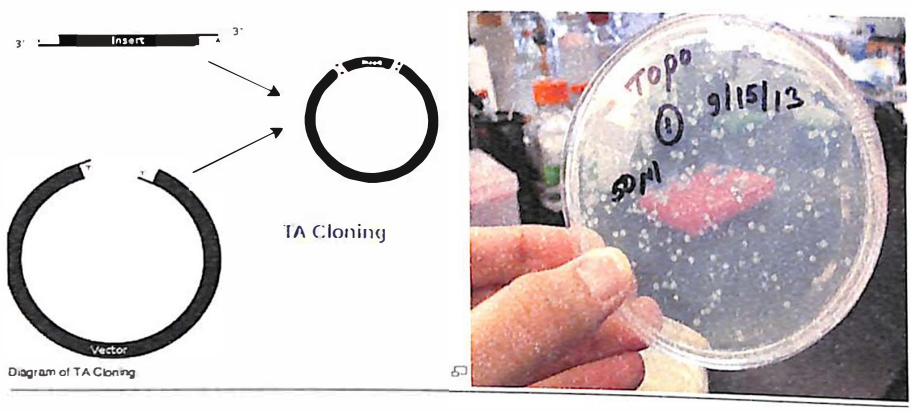

Figure 9. Cloning of 16S rRNA inserts into TOPO TA PCR-4 vector. On LB-kanamycin agar only successful ligated colonies were grown in white colonies.

Three colonies were chosen from each sample (plate) to confirm the PCR insert. Colonies 1b, 2b, 3a, 8c did not work, while 3b and 3c had primer dimer as well as the right band. On archeal 16S rRNA PCR, it showed the same result. All other colony PCR showed the correct band on 1% gel electrophoresis (Fig. 10).

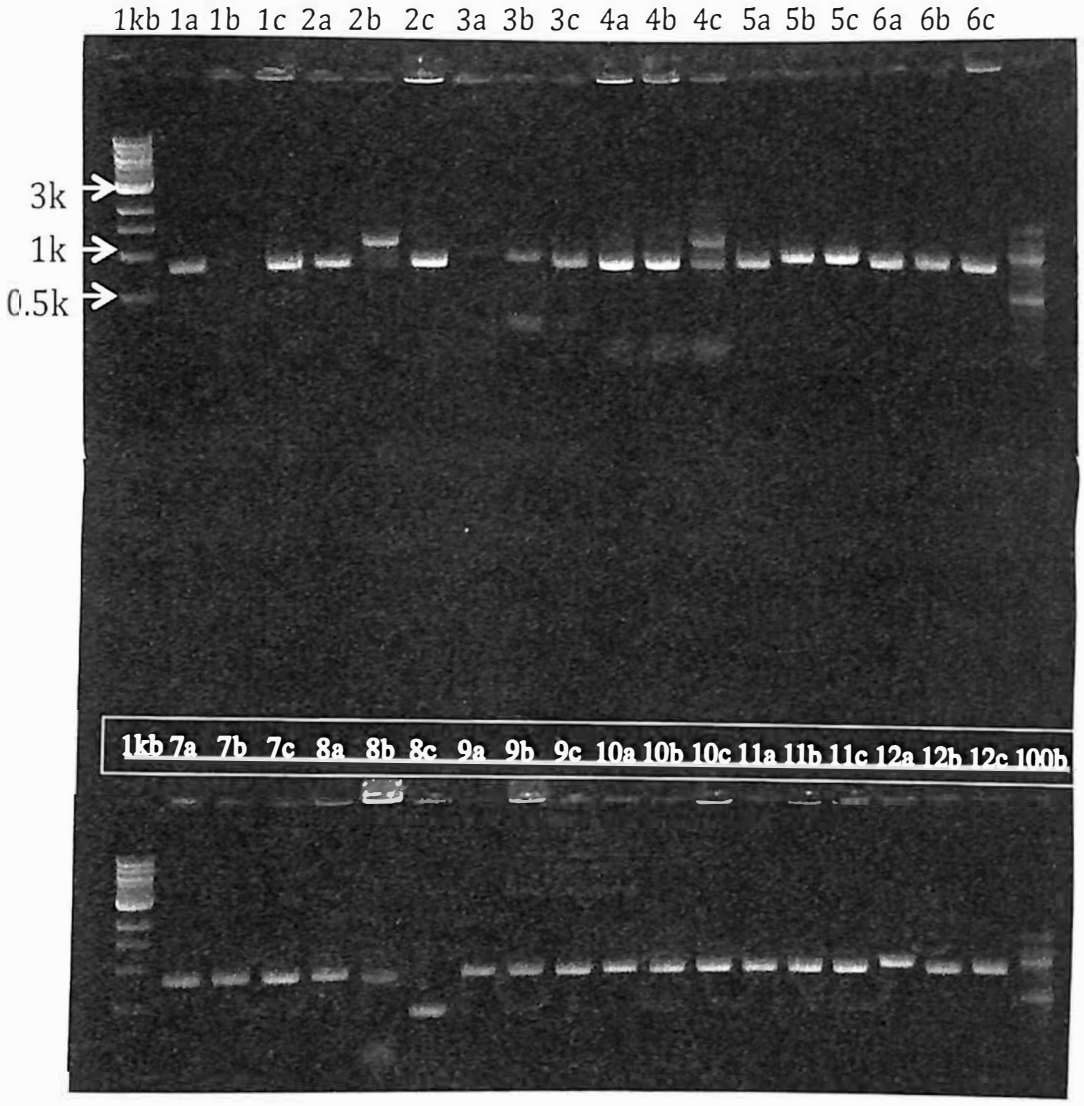

Figure 10. Representative figures of positive clones screened by Colony PCR the primer using 13M and 13F. Sample 1-12. From each plate, 3 colonies were chosen (a, b, c). 1kb and 100bp ladder were used for identification of the molecular weight.

Restriction digestion with *EcoR1* enzyme was used for confirmation of the insert. TOPO PCR-4 vector had *EcoR1* restriction in either side of the region where 16S rRNA was inserted. The vector had size of 3956 bp and the 16S rRNA insert had the 655 bp

(Fig. 11). Based on the molecular weight on gel electrophoresis, it can be confirmed if the right size of insert is there. The uncut plasmid DNA has 3 bands that represent linear, supercoiled, circular DNA. The cut plasmid DNA had 2 bands that had a vector size of 3956 bp and 16S rRNA product 655 bp (Fig. 10 and 11). Figure 11 illustrates the clearer image of 2 bands by *EcoR1* cut. It has lower bands at around 655 bp and the upper band around 4kb.

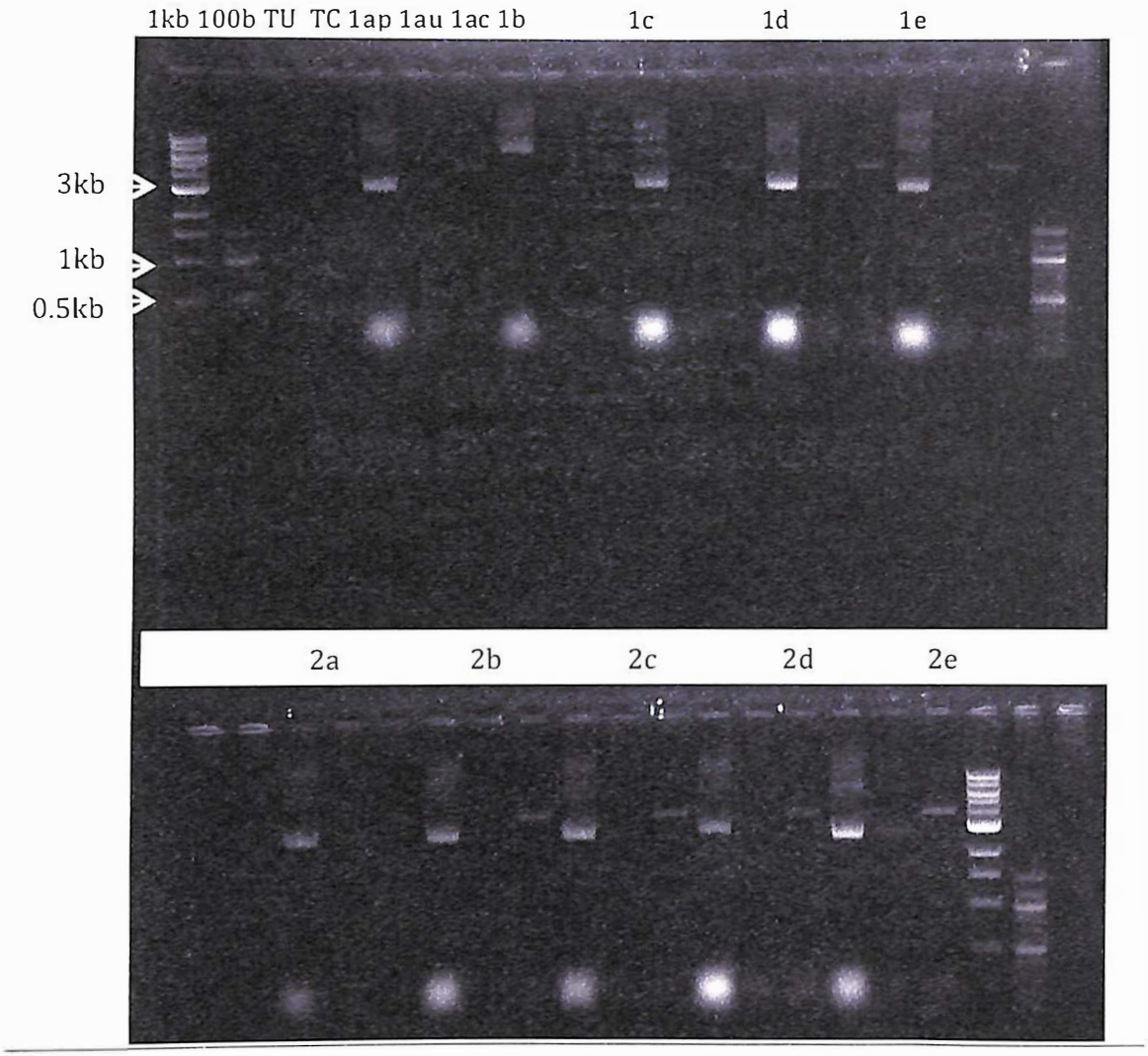

Figure 11. Restriction digestion using *EcoR1* enzyme. TU - TOPO TA plasmid uncut; TC - TOPO TA plasmid cut; 1ap - 1a plasmid; 1au - 1a uncut; 1ac - 1a cut; 1b - plasmid, uncut, cut; 1c - plasmid, uncut, cut, etc.

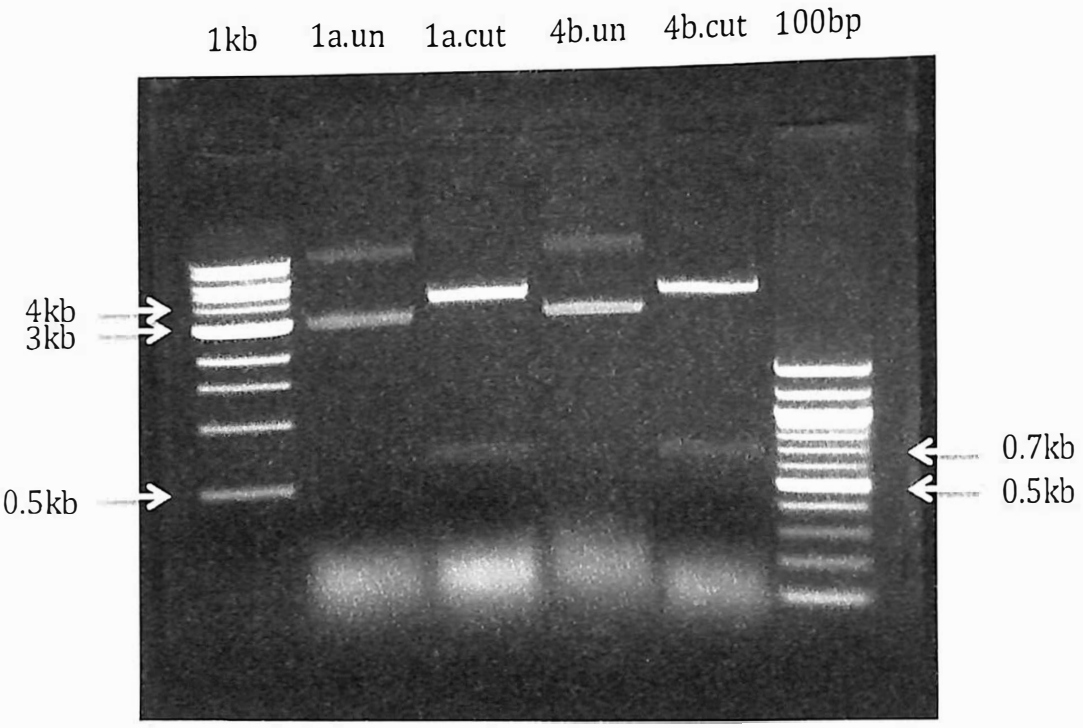

Figure 12. Restriction digestion using *EcoR1* enzyme. 1a.un - sample 1a uncut; 1a.cut - sample 1a cut; 4b.un - sample 4b uncut; 4b.c - sample 4b cut.

A total of 39 plasmid DNA were isolated after screening the right insert to the vector. The concentration of plasmid DNA after the isolation were ranged 1260 to 2460 ng μL^{-1} and it diluted to the range between 58 to 86 ng μL^{-1}. The average concentration was 73.4 ng μL^{-1} that required for sequencing (range 50 to 75 ng μL^{-1}). The quality of isolated plasmid DNA was satisfactory in all samples and did not need further purification.

Table 6. Concentration of plasmid DNA after dilution

Sample ID	Nucleic Acid Conc. ng μL^{-1}	A260	A280	260/280	260/230
1a	73.3	1.467	0.828	1.77	2.46
1d	75.3	1.505	0.819	1.84	2.56
1e	63.1	1.262	0.694	1.82	2.51
2b	81.5	1.631	0.878	1.86	2.64
2c	78.3	1.567	0.853	1.84	2.59
2e	69.4	1.388	0.765	1.81	2.59
3b	81.4	1.627	0.83	1.96	2.31
3c	74.1	1.483	0.805	1.84	2.56
3e	71.6	1.433	0.777	1.84	2.53
4a	87.9	1.758	1.056	1.66	1.81
4d	69.9	1.397	0.774	1.81	2.58
4e	70.7	1.414	0.776	1.82	2.47
5b	74	1.481	0.81	1.83	2.53
5c	84.7	1.694	0.924	1.83	2.51
5e	78.5	1.57	0.84	1.87	2.49
6a	86.5	1.731	0.913	1.9	2.52
6b	78.8	1.575	0.862	1.83	2.58
6c	64.3	1.286	0.693	1.85	2.52
7a	70.2	1.405	0.765	1.84	2.54
7b	72.4	1.449	0.801	1.81	2.61
7c	74.7	1.494	0.793	1.88	2.46
8a	78.1	1.563	0.862	1.81	2.55
8b	79	1.58	0.838	1.89	2.57
8d	78.5	1.571	0.882	1.78	2.62
9a	68.1	1.363	0.763	1.79	2.65
9b	70.6	1.413	0.781	1.81	2.6
9d	62	1.241	0.701	1.77	2.65
10a	71.8	1.437	0.797	1.8	2.55
10b	66.4	1.328	0.743	1.79	2.63
10c	72.7	1.455	0.784	1.86	2.52
11a	65.9	1.318	0.75	1.76	2.61
11b	65.1	1.302	0.721	1.81	2.54
11d	69.5	1.389	0.783	1.77	2.6
12b	77	1.539	0.839	1.83	2.49
12c	74.3	1.486	0.819	1.81	2.58
12d	72.1	1.442	0.785	1.84	2.54
13a	68.2	1.363	0.762	1.79	2.58
13b	58.3	1.166	0.643	1.81	2.59
13c	84.4	1.688	0.929	1.82	2.62

The archeal sequences were obtained from the recombinant libraries. The clone libraries were affiliated (NCBI BLASTN) with archeal environmental sample sequences. All the sequence resulted in uncultured archeal 16S rRNA sequence. But there were no specific archeal species identified. There were some difference in the sequences but it is hard to tell if that sequence represent the different archeal species. The common possible archaeal bacteria in acidic possible heavy metal contaminated soil are Euryarchaea (e.g., Ferroplasma) (Maezato and Blum, 2012). These archaeal bacteria were found in 80% 16S rRNA sequencing of Acid Mine Drainage (AMD) and mining sites (Maezato and Blum, 2012).

Table 7. The highest percentage identity matches of each the samples following BLASTN comparison of archeal 16S rRNA environmental samples and the NCBI collection database.

Sample ID	Sequence result	Sequence similarity, %	Length (bp)	Score (Bits)	Value
1A	Uncultured archaeon clone R125 16S rRNA gene, partial sequence	83	898	592	9E-168
2B	Uncultured archaeon clone 16S rRNA gene, partial sequence	84	1416	634	1E-180
3B	Uncultured archaeon clone R7 16S rRNA gene, partial sequence	99	906	1287	0
4A	Uncultured archaeon clone R125 16S rRNA gene, partial sequence	85	898	634	1E-180
5B	Uncultured archaeon clone R7 16S rRNA gene, partial sequence	98	906	1271	0
6A	Uncultured archaeon clone CRYS_SPR_AO7C 16S rRNA gene, partial sequence	92	1214	458	0
7A	Uncultured archaeon clone CRYS_SPR_AO7C 16S rRNA gene, partial sequence	92	1214	846	0
8A	Uncultured archaeon clone 16S rRNA gene, partial sequence	96	659	1072	0
9A	Uncultured archaeon clone CRYS_SPR_A07C 16S rRNA gene, partial sequence	93	1214	880	0
10A	Uncultured archaeon clone R158 16S rRNA gene, partial sequence	94	876	966	0
11A	Uncultured archaeon clone 081028-OL-KR13:1:2 16S rRNA gene, partial sequence	91	962	894	0
12B	Uncultured archaeon clone R158 16S rRNA gene, partial sequence	91	876	872	0
Initial soil A	Uncultured archaeon clone R106 16S rRNA gene, partial sequence	91	863	889	0

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>gb|FJ184665.1| Uncultured archaeon clone R125 16S ribosomal RNA gene, partial
sequence
Length=898

 Score =   592 bits (320),  Expect = 9e-168
 Identities = 550/659 (83%), Gaps = 23/659 (3%)
 Strand=Plus/Minus

Query  59   ACGGGCGGTGTGTGCAAGGCCCGGGAACGTATTCAACGCAGCGTA-GCTGATCTGCGTTT  117
            ||||||||||||| ||||||||||||| | |||||| |||  |||| |||||  | |||  ||
Sbjct  881  ACGGGCGGTGTGTACAAGGCCCGGGAACATATTCACCGC--CGTATGCTGACCGGCGGTT  824

Query  118  ACTAGCGATTCCGACTTCATGAAGGCGAGTTGCAGCCTTCAATCCGAACTGAG-CGCGCG  176
            ||||||  | ||||  |||||||  ||||||||||||||||  ||||||||||||  |  ||
Sbjct  823  ACTAGCAACTCCGGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGACCGGC-  765

Query  177  TTTCAGAGATTGGCT-TACTTTCACAAGTTCGCTGCTCGCTGT-CGCGCGCATTGTAGCA  234
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Sbjct  764  TTTAAGGGATTCGCTCCAC-CTCGCGGTTTCGCCGCCCGTTGTACCGGC-CATTGTAGCG  707

Query  235  CGTGTGTAGCCCGGGACGTAAGGGCCATGCTGACTTGACGTCATCCCCACCTTCCTCCG-  293
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Sbjct  706  TGGGTGTAGCCCAGGACGTAAGGGCCATGCTGACTTGACGTCATCCCCACCTTCCTCCGA  647

Query  294  GCTTCTCACCGGCGGTCTCGTGTGAGAGATCTTGTGGACCAACACACGACAAGGGTTGCG  353
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Sbjct  477  TCCTGGTAAGGTTCTTCGCGTTGCGTCGAATTAAACCCCACGCTCCGCTGCTTGTGCGGG  418

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Sbjct  417  CCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGACACTTAA  358

Query  592  TGTGTTAACTACGGCACATATGGAGTTGATACCACATACACCTAGTGTCCATCGTTTACG  651
            ||  ||||  ||  ||||||    ||  ||||||||||  ||||||||||||||||||||||
Sbjct  357  TGCGTTAGCTTCGGCACTGGCGGGGTTGATACCACCAACACCTAGTGTCCATCGTTTACG  298
```

Figure 13. Example of MegaBLAST result for Sample 1A. The highest percentage identity matches following BLASTN comparison of Archeal 16S rRNA environmental samples and the NCBI collection database.

```
>gb|FJ184665.1| Uncultured archaeon clone R125 16S ribosomal RNA gene, partial
sequence
Length=898

 Score =   592 bits (320),  Expect = 9e-168
 Identities = 550/659 (83%), Gaps = 23/659 (3%)
 Strand=Plus/Minus

Query  59   ACGGGCGGTGTGTGCAAGGCCCGGGAACGTATTCAACGCAGCGTA-GCTGATCTGCGTTT  117
            ||||||||||||||| ||||||||||||||| |||||| |||  |||| ||||| | ||| ||
Sbjct  881  ACGGGCGGTGTGTACAAGGCCCGGGAACATATTCACCGC--CGTATGCTGACCGGCGGTT  824

Query  118  ACTAGCGATTCCGACTTCATGAAGGCGAGTTGCAGCCTTCAATCCGAACTGAG-CGCGCG  176
            ||||||  | ||||  |||||||  ||||||||||||||||  ||||||||||||||  |  ||
Sbjct  823  ACTAGCAACTCCGGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGACCGGC-  765

Query  177  TTTCAGAGATTGGCT-TACTTTCACAAGTTCGCTGCTCGCTGT-CGCGCGCATTGTAGCA  234
            |||  ||  ||||  |||  ||  ||  |  |||||  ||  ||  |||  |  ||  |||||||||
Sbjct  764  TTTAAGGGATTCGCTCCAC-CTCGCGGTTTCGCCGCCCGTTGTACCGGC-CATTGTAGCG  707

Query  235  CGTGTGTAGCCCGGGACGTAAGGGCCATGCTGACTTGACGTCATCCCCACCTTCCTCCG-  293
              |  |||||||||  ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  706  TGGGTGTAGCCCAGGACGTAAGGGCCATGCTGACTTGACGTCATCCCCACCTTCCTCCGA  647

Query  294  GCTTCTCACCGGCGGTCTCGTGTGAGAGATCTTGTGGACCAACACACGACAAGGGTTGCG  353
            |  |||||  ||||  |||  ||||||||  |||        |  ||||||||   |  ||||||||
Sbjct  646  GTTTCTC-TCGGCAGTCCCGTGTGAGTGAT-------A-CAACACACAGTAGGGGTTGCG  596

Query  354  CTCGTTGCGGGACTTAACCCGACACCTCACGGCACGAGCTGACGACAGCCATGCAGCACC  413
            ||||||||||||||||||  |||  ||||||  ||||||||||||||||||||||  ||||
Sbjct  595  CTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAACACC  536

Query  414  TGTCTTGCGGTCGGGTTGCCCCGAAGCCCAC-TTT-CATGGGATTTCCGCAGATGTCAAG  471
            |||    |  |  |||  |  |  |||  ||  |||  |  |||||  |  |  ||||||||
Sbjct  535  TGT-GGCCAGGCCCCTTG-CGGGGCGCCGACGTTTCCGCCGGATTCCTGGTCATGTCAAG  478

Query  472  CCCCGGTAAGGTTCTTCGCGTTGCGTCGAATTGAACCACATGCTCCACCGCTTGTGCGGG  531
              ||  ||||||||||||||||||||||||||||  ||||  ||  |||||  |  ||||||||||||
Sbjct  477  TCCTGGTAAGGTTCTTCGCGTTGCGTCGAATTAAACCCCACGCTCCGCTGCTTGTGCGGG  418

Query  532  CCCCCGTCAATTCCTTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGGACACTTAT  591
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Sbjct  417  CCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGACACTTAA  358

Query  592  TGTGTTAACTACGGCACATATGGAGTTGATACCACATACACCTAGTGTCCATCGTTTACG  651
            ||  ||||  ||  ||||||    ||  ||||||||||||  ||||||||||||||||||||||
Sbjct  357  TGCGTTAGCTTCGGCACTGGCGGGGTTGATACCACCAACACCTAGTGTCCATCGTTTACG  298
```

Figure 13. Example of MegaBLAST result for Sample 1A. The highest percentage identity matches following BLASTN comparison of Archeal 16S rRNA environmental samples and the NCBI collection database.

5.5. Plant growth promoted by PL-biochar amendment

5.5.1. Biomass yield

The yield in both dry and fresh biomass samples increased in positive proportion with the PL biochar amendment rate. The poverty oatgrass grown in the Control soils yielded 17.48 g of fresh biomass per pot. The yield increased to 71.68, 95.33 and 93.52 g for the pots containing 1%, 2% and 3% PL biochar-amended soils, respectively (Fig. 14). Based on the results, 2% PL biochar provided the higher yield of fresh and dry biomass than the 3% amendment. In the control soil, the dry biomass yield was 3.53 g and it increased to 22.69, 30.63 and 28.66 g in 1%, 2% and 3% PL-biochar amended soils, respectively (Fig. 14, 15 and 16). Vegetative cover was established in the first two months. But the control soil and the1% PL-biochar amended soils had poor establishment. Further, the bulk diameter of the shoots was relatively smaller than the 2% and 3% PL biochar amended soils. The root formation was shallow and poor in the control and the 1% PL-biochar amended soils as compared to the 2% and 3% biochar amendments. Evidently, 2% (w/w) PL-biochar amendment did significantly improve the biomass yield. A higher amendment rate, however, did not further increase the biomass yield. In contrast, Schnell et al. (2012) found no differences in the growth of sorghum with the amendment of sorghum biochar. Likely, sorghum biochar would furnish much less nutrients to plants relative to PL-biochar. Furthermore, the employed biochar amendment rates were 1.5 and 3.0 Mg ha^{-1}, significantly lower than the levels of the present studies.

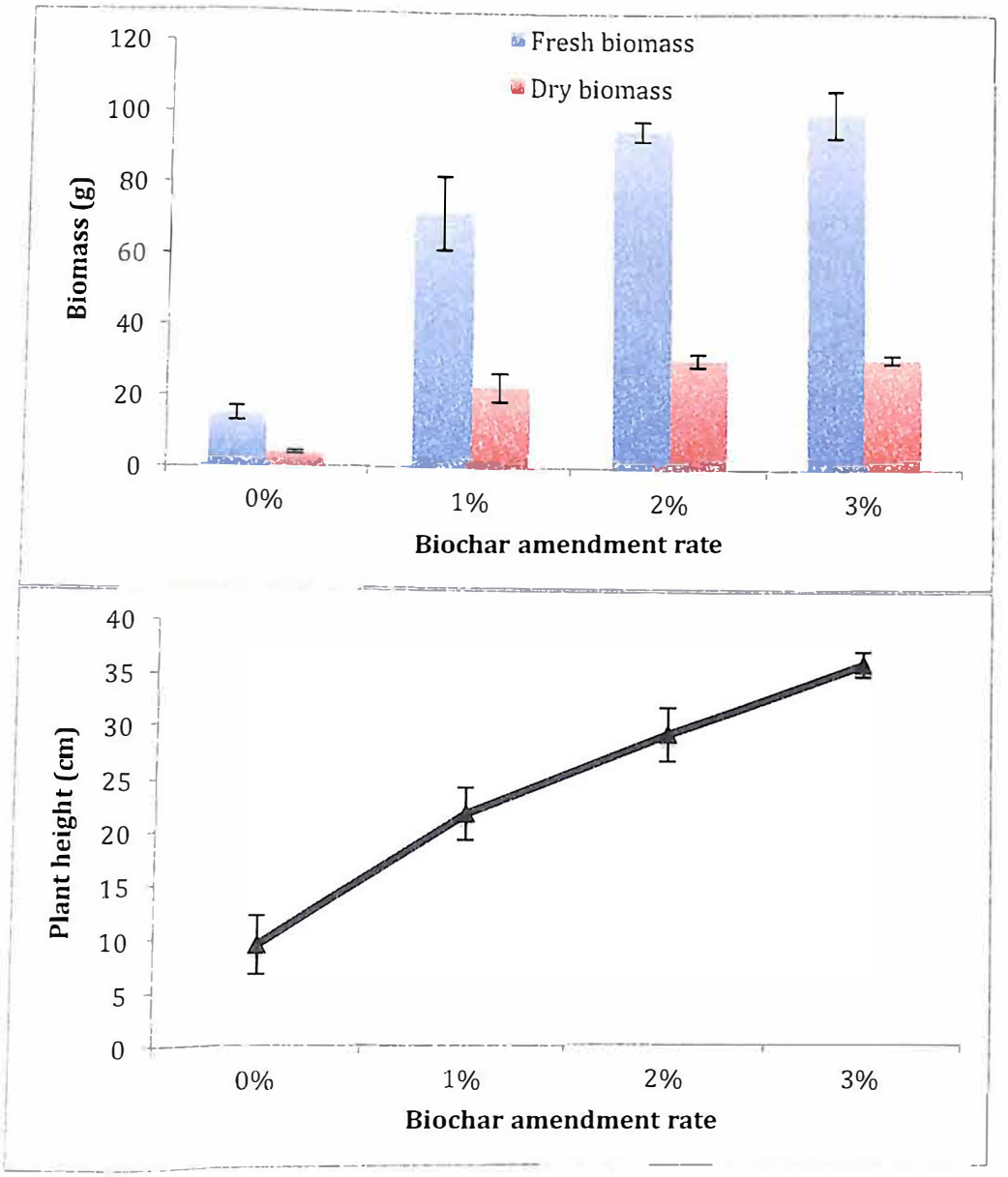

Figure 14. Dry and fresh biomass yields of poverty oatgrass grown in PL-biochar amended AML soils.

5.5.2. Plant height

The final plant height was 9.42 cm for the poverty oatgrass grown in the control soils. The height increased to 21.7 - 35.9 cm in 1% - 3% PL- biochar amended soils after the 6 months of greenhouse potting experiment (Fig. 14). The ANOVA and LSD

analyses indicated significant differences in plant height for PL-biochar amended soils from the control soils (Confidence level 95%).

Figure 15. Growth of poverty oatgrass in AML soils amended with 0%, 1%, 2%, and 3% PL-biochar. The greenhouse potting experiments lasted for 180 days.

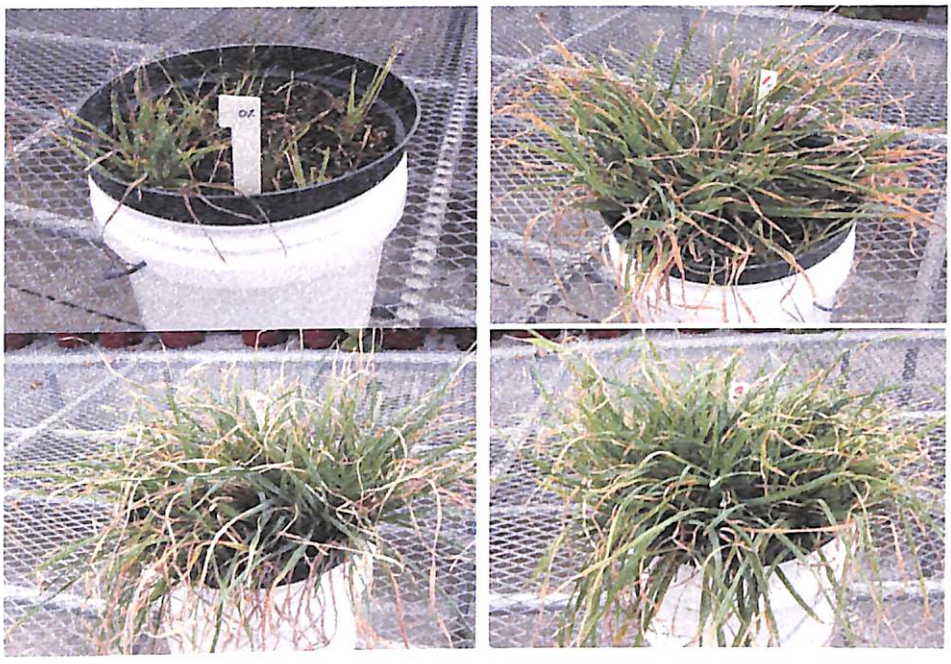

Figure 16. Comparison of poverty oatgrass growth in AML soils amended with PL-biochar at different rates.

6. Conclusions

Overall, PL-biochar amendment significantly improved soil physiochemical characteristics. The increases in soil pH and electric conductivity were noticeable. The pH increased 2.5 units (from 4.4 to 6.9) when the AML soil was amended with PL-biochar at 3%. Meanwhile, the soil changed from "low in salinity" to "moderately saline," as indicated by the significant EC elevation. Clearly, PL-biochar is a fertility enhancer for acidic, low saline soils. Both Mechlich-3 extractable P and TP contents were linearly increased with the PL-biochar amendment rate. With 3% PL-bichar amendment, the soil TN increased 3 folds and TDN 2.7 folds as compared with the control soil. The organic matter content, cation exchange capacity, and plant-available N, P, K, Ca, Mg, and S contents of the amended soils were also substantially elevated by PL-biochar amendment. It is evident that at 2-3% amendment rates the PL biochar provided sufficient nutrients to support plant growth.

In microbial activity analysis, there was no significant difference between unamended and PL-biochar amended soils at 95% confidence level. In microbial community analysis, it was difficult to compare the archeal community based on these results. No specific archeal species were identified among the 16S rRNA sequences.

The biomass yield was significantly higher in PL-biochar amended soils than in the control soil. However, there was no significant difference between 2% and 3% PL-biochar amendment treatments, though the plant height was linearly increased with the PL-biochar amendment rate.

In conclusion, the results support the hypotheses that PL-biochar amendment improved the physical and chemical properties of AML soils and the improvement in soil quality was related to the biochar application rate. At 2-3% amendment rates, PL-biochar served as an ideal conditioner and quality enhancer for poor, acidic AML soils.

References

Altantsetseg, B. 2011. Mining sector and sustainable human development. Mongolia Human Development report 2011, Ulaanbaatar, Mongolia.

Angst, T.E., C.J. Patterson., D.S. Reay., P. Anderson., T.A. Peshkur. And S.P.Sohi. 2013. Biochar diminishes nitrous oxide and nitrate leaching from diverse nutrient sources. Journal of Environmental Quality 42: 3: 672-682.

Barth, R.C. and B.K. Martin. 1984. Soil depth requirements for revegetation of surface-mined areas in Wyoming, Montana, and North Dakota. Journal of Environmental Quality 13:399-404.

Brady, N.C. and R.R. Weil. 2010. Elements of the Nature and Properties of Soils. 3rd edition. Pearson Prentice Hall: Upper Saddle River, NJ.

Burger, J.A. and J.L. Torbert. 1990. Mined land reclamation for wood production in the Appalachian region. Proceedings of the 1990 Mining and Reclamation Conference and Exhibition 1:159-163.

Chambers, J.C., R.W. Brown, and B.D. Williams. 1994. An evaluation of reclamation success on Idaho's phosphate mines. Restoration Ecology 2:4-16.

CODNR. 2002. Best Practices in Abandoned Mine Land Reclamation. Colorado Department of Natural Resources, Denver, CO.

Dangi, S. R., Stahl, P.D., Wick, A.F., & Ingram, L.J. 2011. Soil microbial community recovery in reclaimed soils on a surface coal mine site. Soil Science Society of America Journal, *76*, 916-924. Doi:10.2136/sssaj 2011.0288.

Dere, A.L, R.C. Stehouwer, and K.E. McDonald. 2011. Nutrient leaching and switchgrass growth in mine soil columns amended with poultry manure. Soil Science 176:84-90.

DOI. 2012. Reclaiming abandoned mine lands. Office of Surface Mining Reclamation and Enforcement, US Department of the Interior: Washington, D.C. Available online http://www.osmre.gov/aml/aml.shtm (verified March 28, 2012).

Gee, G.W. and D. Or. 2002. Methods of Soil Analysis. Part 4- Physical Methods. SSSA Book Series 5. In J. Dane, & G. Topp (Eds.), Particle Size Analysis. Soil Science Society of America, Inc.

Gillman, G.P. and E.A. Sumper. 1986. Modification to the compulsive exchange method for measuring exchange characteristics of soils. Australian Journal of Soil Research. 24:61-66

Guo, M., J. Chorover, and R. Fox. 2001. Effects of spent mushroom substrate weathering on the chemistry of underlying soils. J. Environ. Qual. 30:2127–2134.

Guo, M., N. Tongtavee and M. Labreveux. 2009. Nutrient dynamics of field-weathered Delmarva poultry litter: implications for land application. Biology and Fertility of Soils 45:829-838.

Guo, M., Y. Sheng, and Z. He. 2012. Poultry litter-based biochar: preparation, characterization, and utilization. In Z. He (Ed.) Applied Research in Animal Manure Management: Challenges and Opportunities beyond the Adverse Environmental Impacts. Nova Science Publishers: Hauppauge, NY.

Guo, M., W. Song, and R. Kazda. 2012a. Fertilizer value of lime-stabilized biosolids as a soil amendment. Agron. J. 104:1679–1686.

Haering, K.C., W.L. Daniels, and S.E. Feagley. 2000. Reclaiming mined lands with biosolids, manures, and papermill sludges. In R. Barnhisel, R. Darmody, W.L. Daniels (ed) Reclamation of Drastically Disturbed Lands. pp.615-644. Agronomy Monograph 41. American Association of Agronomy: Madison, WI.

Harris, M.A. 2001. The effect of sewage sludge and green manure on the erodibility of two mine tailing materials. Environmental Geology 40:1367-1376.

Hass, A., J.M.Gonzales., I.M. Lima., H.W. Godwin., J.J. Halvorson, and D.G. Boyer. 2012. Chicken manure biochar as liming and nutrient source for Appalachian soil. Journal of Environmental Quality 41: 1096-1106.

Kundu, N.K. and M.K. Ghose. 1997. Soil profile characteristic in Rajmahal Coalfield area. Indian Journal of Soil and Water Conservation 25:28-32.

Lamey, F.J. and D.A. Angers. 2012. The role of organic amendments in soil reclamation: A review. Canadian Journal of Soil Science 92:19-38.

Lehmann, J., J.P. da Silva, C. Steiner, T. Nehls, W. Zech and B. Glaser. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant and Soil 249:343-357.

Lehmann, J., and S, Joseph. 2009. Biochar for environmental management: science and technology. Earthscan UK and US: London, UK

Lehmann, J., M.C. Rillig, J.Thies, C.A. Masiello, W.C. Hockaday and D.Crowley. 2011. Biochar effects on soil biota- A review. Soil Biology and Biochemistry 43: 1812-1836.

Maezato, Y., and P, Blum. 2012. Survival of the fittest: Overcoming oxidative stress at the extremes of acid, heat and metal. A review. Life 2: 229-242.

Marashi, A.R.A and J. Scullion. 2004. 13th International Soil Conservation Organisation Conference. Brisbane.

MonAme. 2009. Land Reclamation, Mongolian Citizen Guide. MonAme Scientific Research Center, Ulaanbaatar, Mongolia.

Munshower, F.F. 1994. Practical Handbook of Disturbed Land Revegetation. New York: Lewis.

NAAMLP. 2012. Hazards associated with abandoned mine lands. Available online http://naamlp.net/hazards.htm (Verified 25 April, 2012).

Murphy, J. and H.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Ana. Chim. Acta 27:31–36.

Navarrete-Tindall, N. E. and J. W. Van Sambeek. 2010. Evaluating poverty grass (*Danthonia spicata*) for golf courses in the Midwest. *USGA* Turfgrass and Environmental Research Online (9):1-8.

Penn State Agricultural analytical service lab. Soil test results and recommendations by county, Agronomic samples (2012-2013). http://agsci.psu.edu/aasl/soil-testing/soil-fertility-testing/handbooks/agronomic/summaries/2012-2013/average-soil-test-results-and-recommendations-by-state-county/view (Verified 25 October 2013)

Penn State University Climatologist website. 2012. Climate data for Clearfield country, Pennsylvania. http://climate.met.psu.edu/www_prod/data/city_information/index (Verified 20 September, 2012)

Redente, E.F., T. Mclendon, and W. Agnew. 1997. Influence of topsoil depth on plant community dynamics of a seeded site in northwest Colorado. Arid Soil Research and Rehabilitation 11:139-149.

Reed, D. 2007. Economic and societal benefits of soil carbon management: policy implications and recommendations. In J.M. Kimble (Ed.) Soil Carbon Management: Economic, Environmental and Societal Benefits. CRC Press: Boca Raton, FL.

Riley, J.D., D.L. Rimmer, and D. Brignall. 2003. Restoration of a magnesian limestone grassland community on former quarry sites. In H.M. Moore, R.H. Fox, and S. Elliott (ed.) Proceedings of the 7th International Conference of the International Affiliation of Land Reclamationists. pp. 243-249. Runcorn, UK.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY.

Schloss, P.D. and J. Handelsman. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Applied Environmental Microbiology 71:1501-1506.

Schnell, R.W., D.M. Vietor., T.L. Provin., C.L.Munster. and S. Capareda. 2012. Capacity of biochar application to maintain energy crop productivity: Soil chemistry, sorghum growth, and runoff water quality effect. Journal of Environmental Quality 41: 1044-1051.

Schomberg, H.H., J.W. Gaskin., K. Harris., K.C. Das., J.M. Novak., W.J. Busscher., D.W.Watts., R.H.Woodroof., I.M. Lima., M. Ahmedna., D.Rehrah, and B. Xing. 2012. Influence of biochar on nitrogen fractions in a coastal plain soil. Journal of Environmental Quality 41:1087-1095.

Schuman, G.E., E.M. Taylor, F. Rauzi, and B.A. Pinchak. 1985. Revegetation of mined land: Influence of topsoil depth and mulching method. Journal of Soil and Water Conservation 40:249-252.

Sharma P.D. and F.S. Carter. 1996. Compaction effects on mineland soil quality. 13th Annual Meetings of American Society of Surface Mining and Reclamation. Knoxville, TN.

Sheoran, V., A.S. Sheoran, and P. Poonia. 2010. Soil reclamation of abandoned mine land by revegetation: A review. International Journal of Soil, Sediment and Water 3/2/13.

Shrestha, R. and R. Lal. 2006. Ecosystem carbon budgeting and soil carbon sequestration in reclaimed mine soil. Environmental International 32:781-796.

Song, W. and M. Guo. 2012. Quality variations of poultry litter biochars generated at different pyrolysis temperatures. Journal of Analytical and Applied Pyrolysis 94:138–145.

Sopper, W.P. 1992. Reclamation of mine land using municipal sludge. Advances in Soil Science 17:351-431.

Sparks, D.L. (1996). Methods of Soil Analysis. Part 3 - Chemical Methods. SSSA Book Series 5. Soil Science Society of America, Inc.: Madison, WI.

Spokas, K.A. 2010. Review of the stability of biochar in soils: predictability of O:C molar ratios. Carbon Management 1:289-303.

Streubel, J.D., H.P. Collins, M. Garcia-Perez, J. Tarara, D. Granatstein and C.E. Kruger. 2011. Influence of contrasting biochar types in five soils at increasing rates of application. Soil Science Society of America Journal 75:4 : 1402-1413.

Sydnor W.M.E. and E.F. Redente. 2002. Reclamation of high-elevation, acidic mine waste with organic amendments and topsoil. Journal of Environmental Quality 31:1528-1537.

Troeh, F.R., J.A. Hobbs, and R.L. Donahue. 2004. Soil and Water Conservation for Productivity and Environmental Protection (4th ed.). Prentice Hall, Upper Saddle River, NJ.

United States Environmental Protection Agency. 2008. Drinking Water Contaminants. [Online] Available at: http://www.epa.gov/safewater/contaminants/index.html#listmcl (Verified 22 October 2013).

University of Delaware Extension center. 2013. Recommended soil test procedures for Northeastern United States. http://extension.udel.edu/lawngarden/files/2012/10/CHAP10.pdf (Verified 23.October 2013).

Vogel, W.G. 1980. Revegetating surface-mined lands with herbaceous and woody species together. General Technical Report No. NE-61. Forest Service, United States Department of Agriculture: Washington, D.C.

Vogel, W.G. 1981. A Guide for Revegetating Coal Mine Soils in the Eastern United States. General Technical Report NE-68. Forest Service, United States Department of Agriculture: Washington, D.C.

Wick, A.F., S.D. Merrill, T.J. Toy, and M.A. Liebig. 2011. Effect of soil depth and topographic position on plant productivity and community development on 28-year-old reclaimed mine lands. Journal of Soil and Water Conservation 66:201-211.

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