Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.10, 2014

The Efficacy of compost, limestone and growth of *Leucaena leucocephala* (Lam.) de wit, *Senna siamea* (Lam.) and *Eucalyptus grandis* W. Hill ex Maid. for the restoration of bacterial functional diversity in the rhizosphere in copper tailings and pyrite soils

Jamilu Edirisa Ssenku[1*] Muhammad Ntale[2] Hannington Oryem-Origa[1]

1. *Department of Biological Sciences, College of Natural Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda*
2. Department of Chemistry, College Natural Sciences, Makerere University, *P. O. Box 7062, Kampala, Uganda*

*Email of the corresponding author: jssenku@gmail.com

Abstract

The potential of compost, limestone and growth of *Leucaena leucocephala* (Lam.) de wit, *Senna siamea* (Lam.) H.S Irwin & Barneby, and *Eucalyptus grandis* W. Hill ex Maid for the restoration of rhizospheric bacterial functional diversity of pyrite soil and copper tailings was assessed in the field. Pyrite soil and copper tailings were amended with limestone, compost followed by planting of experimental trees after homogenisation. The experimental setup was of split block design with site as a blocking factor; amendment application and growth of trees as the treatment factors. After 12 months of growth, background and rhizospheric pyrite and copper tailings were sampled and their physico-chemical characteristics analysed. The Community Level Physiological Profiles (CLPP) of the same samples were determined using Biolog EcoPlate™. The functional diversity was assessed from the Biolog data using various indices including Average Well Colour Development (AWCD), substrate richness (S) and Shannon-Wiener index (*H*).

Background and rhizospheric untreated pyrite and copper tailings were extremely acidic, with low organic matter content, available phosphorous, total nitrogen, and relatively higher concentrations of available heavy metals and low bacterial functional diversity. Application of amendments and growth of the tree species effectively increased the pH, organic matter content, available phosphorous, total nitrogen, growth of understory plant species, bacterial functional diversity and lowered the available concentrations of heavy metals. AWCD, bacterial species diversity and richness were higher in rhizospheres of leguminous tree species than the non leguminous *Eucalyptus grandis*, suggesting the suitability of the former for remediation of pyrite and copper tailings.

Keywords: efficacy, compost, restoration, bacterial functional diversity, rhizosphere, Biolog Ecoplate™

1. Introduction

Copper mining activities in Kilembe that lasted for close to 30 years from 1956 to 1982 generated 1.13 million metric tonnes of cobaltiferous pyrite wastes which were stockpiled near Kasese town 11 Km east of the mines (Oryem-Origa *et al*. 2007). Flotation tailings to the tune of 15 million metric tonnes from the mines were dumped in four different areas in Kilembe valleys in which the fast flowing River Nyamwamba is located (Muwanga *et al*. 2009). After definitive closure of the mines in 1982 both the cobaltiferous stockpile and the tailings dams were abandoned. They consequently inflicted a wide range of catastrophic environmental impacts in parts of Queen Elizabeth Conservation Area (QECA) and the environs of the tailings dams respectively, through heavy metal pollution and acid mine drainage. Soil contamination with heavy metals is currently the most serious environmental hazard in the area, which according to Lin *et al.* (2010) is very costly to remediate.

Heavy metal pollution exerts a negative impact on soil microbial activity, which greatly alters nutrient cycles (Valsecchi *et al*. 1995). They negatively affect microbial activity and growth by damaging proteins and/or by disrupting cell membranes (Leita *et al*. 1995). Soils contaminated with heavy metals associated with mining activities usually lack a proper structure and aeration and have low fertility and organic matter (OM) content that result in a low microbial biomass in these soils (Clemente *et al*. 2006), together with poor plant growth potential. It is generally accepted that accumulated heavy metals reduce the amount of soil microbial biomass (Brookes and McGrath 1984; Chander *et al*. 1995) and various enzyme activities, leading to a decrease in the functional

diversity in the soil ecosystem (Kandeler *et al.* 1996) and changes in the microbial community structure (Frostegard *et al.* 1993; Pennanen *et al.* 1998). Many microbial processes are affected by heavy metal contaminants (Leyval *et al.* 1997) including carbon and nitrogen mineralisation (Doelman *et al.* 1986), general organic matter turnover rates (Chandler and Brookes 1991) (Chandler 1991) heterotrophic and autotrophic nitrogen fixation (Brookes *et al.* 1986). Since heavy metals are non-biodegradable, their presence in the soil presents a permanent threat to the functioning of soil ecosystem, most likely through the disturbance of the microbial community (Jia *et al.* 2013).

Microbial activities and diversity are crucial components of ecosystem function, as they are important driving forces between biological, physical and geochemical systems (Finlay *et al.* 1997). The productivity of soil system is known to depend greatly upon the structure and functions of soil microbial communities, which regulate and influence many ecosystem processes such as nutrient transformation, litter decomposition, soil structure and plant health (Garllardo and Schlesinger 1994; Kennedy 1999; Zak *et al.* 2003; Garbeva *et al.* 2004). The synergistic impacts of plant-associated bacteria in a phytoremediation process have been reported by different authors. Several of the plant-associated bacteria can play a significant role in accelerating phytoremediation in heavy metal-contaminated soils by promoting plant growth and health (Ma *et al.* 2009; Compant *et al.* 2010; Dary *et al.* 2010). Rhizosphere microbial communities carry out fundamental processes that contribute to nutrient cycling and plant growth (Wang *et al.* 2007). The rhizosphere microbial community is an important factor in determining the survival and sustainable growth of plants (Zhang *et al.* 2011). Therefore, restoration of heavy metal-contaminated environments requires a functional microbial community for successful plant community establishment, soil development, and biogeochemical cycling (Moynahan *et al.* 2002), yet they are non-existent in such environments. This rendered development of cost effective and ecologically sound techniques for bacterial functional diversity revitalization in phytoremediation paramount.

In the current study, the focus was on testing locally available limestone, compost from domestic organic food wastes and locally growing selected tree species. Organic amendments have been found to immediately decrease heavy metal bioavailability, provide a slow release of mineral nutrients and to serve as a microbial inoculum (Mendez *et al.* 2007). Lime application can improve soil pH and ultimately the reestablishment of vegetation (Oryem-Origa *et al.* 2007) and recolonization of mine waste polluted habitats. It is well known that different plant species can associate with microbial communities with unique characteristics (Chen *et al.* 2002; Viketoft *et al.* 2005), probably due to differences in amount and quality of root exudates (Nguyen 2003). It was hoped that the growth of particular tree species would re-enforce the recolonization of the polluted soils by different bacterial species through the production of root exudes that attract them. One localized leguminous tree species *Leucaena leucocephala* (Lam.) de wit. (Family Fabaceae), one exotic leguminous tree species *Senna siamea* (Lam.) H.S Irwin & Barneby (Family Fabaceae) and one non leguminous timber tree species *Eucalyptus grandis* W. Hill ex Maid (Family Myrtaceae) were experimented on in the field to assess their effectiveness in boosting bacterial functional diversity. *Leucaena leucocephala* is a legume capable of enhancing nitrogen fixation hence improving soil fertility of the nutritionally impoverished tailings and polluted soils. *Eucalyptus grandis* is known to exhibit great environmental plasticity with ability to grow in impoverished soils (Arriagada *et al.* 2004) while *Senna siamea* is a non nodulating woody legume (Fagbola *et al.* 1998), growing vigorously within the environmental settings of the area, tolerant to both limestone and moderately acid soils (Hossain 1999) and capable of growing on degraded infertile soils (Jøker 2000). Despite use of trees in aided phytoremediation gaining popularity, their contribution to microbial functional diversity restoration is not known. We aimed at assessing the changes in the physico-chemical characteristics of pyrite soils and copper tailings and bacterial functional diversity following amendment application and growth of the selected tree species.

2. Materials and Methods

2.1 Description of study site

The study area comprised of the pyrite trail in Queen Elizabeth Conservation Area (QECA) located at the geographical coordinates of latitude 0° 8'53.03"N, longitude 30° 4'27.53"E and altitude of 949 meters above sea level and the four tailings dams in the vicinity of Kilembe Town area located at latitude of 0°11'16.12"N, longitude of 30° 1'11.43"E and altitude of 1243 meters above sea level (Figure 1).

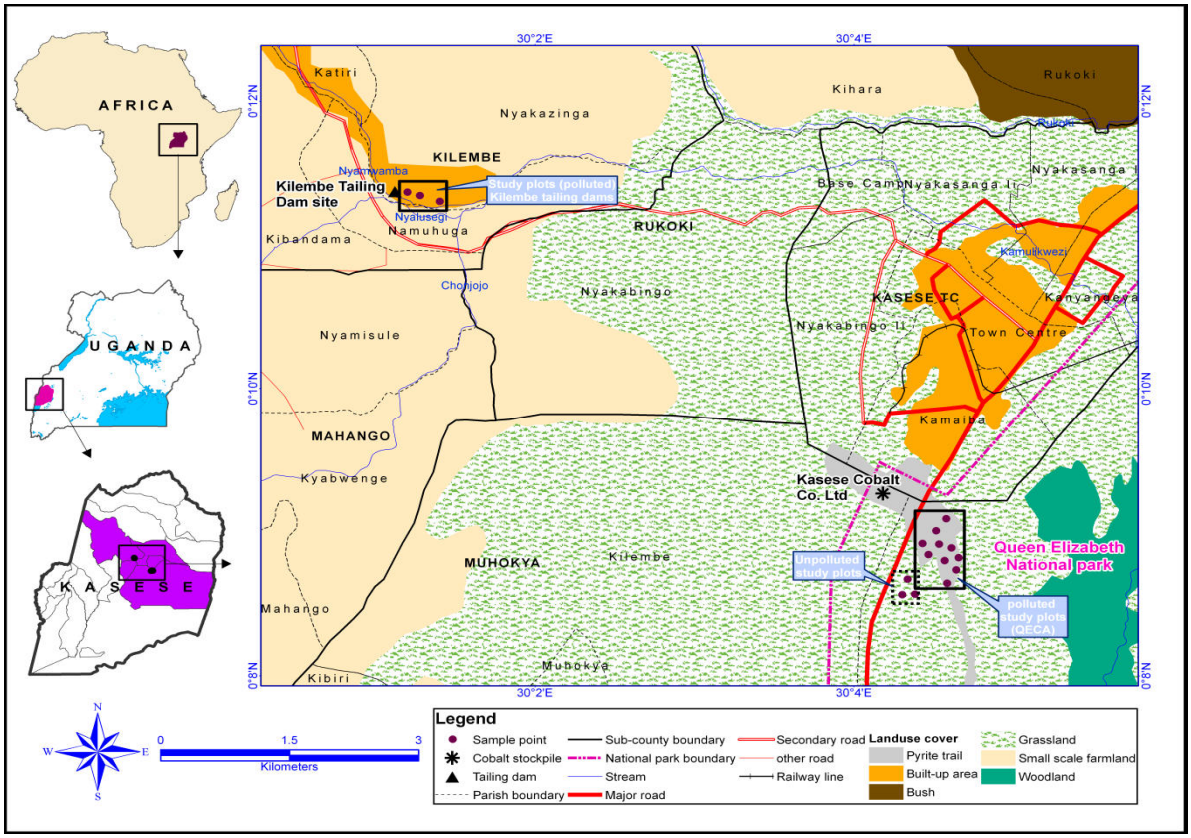

Figure 1. Location of the experimental sites at the tailings dams in Kilembe and the pyrite trail in QECA

It experiences a tropical climate with rainfall which is bi-modally distributed with the wetter periods occurring from March to May and August to November. During the study period the temperatures for pyrite trail site showed mean minimum temperature of 18.5 ^{0}C and a mean maximum temperature of 30.8 ^{0}C. Records of temperatures for the Kilembe tailings dams were not available, but being located at higher altitude it is always cooler than the pyrite trail site. The tailings dams are flattened at the top, characterised by longitudinal rows of depressions and elevations that were formed during the dumping process and gullies formed as result of water erosion. The flattened top is characterised on the surface with very fine polluted powdery soils that are easily transferred into nearby gardens and River Nyamwamba by eolian dispersal during the dry season. The pyrite trail is characterised by bare patches dotted with shrubs of *Capparis tormentosa* Lam., tree species of *Acacia gerrardii* Benth and *Balanites aegyptiaca* (L.) Del. and islets of vegetation composed of *Phytolacca dodecandra* L Hérit, *Fimbristylis ferruginea* (L.) Vahl, *Imperata cylindrica* (L.) P.Beauv, *Sporobolus pyramidalis* P. Beauv., *Typha latifolia* L. *Cynodon dactylon* covers most of the regenerated part of the pyrite trail. The surrounding vegetation consists largely of *Acacia* savannah woodland.

2.2 Experimental design

The study area was categorised into four study sites coded as Kilembe tailing dams site (KTDS), low polluted pyrite trail site (LPPTS), highly polluted pyrite trail site (HPPTS) and unpolluted site (UPS). The categorisation was based on the results of the baseline geochemical survey of the eight zones that were mapped out covering the entire study area. A split block experimental design was used with site as a blocking factor and amendment types categorised into unamended (UA), limestone (LS), compost (Comp) and limestone+compst (LS+Comp) and the tree species grown as treatment factors. Establishment of plots, amendment application in the sub-plots and planting of experimental tree species was done as per the description of Ssenku *et al.* (2014).

2.3 Soil sampling technique

After one year of amendment application in November 2012, three separate collections of soil samples were made. Each soil sample was collected from the rhizosphere of particular species growing under a given treatment (sub-plot). From each sub-plot three sub-soil samples were collected from the rhizosphere of three randomly selected plants from the interior of a sub-plot using a sterile trowel. The subsamples were thoroughly mixed to

form a composite sample for a particular sub-plot. Approximately 10g of the composite sample was transferred into an autoclaved MacKateney bottle that was immediately sealed off and transferred to a cold box maintained at 4^0C to avoid any changes in the microbial community structure. Upon completion of sampling from a particular sub-plot the trowel was sterilized by dipping it in 100 percent ethanol and heated strongly. The remaining portion of the composite sample was packed in a polythene bag and taken to the laboratory for physico-chemical characterization.

2.4 Physico-chemical characterization of rhizosphere soil samples

Physico-chemical characterisation of the soil samples was done at National Agricultural Research Laboratories (NARL) at Kawanda following standard procedures. Soil pH (soil: deionised water=1:2.5 w/v) was determined with a calibrated pH meter (Orion 550 Bench pH meter) and organic matter content by Walkley-Black potassium dichromate wet oxidation (Nelson and Sommers 1982) as described in Okalebo *et al*. (2002) while total nitrogen by the semi-micro Kjeldahl method (Bremner and Mulvaney 1982). Extraction of available phosphorous and heavy metals was done using Mehlich 3 extractant. The available phosphorous in the extract was determined following Ammonium Molybdate-Ascorbic acid method (Knudsen and Beegle 1988) using a UV/Visible spectrophotometer at 860 nm while available heavy metal concentrations by using an atomic absorption spectrophotometer (SHIMADZU AA-6800).

2.5 Determination of functional diversity of bacterial communities in the rhizosphere of selected tree species

The pattern of potential carbon source utilization by soil microbial communities under different amendments and plant species was assessed by Biolog Ecoplates^{TM} system containing triplicates of 31 different environmentally relevant carbon sources and control well (Biolog Inc., CA, USA, (Choi and Dobbs 2003; Douterelo *et al*. 2010; Chakraborty *et al*. 2011). The wells also contain an indicator substance; tetrazolium dye, that changes colour with substrate consumption. The reduction of the tetrazolium dye due to cell respiration turns the contents of the wells purple. The Biolog Ecoplates^{TM} are based on the capacity of bacteria to utilize different substrates and thus leaving a metabolic fingerprint providing information on functional biodiversity in the soil over time (Preston-Mafham *et al.* 2002). The analysis was performed with the protocols described by (Classen *et al.* 2003). In brief, a soil suspension was prepared by votexing 1.5 g of soil (on dry weight basis) in 15 ml of sterile phosphate buffered saline (BPS) and allowed to settle for 2 hours. The supernatant was serially diluted to obtain 10^{-3} dilution which was used to inoculate the Biolog Ecoplates^{TM}. Aliquots of 150 µl of the 10^{-3} dilution for each sample was inoculated to each well of Biolog Ecoplate using multi 8-channel pipette and later incubated in oven at 28^0C. Colour development of each well was measured as absorbance (A) at 590 nm with the Biolog microplate reader (BioTeK ELx800) at 24 hour intervals for until 168 hours.

2.6 Ecoplate data analysis

Individual absorbance values of the 31 single substrates were corrected by subtraction of the blank control value (raw difference). Well optical density values that were negative or under 0.06 were adjusted to zero (Classen *et al.* 2003). To minimize the effects of different inoculum densities, data were normalized by dividing the raw difference values by their respective average well colour development (AWCD) values. The number of active wells on a given plate was determined by quantification of the number of positive wells (>0.06 absorbance units above the time zero reading) in each sample (Li *et al.* 2011). The microbial activity in each microplate was expressed as the Average Well Colour Development (AWCD) that was determined using the expression below (Frac *et al.* 2012):

$$AWCD = \sum \frac{OD_i}{31} \quad (1)$$

Where OD_1 is the optical density value from each well. The Shannon-Wiener Diversity Index (H) was calculated using the OD of 0.06 as threshold for positive response using the following expression (Harch *et al.* 1997; Yan *et al.* 2000; Fowler *et al.* 2006; Frac *et al.* 2012).

$$H = -\sum_{i=1}^{N} p_i \ln p_i \quad (2)$$

Where p_i, in this case, is the proportion of AWCD of a particular substrate to the AWCD of all substrates of a particular sub-plot.

2.7 Quality assurance

Prior to use, all solutions, transfer equipment, and glassware were sterilized with an autoclave. Weighing of soil samples, serial dilutions and plate inoculation was done under a laminar-flow hood to minimize the risk of contamination from the surrounding. For heavy metal analysis, the glassware used was thoroughly cleaned and all the reagents were of analytical grade. Double distilled water was used throughout the analysis.

2.8 Data analysis

All analyses were performed using R statistical package 2.13.2 (R Development Core Team 2011) and STATA 9.0. Prior to statistical analysis, data distributions were checked for normality and homogeneity of variances. Data with strong deviations from the normal distribution and/or that were heteroscedastic were log-transformed. Analysis of variance (ANOVA) was used to evaluate the effects of treatments and site on Community Level Physiology Profiles (CLPP) followed by separation of means by a post-hoc test (Tukey's Honest Significant Multiple Comparison) with means considered to be significantly different at $p < 0.05$. In addition, Principal Component Analysis (PCA) on correlations matrix of substrate utilization patterns was carried out to cluster the microbial status of the different soil types. Leguminous tree species were each compared with non-leguminous tree species with respect to AWCD, substrate diversity (Shannon-Wiener) and substrate richness (S) using the student t-test of paired samples.

3. Results

3.1 Soil physico-chemical characteristics

The physico-chemical characteristics of soils varied widely across the study area. In background and unamended copper tailings and pyrite soils the pH values were very low ranging between 1.9 and 4.5 (Table 1). Application of amendment materials significantly raised the pH values (Tukey's HSD test, $p<0.05$) to a range of 4.6 to 8.3 that was relatively higher than that of unpolluted soils. Organic matter content, total nitrogen and available phosphorous were also generally lower in untreated and unamended pyrite soils and copper tailings. These improved upon treatment for all tree species and at all sites most especially with the compost and limestone+compost amendment application. Application of compost to pyrite soils and copper tailings led to a significant rise in available phosphorous at all sites and under tree species grown (Tukey's HSD test, $p<0.05$) to levels that were higher than those of unpolluted soils. With the exceptional case of *Eucalyptus grandis* plot at LPPTS and *Senna siamea* at HPPTS compost amendment lead to a significant rise in total nitrogen while for organic matter the rise was significant for all study plots (Tukey's HSD test, $p<0.05$). A regression analysis of organic matter content with total nitrogen and available phosphorous revealed their dependence on organic content ($R^2=0.61$, $p<0.001$) and ($R^2=0.23$, $p<0.01$) respectively (Figure 2).

Table 1. Physico-chemical characteristics of background and rhizospheric soils. BK-Background soils, UA=Unamended, LS=Limestone, Comp=Compost and LC=Limestone+compost, KTDS=Kilembe tailings dams site, LPPTS= Low polluted pyrite trail site and HPPTS=Highly polluted pyrite trail site

Site/Tree species	Treatment	pH	OM(%)	Total N (mgkg^{-1})	Available P (mgkg^{-1})	Melich 3 extractable heavy metal conc. (mg kg^{-1}) Cu	Co	Ni	Pb
KTDS/*E. grandis*	BK	4.4±0.12**b**	1.9±0.10**b**	0.19±0.04**ab**	4.37±0.98**c**	25.17±2.19**a**	20.41±1.87**e**	8.65±0.98**a**	2.52±0.23**c**
	UA	3.8±0.05**b**	2.7±0.06**ab**	0.02±0.00**ab**	3.0±0.25**c**	18.78±2.25**ab**	11.49±1.41**d**	4.68±0.09**b**	1.05±0.07**b**
	LS	6.5±0.15**a**	2.8±0.04**ab**	0.13±0.03**b**	28.3±9.87**b**	17.95±4.84**b**	5.59±0.34**c**	1.71±0.04**c**	0.08±0.10**b**
	Comp	5.1±0.30**a**	4.47±0.13**a**	0.34±0.02**a**	60.9±3.76**a**	10.31±2.81**ab**	9.55±0.22**a**	1.89±0.14**c**	0.32±0.07**a**
	LC	7.5±0.21**a**	2.9±0.06**ab**	0.16±0.09**ab**	56.27±3.10**a**	15.16±1.56**b**	1.31±0.03**b**	1.51±0.07**c**	1.10±0.17**b**
KTDS/*L. leucocephala*	BK	4.2±0.13**c**	4.0±0.11**b**	0.18±0.04**c**	7.37±1.31**c**	10.44±0.59**a**	9.49±1.42**a**	5.32±0.29**a**	1.90±0.08**a**
	UA	4.5±0.29**c**	4.7±0.48**b**	0.23±0.02**bc**	3.12±0.01**c**	11.55±1.20**a**	6.10±1.08**a**	3.61±0.34**a**	1.96±0.21**a**
	LS	8.3±0.23**b**	4.6±0.55**b**	0.23±0.03**bc**	23.61±1.01**b**	5.59±0.94**a**	4.99±0.62**a**	2.77±0.22**a**	trace
	Comp	6.4±0.30**a**	7.0±0.47**a**	0.32±0.03**a**	72.05±4.10**a**	5.26±1.37**a**	3.88±0.12**a**	4.69±0.78**a**	trace
	LC	7.4±0.52**ab**	5.6±0.41**ab**	0.26±0.01**ab**	65.56±2.27**a**	6.99±0.47**a**	4.16±0.13**a**	4.27±0.58**a**	trace
KTDS/*Senna siamea*	BK	4.3±0.57**c**	4.5±0.05**b**	0.17±0.02**a**	4.03±0.13**c**	9.89±2.02**b**	6.78±1.55**a**	4.98±0.26**a**	2.35±0.189**a**
	UA	4.6±0.00**c**	0.96±0.12**c**	0.04±0.01**d**	2.4±0.27**c**	5.04±0.11**a**	4.57±0.33**ab**	4.40±0.37**a**	2.00±0.88**a**
	LS	7.5±0.25**b**	4.7±0.31**ab**	0.28±0.01**c**	24.73±0.98**b**	4.69±0.42**a**	4.01±0.36**b**	3.52±0.21**a**	trace
	Comp	6.2±0.15**a**	5.8±0.18**a**	0.49±0.06**a**	67.90±6.29**a**	3.66±0.62**a**	6.35±1.19**ab**	2.98±0.44**a**	0.30±0.05**b**
	LC	7.9±0.31**b**	5.3±0.15**ab**	0.40±0.03**a**	43.07±4.05**a**	5.08±0.28**a**	5.14±0.70**ab**	1.97±0.06**a**	trace
LPPTS/*E. grandis*	BK	3.3±0.02**c**	3.4±0.11**a**	0.15±0.04**a**	7.00±0.45**c**	21.80±4.63**b**	30.55±5.69**c**	3.22±0.08**a**	3.90±0.85**c**
	UA	2.9±0.14**c**	2.5±0.13**b**	0.17±0.01**a**	3.7±0.03**c**	17.46±1.13**b**	27.12±6.49**c**	6.67±0.67**b**	1.47±0.21**b**
	LS	7.4±0.26**b**	3.5±0.05**a**	0.18±0.02**a**	51.8±6.03**b**	6.20±0.26**a**	12.86±0.53**ab**	3.78±0.48**a**	trace
	Comp	4.6±0.13**a**	5.3±0.28**a**	0.24±0.02**a**	104.4±11.22**a**	9.20±0.26**a**	8.41±0.69**a**	2.73±0.29**a**	0.43±0.03**a**
	LC	7.4±0.15**b**	4.8±0.18**a**	0.23±0.03**a**	44.2±5.60**b**	10.25±0.49**a**	19.94±3.06**bc**	4.38±0.68**a**	trace
LPPTS/*L. leucocephala*	BK	2.9±0.07**c**	1.7±0.12**b**	0.08±0.01**e**	7.00±0.60**d**	57.79±8.28**b**	70.17±9.82**c**	6.12±0.73**c**	1.76±0.02**b**
	UA	4.7±0.21**b**	1.2±0.06**b**	0.11±0.01**d**	7.21±0.20**d**	26.43±0.21**bc**	14.18±1.15**b**	5.06±0.59**c**	1.38±0.04**b**
	LS	7.8±0.21**a**	1.6±0.20**b**	0.18±0.01**c**	16.97±1.23**c**	15.46±0.91**ac**	12.72±0.52**b**	2.75±0.18**ab**	0.27±0.06**a**
	Comp	7.4±0.50**a**	3.2±0.02**a**	0.35±0.01**a**	163.08±2.23**a**	8.10±0.26**a**	12.93±2.65**b**	3.54±0.57**b**	0.44±0.12**a**
	LC	7.9±0.43**a**	1.5±0.01**b**	0.33±0.01**b**	48.28±3.00**b**	17.33±0.31**ab**	4.95±0.758**a**	1.27±0.00**a**	trace

Site/Tree species	Treatment	pH	OM(%)	Total N (mgkg^{-1})	Available P (mg kg^{-1})	Melich 3 extractable heavy metal conc. (mg kg^{-1}) Cu	Co	Ni	Pb
LPPTS/*Senna siamea*	BK	3.5±0.10**c**	3.9±0.12**c**	0.20±0.05**c**	7.90±1.30**d**	17.82±2.38**d**	37.02±4.14**c**	5.27±0.29**a**	1.49±0.19**bc**
	UA	3.1±0.11**c**	8.8±0.94**b**	0.29±0.02**c**	1.35±0.02**e**	14.26±0.18**c**	48.73±0.75**b**	4.74±0.34**a**	1.07±0.37**ac**
	LS	7.0±0.34**b**	8.7±0.77**b**	0.38±0.03**b**	43.40±3.51**b**	4.35±0.48**b**	8.31±0.63**a**	3.65±0.64**a**	1.09±0.18**b**
	Comp	5.9±0.30**a**	13.6±0.20**a**	0.57±0.02**a**	86.7±3.53**a**	5.86±0.55**ab**	5.72±0.65**a**	3.07±0.54**a**	0.30±0.09**a**
	LC	7.6±0.05**b**	9.3±0.85**b**	0.34±0.06**b**	26.0±1.08**c**	8.38±1.00**a**	10.54±1.06**a**	3.56±0.33**a**	1.30±0.08**bc**
HPPTS/*E. grandis*	BK	1.9±0.00**b**	2.4±0.13**b**	0.15±0.05**bc**	5.63±3.11**c**	15.12±2.37**b**	67.73±9.45**b**	7.89±1.18**a**	3.43±0.45**b**
	UA	2.3±0.25**b**	1.3±0.00**b**	0.11±0.00**c**	2.59±0.11**c**	15.13±0.70**b**	24.42±4.68**a**	6.38±0.44**a**	1.29±0.09**b**
	LS	7.4±0.10**a**	1.4±0.02**b**	0.10±0.03**c**	26.12±3.16**b**	8.17±0.70**a**	15.63±1.06**a**	5.53±0.48**a**	0.40±0.10**a**
	Comp	7.1±0.21**a**	6.8±0.19**a**	0.28±0.02**a**	129.1±10.00**a**	9.10±0.14**a**	19.49±1.67**a**	5.94±0.52**a**	0.77±0.03**a**
	LC	7.4±0.21**a**	3.9±0.14**b**	0.19±0.03**b**	93.21±4.59**a**	9.40±1.59**a**	17.68±1.58**a**	8.06±1.01**a**	1.00±0.01**a**
HPPTS/*L. leucocephala*	BK	2.3±0.12**b**	1.3±0.04**ab**	0.10±0.03**b**	4.46±0.13**c**	82.29±4.18**d**	90.51±0.17**b**	17.55±3.07**c**	0.56±0.17**a**
	UA	2.6±0.17**b**	2.1±0.12**ab**	0.12±0.02**b**	3.16±2.44**c**	70.20±0.12**c**	41.76±3.61**a**	9.73±0.75**b**	0.63±0.12**a**
	LS	8.1±0.17**a**	1.1±0.05**b**	0.11±0.01**b**	17.78±0.62**c**	6.76±1.11**b**	20.66±0.07**a**	4.95±0.27**a**	0.30±0.01**a**
	Comp	6.1±0.60**a**	4.1±0.06**a**	0.22±0.03**a**	234.3±29.03**a**	16.76±2.90**a**	6.42±0.73**a**	6.67±2.72**ab**	0.46±0.14**a**
	LC	7.5±0.21**a**	3.0±0.05**ab**	0.17±0.02**ab**	154.21±2.79**b**	15.16±1.25**a**	19.36±1.11**a**	5.26±0.09**ab**	0.30±0.05**a**
HPPTS/*Senna siamea*	BK	2.4±0.05**c**	2.1±0.11**b**	0.11±0.04**a**	3.33±0.45**d**	32.91±6.10**a**	187.9±10.43**c**	29.71±2.42**b**	2.65±0.32**b**
	UA	4.9±0.51**a**	1.5±0.14**a**	0.11±0.01**a**	3.6±0.09**d**	53.50±4.60**c**	31.63±1.06**ab**	15.33±4.38**a**	0.63±0.11**a**
	LS	7.7±0.49**b**	2.1±0.17**a**	0.12±0.01**a**	19.98±0.92**c**	50.00±1.05**c**	24.53±0.55**b**	12.10±0.81**a**	0.40±0.02**a**
	Comp	5.8±0.10**a**	5.7±1.21**a**	0.12±0.04**a**	129.98±1.62**a**	33.50±0.53**a**	45.81±2.78**a**	8.42±0.75**a**	0.50±0.01**a**
	LC	7.7±0.05**b**	2.9±0.14**a**	0.11±0.01**a**	53.51±2.57**b**	20.47±3.68**b**	21.95±2.53**b**	7.79±0.96**a**	0.67±0.15**a**
Unpolluted									
E.grandis		6.3±0.10	11.5±0.98	0.34±0.03	86.6±2.78	6.90±0.36	trace	4.39±0.38	trace
L. leucocephala		6.4±0.12	7.9±0.09	0.46±0.06	93.6±9.65	7.17±1.36	2.63±0.66	trace	trace
Senna siamea		6.20±0.00	7.9±0.75	0.38±0.01	80.59±4.12	10.40±0.17	2.16±0.08	trace	trace

Means with different letters within a column for a particular species and site indicate significant difference between values at $p<0.05$; post hoc Tukey's HSD test.

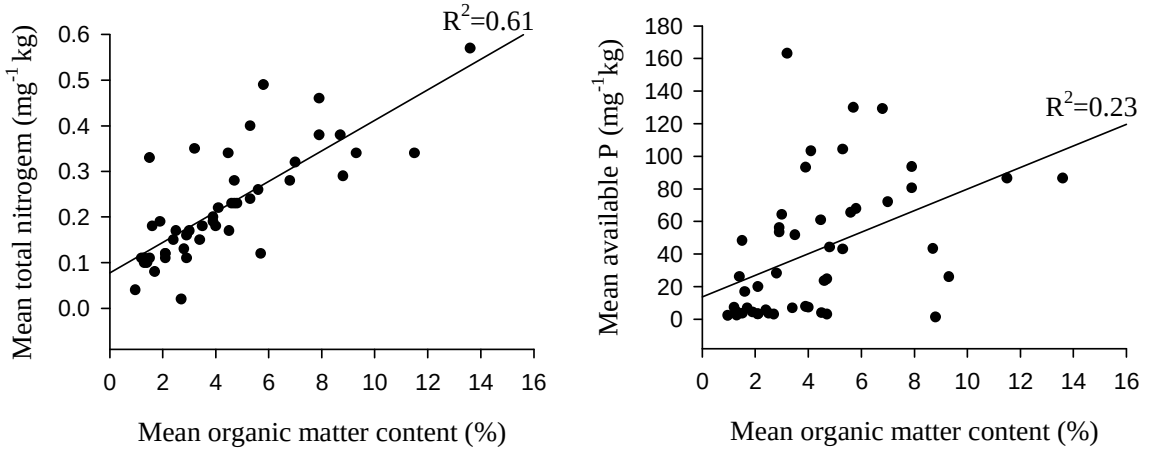

Figure 2. Relationship between organic matter content and total nitrogen and available phosphorous content

Background and unamended pyrite soils and copper tailings were characterised with significantly higher available concentrations of heavy metals at all polluted sites as compared to the unpolluted soils (Tukey's HSD test, $p<0.05$). Amendment applications led to a reduction in their concentrations at all sites. The abundance of heavy metals in the entire study area was in the order of cobalt>copper>nickel>lead. However copper was more abundant than cobalt in the tailings at KTDS while cobalt was more abundant than copper in pyrite soils at both LPPTS and HPPTS.

3.2 Bacterial functional diversity

3.2.1Average well colour development (AWCD)

The microbial activity as expressed by AWCD varied significantly across amendments applied to the copper tailings and pyrite soils (ANOVA, $p<0.001$). Rhizospheric soil samples from the unpolluted site and amended copper tailings and pyrite soils showed higher microbial activity than those from background and unamended polluted sub-plots (Figure 3). Amongst the treated soils, the mean average well colour development for *Senna siamea* was in the range of 0.355±0.088 to 0.881±0.115 for compost and limestone+compost treated soils at LPPTS respectively. Amended soil samples from *Leucaena leucocephala* plots had AWCD in the range of 0.222±0.037 to 0.844±0.116 for limestone treated and compost treated soils at HPPTS and LPPTS respectively. The microbial activity of the treated soils for *Leucaena leucocephala* at the HPPTS was generally lower than that of their respective counterparts at the KTDS and LPPTS ((Tukey's HSD test, $p<0.05$). For *Eucalyptus grandis*, it ranged between 0.303±0.050 to 0.683±0.151 for compost treated and limestone+compost treated soils at LPPTS and KTDS respectively. The highest activity for treated soils of each tree species was higher than that shown by the unpolluted soils of their respective species but were not significantly different ($P>0.05$). The two soil samples with relatively much higher AWCD were from the plots of the two leguminous tree species *Senna siamea* and *Leucaena leucocephala* but there was no significant differences between the AWCD of the respective leguminous tree species and the non-leguminous *Eucalyptus grandis* (t-test, $P>0.05$). Similarly, there was no significant overall effect of tree species on AWCD (ANOVA, $F=1.98$, $p>0.05$), but site ($F=3.46$, $p<0.05$) and treatment ($F=17.71$, $p<0.001$) significantly affected it. The interaction of site*species ($F=1.36$, $p>0.05$), site*treatment ($F=0.61$, $p>0.05$) and species*treatment ($F=0.56$, $p>0.05$) didn't have significant effect on AWCD.

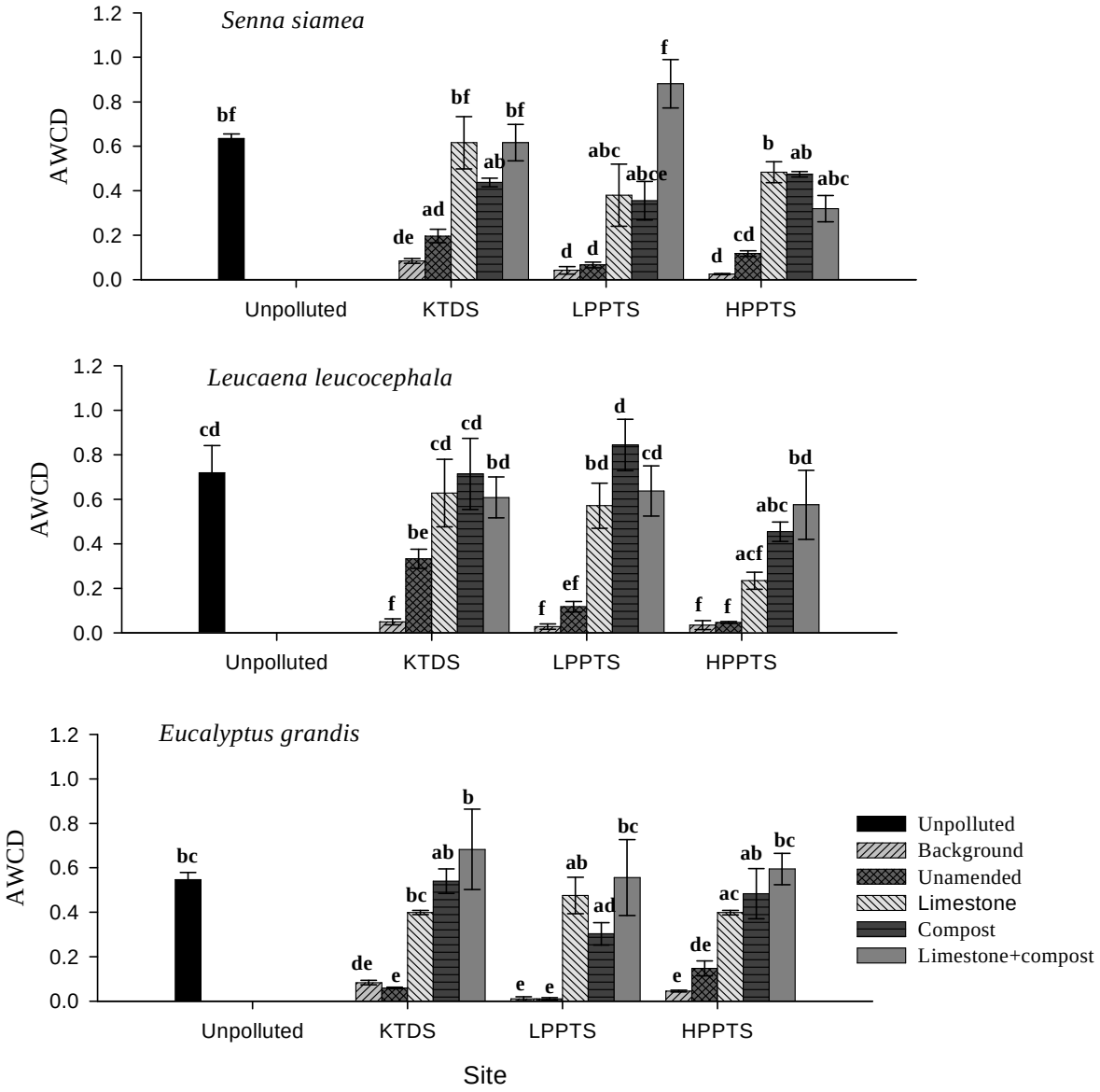

Figure 3. Mean Average Well Colour Development (n=3) measured at 96 hours for different soil samples. Bars followed with different letters are significantly different at $p<0.05$ (post hoc Tukey's HSD test).

3.2.2 Substrate diversity and richness

Bacterial species diversity as expressed by substrate diversity and richness were lower in the background and untreated soils at all sites (Table 2). Their treatment boosted them at all sites and for every species. For *Eucalyptus grandis* plots, diversity varied slightly and the variations were not significantly different at all sites. A similar trend is reflected for substrate richness. The unpolluted soils had the highest diversity of 2.850±0.04 but it was not significantly higher than the diversity of all the treated soils ($p>0.05$). Diversity was highest in treated soils at LPPTS for *Senna siamea* but not significantly higher than that of the unpolluted soils ($p>0.05$). However, substrate richness was higher in the unpolluted soils than in any other amended soils. The trend was similar with soils from *Leucaena leucocephala* plots but the highest value was observed in limestone+compost plots at KTDS and was higher than that of *Senna siamea.* Substrate richness was highest in unpolluted soils. Substrate diversity and richness for rhizospheric soils from the plots of leguminous tree species *Senna siamea* and *Leucaena leucocephala* were not significantly different (t-test, $p>0.05$), but significantly different for each leguminous species from those of non-leguminous *Eucalyptus grandis* (t-test, $p>0.05$) .

Table 2. Values (mean+SEM, n=3) Shannon-Wiener Diversity Index (*H*) and Substrate richness(S) calculated from Biolog data measured at 96 hours of incubation

Site	Treatment	Experimental tree species *Eucalyptus grandis*		*Senna siamea*		*Leucaena leucocephala*	
		H	S	*H*	S	*H*	S
KTDS	Back ground	1.375±0.12**bcd**	11±0.00**c**	1.212±0.17**cd**	9±1.15**ef**	1.099±0.04**c**	5±1.00**ef**
	Unamended	1.539±0.26**ce**	9±1.00**ce**	2.223±0.08**ab**	11±2.08**df**	2.453±0.08**ab**	13±1.52**dg**
	Limestone	2.426±0.12**ae**	18±2.65**ab**	2.963±0.07**b**	20±0.57**abc**	3.017±0.15**b**	22±2.00**ab**
	Compost	2.058±0.41**abe**	19±2.31**ab**	2.987±0.12**b**	22±2.00**abc**	2.823±0.22**b**	26±1.73**bc**
	LS+Comp	2.195±0.15ade	17±2.30**a**	2.740±0.544**b**	22±1.00**abc**	3.363±0.13**b**	23±1.53**ab**
LPPTS	Back ground	1.117±0.02**bc**	4±1.15**de**	0.735±0.02**c**	4±1.15**f**	0.772±0.27**c**	4±0.57**ef**
	Unamended	0.920±0.02**c**	3±0.57**d**	2.330±0.14**ab**	10±1.00**df**	1.677±0.05**ac**	10±3.00**fg**
	Limestone	2.677±0.37**a**	18±2.10**ab**	2.600±0.31**ab**	22±4.32**abc**	2.967±0.16**b**	22±3.06**ab**
	Compost	2.413±0.11**ae**	18±3.06**ab**	3.046±0.13**b**	25±3.21**bc**	3.067±0.21**b**	23±3.51**ab**
	LS+Comp	2.810±0.09**a**	21±1.15**ab**	3.033±0.24**b**	24±1.52**abc**	3.007±0.21**b**	24±3.06**ab**
HPPTS	Back ground	1.483±0.28**bc**	7±1.00**cd**	0.854±0.09**cd**	4±0.57**f**	0.960±0.07**c**	5±1.15**ef**
	Unamended	1.200±0.12**bc**	8±1.15**cd**	1.733±0.06**ac**	7±2.51**d**	0.853±0.03**c**	2±0.00**e**
	Limestone	2.710±0.19**a**	19±2.31**ab**	2.657±0.35**ab**	16±1.73**ade**	2.730±0.07**b**	19±1.53**ad**
	Compost	2.807±0.15**a**	21±2.65**ab**	2.593±0.41**ab**	21±2.31**abc**	2.660±0.14**ab**	21±2.31**ab**
	LS+Comp	2.797±0.32**a**	23±1.15**b**	2.750±0.36**b**	18±2.51**bd**	2.777±0.15**b**	21±4.04**ac**
UPS		2.850±0.05**a**	23±3.21**b**	2.993±0.06**b**	28±1.00**c**	2.983±0.12**b**	28±1.53**b**

Means with different letters within a column for a particular species and site indicate significant difference between values at p<0.05; post hoc Tukey's HSD test.

There were significant differences in substrate diversity across the species of the trees (ANOVA, F=7.78, p<0.01) and treatment (F=52.55, p<0.001) and no significant variation across sites (F=2.21, p>0.05). Test for the impact of interaction of factors on substrate diversity revealed a significant effect of site*species (F=3.39, p<0.05) but no significant effect of site*treatment (F=2.59, p>0.05) and species treatment (F=1.86, p>0.05). With the exceptional case of site that had no significant effect on diversity, all the other factors and their interactions had the same effect on substrate richness as that of substrate diversity. Substrate richness varied significantly across sites (F=11.15, p<0.001), species (F=10.81, p<0.01) and treatment (F=138.96, p<0.001). Test for interactive effects revealed significant effect of site*species (F=5.87, p<0.01) and no significant effects of site*treatment (F=2.18, p>0.05) and species*treatment (F=0.78, p>0.05).

3.3 Relationship between AWCD, S, H and soil physico-chemical characteristics.

Soil microbial activity and soil physico-chemical properties were very much interrelated. Speramans rank-order tests between soil physico-chemical characteristics and AWCD, S and *H* values calculated from Biolog Ecoplates™ readings. The latter values were positively and strongly correlated with pH, organic matter, total nitrogen, and phosphorous and strongly and negatively correlated with available heavy metal concentrations (p<0.01) (Table 3).

Table 3. Pearson correlation coefficients (r) between soil physico-chemical characteristics of soil and AWCD, substrate richness and Shannon-Wiener diversity index for the Ecoplates

Parameter	AWCD	Shannon-weiner diversity index (*H*)	Substrate richness (S)
pH	0.779***	0.739***	0.670***
Organic matter	0.431**	0.499***	0.591***
Total nitrogen	0.557***	0.631***	0.709***
Phosphorous	0.681***	0.669***	0.765***
Copper	-0.584***	-0.579***	-0.585***
Cobalt	-0.676***	-0.593***	-0.656***
Nickel	-0.494***	0.467***	-0.485***
Lead	-0.706***	0.646***	-0.634**

Values marked by ** and *** are significant at p<0.01 and p<0.001 levels of significance testing.

Principal Component Analysis (PCA) to identify similarity/dissimilarity in utilization of carbon sources showed

two clear cut clusters of soils (Figure 4). The first principal component axis (PC1) explained 55.5% of the overall variance in the data while the second principal component axis (PC2) explained 13.42%. Substrate utilization patterns were largely separated on the PC1 axis. All the treated and unpolluted soil samples were distributed on the positive terminal of PC1 along with available phosphorous, total nitrogen, pH and organic matter content whereas all the background and untreated soils were distributed to the negative terminal of the axis along with the heavy metals. Along PC2 axis the samples from HPPTS were majorly drawn to the positive terminal while majority of the samples from KTDS, LPPTS and UPS were drawn to the negative terminal.

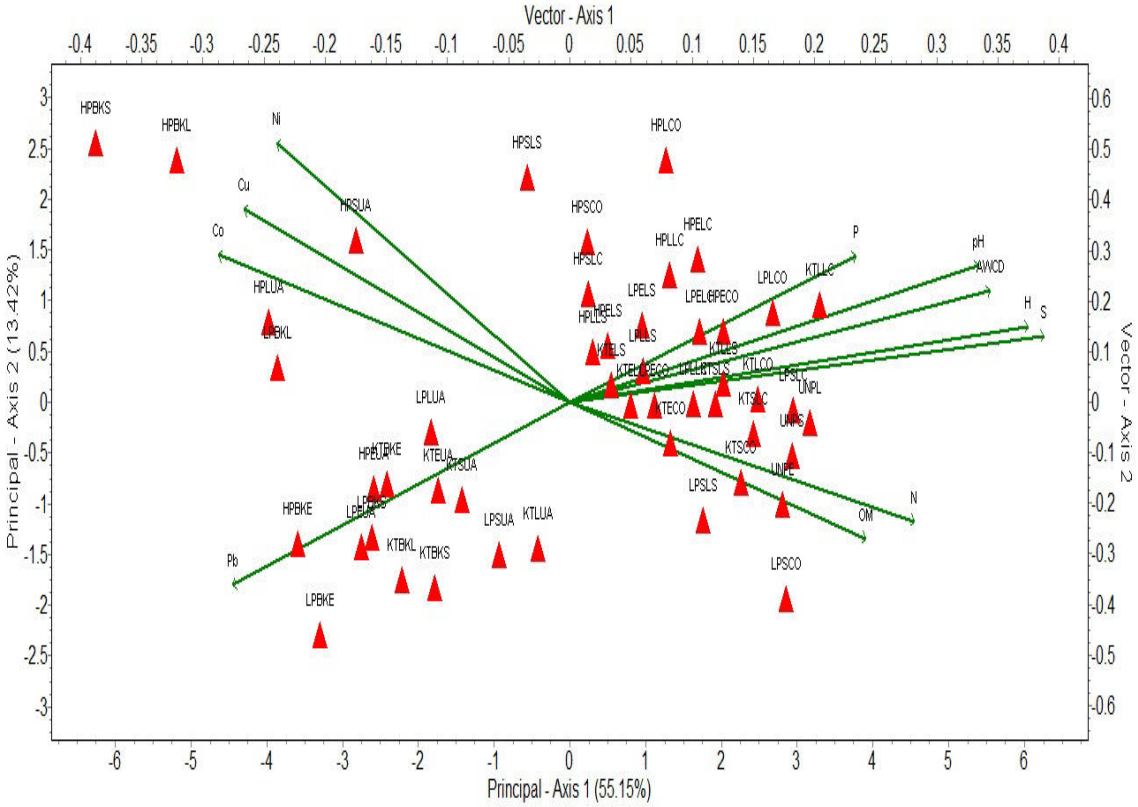

Figure 4. PCA correlation of carbon substrate utilization patterns with physico-chemical characteristics of soil samples. Soil types defined in order by site (HP-highly polluted, LP-lowly polluted,KT-Kilembe tailings dams and BK-Background), species (S-*Senna siamea*, *Leucaena lecocephala* and E-*Eucalyptus grandis*) and treatment (UA-unamended, LS-limestone, CO-compost and LC-limetsone+compost).

3. Discussion

3.1 Physico-chemical characteristics of rhizospheric pyrite soils and copper tailings

Background and rhizospheric unamended pyrite soils and copper tailings had extremely low pH and relatively higher concentrations of available heavy metals. This was due to the exposure of pyretic material (metal sulphide) to air and water leading to its oxidation and hydrolysis ultimately causing the acidification and solubilisation of heavy metals (Simón *et al.* 2001). The mean pH range determined in this study for background and unamended rhizospheric soils respectively falls within the range characterised to be extremely acid (USDA 1998), associated with increased metal toxicities and reduced populations of nitrogen fixing bacteria (Sheoran *et al.* 2010). The pH values recorded are in agreement with those determined for a site of comparable nature in the range of 2.8-4.8 (Anawar *et al.* 2013) and those reported in earlier studies at the study site by Oryem-Origa *et al*. (2007) and Muwanga *et al*. (2009).

After amendment applications, soil pH significantly increased, with limestone and limestone+compost application showing higher impact relative to lone compost application. All Limestone treated soils and unpolluted soils were in the range of 6.1-7.5 classified as being neutral (USDA 1998) whereas majority of the limestone+compost treated soils were within the neutral range and very few slightly alkaline. For lone compost application regime, the variability in pH values were wide ranging from acid through neutral to slightly alkaline. This variability is indicative of inefficiencies that may arise with application of compost alone in

phytoremediation process. Such inefficiency of compost in the control of soil pH has also been reported Walker *et al.* (2004). The acidity may have been developed with time after compost application due to the mineralisation of the labile organic matter in compost which altered the redox state of the soils to less oxidizing conditions thus minimizing the oxidation of sulphide to sulphate and consequently further acidification (Walker *et al.* 2003). Therefore, monitoring the physico-chemical characteristics of soils following organic matter application is vital, as changes occur with time, due to microbiologically-mediated transformations of organic compounds

Available concentrations of heavy metals were lower in unpolluted soils and treated pyrite soils and copper tailings with high pH and organic matter content relative to the unamended pyrite soils and copper tailings with extremely low pH and organic matter content. Soil pH is the most important parameter influencing metal solution and soil surface chemistry and determines the cation and anion adsorption onto mineral oxides (Bradl 2004). The availability of heavy metals is usually high at low pH because most metals exist in a free ionic state, as opposed to precipitates of oxides, hydroxides and oxyhydroxides (Wakelin *et al.* 2012). The rise in pH and reduction in available concentrations of heavy metals could have been due to the release of hydroxyl ions by hydrolysis of $CaCO_3$ (Lee *et al.* 2004) and precipitation of metals as carbonates (Khan and Jones 2008).

The organic matter content majorly increased due to amendment with compost and was at the time of sampling still higher than in the background, unamended and limestone treated pyrite soils and tailings, despite probable mineralization of its easily degradable component. This may be attributed to the presence of understory plant cover that emerged after amendment application and growth of trees that compensated carbon losses through mineralization by organic inputs such as root exudates and plant remains (Santibanez *et al.* 2012). Regressing total nitrogen and available phosphorous with organic matter revealed a significant positive relationship. In such nutrient poor soils, revitalisation of microbial activity after amendment application could have enhanced the supply of growth limiting N and P to both understory plants and tree species (Van der Heijden *et al.* 2008), leading to their robust growth and further supplies of organic matter. In such pyrite soils and tailings with nutrient deficiency, up to 90% of N and P for plant growth might be provided by soil microbes, hence their importance for plant productivity in such soils (Van der Heijden *et al.* 2008). Organic matter was significantly and positively correlated with AWCD ($r=0.431$, $p<0.01$), bacterial species diversity ($r=0.499$, $p<0.001$) and richness ($r=0.591$, $p<0.001$). This may be ascribed to the abundance and quality of food resources availed to the bacterial communities by organic matter and the efficient immobilisation of heavy metals by strong binding to it (Stefanowicz *et al.* 2012).

3.2 Bacterial functional diversity

The average well colour development was used to assess the bacterial activity while the Shannon-Weaner index and substrate diversity were used to reflect on the diversity and homogeneousness of the rhizospheric bacterial communities. Generally, AWCD, bacterial diversity and richness were higher in unpolluted than in the pyrite and copper tailings. It is indeed a usual trend for indigenous microbial communities associated with mine tailings to often show limited density and diversity, relative to the undisturbed sites (de la Iglesia *et al.* 2006) and mainly corresponding to iron-/sulphur-oxidizing bacteria (Diaby *et al.* 2007). The PCA correlation analysis revealed a strong association between higher concentrations of heavy metals with background, unamended pyrite soils and copper tailings samples with low bacterial activity. Further still, AWCD, S and *H* were significantly and inversely correlated with all heavy metals understudy ($p<0.001$). Most probably, heavy metals pose a restraint effect to the natural revitalisation of microbial activities in the pyrite soils and tailings. Similarly, the extremely acid condition that characterise the untreated pyrite and tailings do have a similar effect.

Average well colour development (AWCD) measures the overall potential for heterotrophic microbial activity (Wakelin *et al.* 2012). Microbial utilization of the substrates in the Biolog plates was to some extent also observed for background and unamended rhizospheric pyrite soils and copper tailings. This indicates potential for heterotrophic processes (Wakelin *et al.* 2012), despite low total nitrogen and low available phosphorous levels they contain. Their low content could have been due lack or presence of minimal amounts of the organic substratum to be mineralised and elevated concentrations heavy metals that are known to have largely deleterious effects on microbial processes associated with the cycling of C and N(Bååth 1989). Their utilisation of substrates also further suggests the existence of bacterial species that can thrive in extremes of low soil pH and higher heavy metal concentrations. This is supported by earlier studies by Oryem-Origa *et al*, (2007) at the same site who isolated bacterial strains from extremely acid pyrite soils. Bacteria can persist in such media with low pH and elevated concentrations of heavy metals by forming organic metal-complexing agents (Higham *et al.* 1984) and precipitation or redox transformation of metals (Southam 2002)

Using the Biolog EcoPlate™ assay with carbon substrates allowed detection of the changes in the bacterial functional diversity after application of limestone, compost and growth of trees on pyrite soils and copper tailings. The treatment factor had a significant effect on AWCD, bacterial species diversity and richness. The rise in pH and decrease in the heavy metal soluble concentration could be responsible for the increase in the rhizospheric microbial activity and diversity (Mench *et al.* 2006; Perez-de-Mora *et al.* 2006). The difference in spatial distribution amongst soil samples in the PCA indicates that bacterial functional diversity of pyrite soils and copper tailings was largely influenced by application of amendments. All the amendment regimes significantly increased the average well colour development for a particular species and site relative to their respective background and unamended pyrite and copper tailings. Soil pH is a key factor in determining soil microbial community structure (Xue *et al.* 2010) and it had strong positive correlation with AWCD, *H* and S. Thus the change in pH following amendment application could have led to an influx of diverse arrays of bacterial species into the pyrite soils and copper tailings.

Several studies have demonstrated that root exudation is a major factor controlling microbial activity and community structure in the rhizosphere (Kuzdroj and van Elisa 2000; Baudoin *et al.* 2003; Kumpiene *et al.* 2009). Bacterial species richness and diversity for each of the leguminous tree species as measured by Biolog™ was significantly higher than that of *Eucalyptus grandis* (t-test, $p<0.05$). Nevertheless, AWCD was relatively higher in leguminous tree species than in *Eucalyptus grandis* but not significantly different. The response of rhizospheric bacterial communities to AWCD, S and *H* denotes higher functional diversity of leguminous tree species than the non leguminous tree species. This is supported by earlier studies in which rhizodeposits of leguminous *Pisum sativum* promoted a greater microbial abundance and activity than rhizodeposits of non-leguminous *Triticum valgare* (Castaldi *et al.* 2009). Bacterial communities can play a cardinal role in the regeneration of soil functional qualities through recycling of nutrient and nutrient enrichment. Thus the efficiency demonstrated by the leguminous tree species *Senna siamea* and *Leucaena leucocephala* is suggestive of their potential for phytoremediation of the site.

Even though we sampled targeting the rhizosphere of the tree species, probable influence of plants that grew close to the trees can't be overlooked. Diversity of plant species has been shown to have a positive effect on the amount of soil bacteria (Han *et al.* 2011). The number of plant species determines available inhabitation, food and energy for microbes in the soil (Wardle *et al.* 2004; Ushio *et al.* 2008), thus, the rich plant species can produce a high diversity of litter and consequently, lead to the high amount of soil bacteria (Bartelt-Ryser *et al.* 2005), that may inhabit the rhizospheres of the trees. These plants could ensure the presence of moisture that is crucial in shaping microbial community structure (Rasche *et al.* 2010; Brockett *et al.* 2012). Therefore besides the probable influence of rhizodeposits, the disparity in emergence of understory plant species could have also contributed to the difference. Legumes were observed to support a higher diversity of understory plant species as opposed to *Eucalyptus grandis* due to its allelopathic influence. Plants have been reported to substantially influence the chemistry of soil solution, making local sources of nutrients (or toxins) available or unavailable to soils microorganisms (Hinsinger *et al.* 2006). However plant species show varying abilities to modulate the soil chemical characteristics. Thus higher diversity of understory plant species of leguminous tree species could have generated higher biochemical diversity and in the process maintaining more diverse and presumably better functioning microbial communities (Stefanowicz *et al.* 2012).

5. Conclusions

Bacterial functional diversity of pyrite soils and copper tailings was poor due to their physico-chemical characteristics that have both direct and indirect adverse effects on bacterial populations. Despite extremely acidic conditions and relatively higher availability of heavy metals, some bacterial strains capable of heterotrophic activities as revealed by the utilisation of some carbon sources do inhabit pyrite soils and copper tailings. Bacterial functional diversity is under control of a multitude of factors with pH being the major one due to its direct and indirect effects mediated through its influence on other factors involved in the control. Rhizospheric soils for leguminous *Senna siamea* and *Leucaena leucocephala* had relatively higher AWCD, species diversity and richness than the non-leguminous *Eucalyptus grandis*. These results are suggestive of higher efficacy for revitalisation of bacterial functional diversity by leguminous species than the non-leguminous *Eucalyptus grandis* and thus revealing their suitability for phytoremediation of the site. Soil amendments significantly restored bacterial functional diversity regardless of site but their stability in controlling the physico-chemical characteristics of tailings and pyrite soils with time needs to be monitored. The metal-tolerant bacteria that can survive in heavily contaminated habitats have shown great potential for the bioremediation of contaminated soils and need to be characterised in future studies.

Acknowledgement

The authors acknowledge Sida for the financial support extended through Makerere University and National Agriculture Research Laboratories (NARL) for the physico-chemical analyses of the soil samples. We also acknowledge Uganda Wild life Authority (UWA) for giving us permission to conduct in QECA. Finally, we are also grateful to Uganda National Council for Science and Technology (UNCST) for granting us permission to carry out this research.

References

Anawar, H.M., Canha, N., Santa-Regina, I. & Freitas, M.C. (2013). Adaptation, tolerance, and evolution of plant species in a pyrite mine in response to contamination level and properties of mine tailings: sustainable rehabilitation. *Journal of Soils and Sediments* 13, 730-741.

Bååth, E. (1989). Effects of heavy metals in soil on microbial processes and populations. *Water, Air and Soil Pollution* 47, 335-379.

Bartelt-Ryser, J., Joshi, J., Schimid, B., Bradl, H. & Balser, T. (2005). Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspective in Plant Ecology, Evolution and systematics* 7, 27-49.

Baudoin, E., Benizri, E. & Guckert, A. (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biology and Biochemistry* 35, 1183-1192.

Bradl, H.B. (2004). Adsorption of heavy metal ions on soil and soil constituents. *Journal of Colloid and Interface Science* 277, 1-18.

Bremner, J.M. & Mulvaney, C.S. (1982). Nitrogen-Total. In: Page A.L. *et al*. (eds) Methods of soil analysis Part 2, 2nd edn. Agron Monograph 9, pp 595-624

Brockett, B.F.T., Prescott, C.E. & Grayston, S.J. (2012). Soil moisture is a major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry* 44, 9-20.

Brookes, P.C. & McGrath, S.P. (1984). Effect of metal toxicity on the size of the soil microbial biomass. *Journal of Soil Science* 35, 341-346.

Brookes, P.C., McGrath, S.P. & Heijnen, C. (1986). Metal residues in soil previously treated with sewage-sludge and their effects on growth and nitrogen fixation by blue-green algae. *Soil Biology and Biochemistry* 18, 383-388.

Castaldi, P., Melis, P., Silvetti, M., Deiana P. & Garau, G. (2009). Influence of pea and wheat growth on Pb, Cd and Zn mobility and soil biological status in a polluted amended soil. *Geoderma* 151, 241-248.

Chakraborty, A., Chakrabarti, K., Chakraborty, A. & Ghish, S. (2011). Effect of long term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil. *Biology and Fertility of Soils* 47, 227-233.

Chander, K., Brookes, P.C. & Harding, S.A. (1995). Microbial biomass dynamics following addition of metal-enriched sewage sludges to a sandy loam. *Soil Biology and Biochemistry* 27, 1409-1421.

Chandler, K.. & Brookes, P.C. (1991). Effects of heavy metals from past application of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and silty loam U.K. soil. *Soil Biology and Biochemistry* 23(10), 927-932.

Chen, X., Fang, Z.G.. & Hu, S. (2002). Phosphate solubilising microbes in rhizosphere soils of 19 weeds in southern China. *Journal Zhejiang University Science* 3, 355-361.

Choi, K.H. & Dobbs, F.C. (2003). Comparison of two kinds of Biolog micro-plates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *Journal of Microbiological Methods* 36, 203-213.

Classen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T. & Hart, S.C. (2003). Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soil. *FEM Microbiology Ecology* 44, 319-328.

Clemente, R.C., Almela, M.P. & Bernal, M.P. (2006). A remediation strategy based on active phytoremediation followed by natural attenuation in a soil contaminated by pyrite waste. *Environmental Pollution* 143, 397-406.

Compant, S., Clemente, C. & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* 42, 669-678.

Dary, M., Chamber-Perez, M.A., Palomares, A.J. & Pajuelo, E. (2010). "In situ" Phytostabilisation of heavy metal polluted soils Using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. *Journal of Hazardous Materials* 177, 323-330.

de la Iglesia, R., Castro, D., Ginocchio, R., van der Lelie, D. & Gonzalez, B. (2006). Factors influencing the composition of bacterial communities found at abandoned copper-tailings dumps. *Journal of Applied Microbiology* 100, 537-544.

Diaby, N., Dold, B., Pfeifer, H.R., Holliger, C., Johnson, D.B. & Hallberg, K.B. (2007). Microbial communities in a porphyry copper tailings impoundment and their impact on the geochemical dynamics of the mine wastes. *Environmental Microbiology* 9, 298-307.

Doelman, P., Jensen, V., Kjoeller, A. & Soerenson, L.H. (1986). Resistance of soil microbial communities to heavy metals. *Microbial Communities in Soil* 33, 369-384.

Jøker, D. (2000). *Senna siamea* (Lam.) Irwin et Barneby. Seed leaflet. Danida Forest Seed Centre, Issue: 29 Pages: 2 pp. Available at: curis.ku.dk/portal-life/files/20648631/senna_siamea_int.pdf. Accessed on 8th/04/2014.

Douterelo, R.M., Goulder, M. & Lillie, M. (2010). Soil microbial community response to land management and depth: Related to the degradation of organic matter in English wetlands: Implications for the in situ preservations of archaeological remains. *Applied Soil Ecology* 44, 219-227.

Fagbola, O., Osonubi, O., & Mulongoy, K. (1998). Contribution of arbuscular mycorrhizal (AM) fungi and hedgerow trees to the yield and nutrient uptake of cassava in an alley-cropping system. *Journal of Agricultural Science, Cambridge* 131, 79-85.

Finlay, B.J., Maberly, S.C. & Cooper, J.I. (1997). Microbial diversity and ecosystem function. *Oikos* 80, 209-213.

Fowler J, Cohen L. & Jarvis P, (2006). Practical statistics for field biology, Second Edition. John Wiley &Sons Ltd, West Sussex, England.

Frac, M., Oszust, K., & Lipiec, J. (2012). Community level physiological profiles (CLPP), characterization and microbial activity of soils amended with dairy sewage sludge. *Sensors* 12, 3253-3268.

Frostegard, A., Tunlid, A. & Baath, A. (1993). Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied Environmental Microbiology* 59, 3605-3617.

Garbeva, P., Veen, J.A.V. & Elsas, J.D.V. (2004). Micro-bial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of phytopathology* 42, 243-270.

Garllardo, A. & Schlesinger, W.H. (1994). Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biology and Biochemistry* 26, 1409-1415.

Han, X.H., Wang, R., Guo, W., Pang, X., Zhou, J., Wang, Q., Zhan, J. & Dai, J. (2011). Soil microbial community response to land use and various soil elements in a city landscape of north China. *African Journal of Biotechnology* 10, 16554-16565

Harch, B.D., Correl, R.L., Meech, W., Kirkby, C.A. & Pankhurst, C.E. (1997). Using the Gini Coefficient with Biolog substrate utilization data to provide an alternative quantitative measure for comparing bacterial soil communities. *Journal of Microbiological Methods* 30, 91-101.

Higham, D.P., Sadler, P.J. & Scawen, M.D. (1984). Cadmium-resistant *Pseudomonas putida* synthesizes novel cadmium proteins. *Science* 225, 1043-1046.

Hinsinger, P., Plassard, C. & Jaillard, B. (2006). Rhizosphere: a new frontier for soil biogeochemistry. *Journal of Geochemical Exploration* 88, 210-213.

Hossain, K.M. (1999). *Senna siamea*: A widely used legume tree. A quick guide to multipurpose trees from around the world. Fact sheet 99-04. Institute of Forestry and Environmental Sciences, Chittagong University, Chittagong 4331, Bangladesh.

Jia, X., Dong, S. & Zhou, C. (2013). Response of microorganism in the rhizosphere of winter wheat seedlings at a low concentration of lead. *Advanced Journal of Food Science and Technology* 5, 633-639.

Kandeler, E., Kampichler, C. & Horak, O. (1996). Influence of heavy metals on the functional diversity of soil microbial communities. *Biology and Fertility of Soils* 23, 299.

Kennedy, A.C. (1999). Bacterial diversity in agroecosystems. *Agriculture Ecosystems and Environment* 74, 65-76.

Khan, M.J. & Jones, D.L. (2008). Chemical and organic immobilisation treatments for reducing phyto-availability of heavy metals in copper-mine tailings. *Journal of Plant Nutrition and Soil Science* 171, 908-916.

Kumpiene, J., Guerri, G., Landi, L., Pietramellara, G., Nannipieri, P. & Renella, G. (2009). Microbial biomass, respiration and enzyme activities after in situ aided phytostabilization of Pb- and Cu- contaminated soil. *Ecotoxicology and Environmental Safety* 72, 115-119.

Kuzdroj, J. & van Elisa, J.D. (2000). Response of the bacterial community to root exudates in soil polluted with heavy metals assessed by molecular and cultural approaches. *Soil Biology and Biochemistry* 32, 1405-1417.

Lee, M.T., Lai, H.Y. & Chen, Z. S. (2004). Effect of chemical amendments on the concentration of cadmium and

lead in long term contaminated soils. *Chemosphere* 57, 1459-1471.

Leita, L., De Nobili, M., Muhlbachova, G., Mondini, C., Marchiol, L. & Zerbi, G. (1995). Bioavailability and effects of heavy metals on soil microbial mass survival during laboratory incubation. *Biology Fertility Soils* 19, 103-108.

Leyval, C., Turnau, K. & Haselwandter, K. (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: physical, ecological and applied aspects. *Mycorrhiza* 7, 139-153.

Li, J., Jin, Z. & Gu, Q. (2011). Effects of plant species on the function and structure of the bacterial community in the rhizosphere of lead-zinc mine tailings in Zhejiang, China. *Canadian Journal of Microbiology* 57, 569-577.

Ma, Y., Rajkumar, M. & Freitas, H. (2009). Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea. Journal Environment Managment* 90, 831-837.

Mench, M., Renella, G., Gelsomino, A., Landi, L. & Nannipieri, P. (2006). Biochemical parameters and bacterial species richness in soils contaminated by sludge-borne metals and remediated by organic soil amendments. *Environmental pollution* 144(1), 24-31.

Mendez, M.O., Glenn, E.P. & Maier, R.M. (2007). Phytostabilization potential of quail bush for mine tailings: growth, metal accumulation, and microbial community changes. *J. Environmental Quality* 36, 245-253.

Moynahan, O.S., Gannon, E.J. & Zabinsiki, A.C. (2002). Microbial community structure and carbon utilization diversity in a mine tailings revegetation study. *Restoration Ecology* 10, 77-87.

Muwanga, A., Oryem-Origa, H., Maksara, A., Hartwig, T., Ochan, A., Owor, M., Zachmann, D. and Pohl, W. (2009). Heavy metals and their uptake by plants in the River Nyamwamba-Rukoki-Kamulikwezi-Lake George System, Western Uganda. *African Journal of Science and Technology (AJST), Science and Engineering Series*10, 60-69.

Nelson, D.W. & Sommers, L.E. (1982). Total carbon, organic carbon, and organic matter. In: Page A..L., Miller, R.H., Keeney, D.R. (Eds.) Methods of soil analysis Part 2: Chemical and microbiological Properties, 2nd Edition, Agronomy, 9. American Society of Agronomy, Madison, WI, pp, 539-594.

Nguyen, C. (2003). Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie* 23, 375-396.

Okalebo, J.R., Gathua, K.W. & Woomer, P.L. (2002). Laboratory Methods of Soil and Plant Analysis. A Working Manual. 2nd Edition, Tropical Soil Fertility Programme, Nairobi.

Oryem-Origa, H., Makara, A., & Tumusiime., F.M. (2007). Propagule establishment in the acid-mine polluted soils of the pyrite trail in Queen Elizabeth National Park, Uganda. *African Journal of Ecology,* 45, 84-90.

Pennanen, T., Fritze, H., Vanhala, P., Kiikkila, O., Neuvonen, S. & Baath, E. (1998). Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. *Applied and Environmental Microbiology* 64, 2173-2180.

Perez-de-Mora, A., Burgos, P., Madejon, E., Cabrera, F. Jaeckel, P. & Schloter, M. (2006). Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and soil amendments. *Soil Biology and Biochemistry* 38, 327-341.

Preston-Mafham, J., Boddy, L. & Randersonp, F. (2002). Analysis of microbial community functional diversity using sole-carbon-sources utilisation profiles-a critique. *FEM Microbiology Ecology* 42, 1-14.

R Development Core Team, (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. (August 13, 2013)

Rasche, F., Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler. B., Zechmeister-Boltenstern, S., Richter, A. & Sessitsch, A. (2010). Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *ISME Journal* 5, 389-402.

Santibanez, C., Fuente, L. M., Bustamante, E., Silva, S., Leon-Lobos, P. & Ginocchio, R. (2012). Potential use of organic- and hard-rock mine wastes on aided phytostabilisation of large scale mine tailings under semiarid Mediterranean climatic conditions: short term field study. *Applied and Environmental Soil Science*, Volume 2012, 15 pages, Article ID 895817. http://dx.doi.org/10.1155/2012/895817

Sheoran, V., Sheoran, A.S. & Poonia, P. (2010). Soil reclamation of abandoned mine land by revegetation: A review. *International Journal of Soil, Sediment and Water* 3(2), 1-21.

Simón, M., Martín, F., Ortiz, I., García, I., Fernández, J., Fernández, E., Dorronsoro, C. & Anguilar, J. (2001). Soil pollution by oxidation of tailings from toxic spill of a pyrite mine. *Science of Total Environment* 279, 63-74.

Southam, G. (2002). Metal stressed environments, bacteria. In: Bitton G (ed) Encyclopaedia of Environmental Microbiology, John wiley, NY, pp 1893-1901

Ssenku, E.J., Ntale, M., Backéus, I. & Oryem-Origa, H. (2014). Assessment of seedling establishment and growth performance of *Leucaena leucocephala* (Lam.) De Wit., *Senna siamea* Lam. and *Eucalyptus grandis* W. Hill ex Maid. in amended and untreated pyrite and copper tailings. *Journal of Biosciences and*

Medicines 2, 34-51.

Stefanowicz, A.M., Kapusta, P., Szarek-Lukaszewska, Grodziǹska, K., Niklińska, M. & Vogt, R.D. (2012). Soil fertility and plant diversity enhance microbial performance in metal-polluted soils. *Science of the Total Environment* 439, 211-119.

USDA, 1998. Soil quality indicators: pH soil quality sheet. Available on line at www.statlab.lastatc.educ. (August 8, 2013)

Ushio, M., Wagai, R., Balser, T.C. & Kitayama, K. (2008). Variation in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? *Soil Biology and Biochemistry* 40, 2699-2702.

Valsecchi, G. & Gigliotti, C.A.F. (1995). Microbial biomass, activity, and organic matter accumulation in soils contaminated with heavy metals. *Biology and fertility of soils* 20, 253-259.

Van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11, 296-310.

Viketoft, M., Palmborg, C., Sohlenius, B., Huss-Danell, K. & Bengtsson, J. (2005). Plant species effects on soil nematode communities in experimental grasslands. *Applied Soil Ecology* 30, 91-103.

Wakelin, S.A., Anand, R.R., Reith, F., Gregg, A.L., Noble, R.R.P., Goldfarb, K..C., Andersen, G.L., DeSantis, T.Z., Piceno, Y.M. & Brodie, E.L. (2012). Bacterial communities associated with a mineral weathering profile at a sulphurdic mine tailings in arid Western Australia. *FEMS Microbiology Ecology* 79, 298-311.

Walker, D.J., Clemente, R. & Bernal, M.P., (2004). Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in a soil contaminated by pyritic mine waste. *Chemosphere* 57, 215-224.

Walker, D.J., Clemente, R., Roig, A. & Bernal, M.P. (2003). The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environmental Pollution* 122, 303-312.

Wang, Y., Shi, J., Wang, H., Lin, Q., Chen, X. & Chen, Y. (2007). The influence of soil heavy metals pollution on soil microbial biomass enzyme activity, and community composition near a copper smelter. *Ecotoxicology Environmental. Safety* 67, 75-81.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van der Putten, W. H. & Wall, D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science* 304, 1629-1633.

Xue, D., Huang, X., Yao, H. & Huang, C. (2010). Effect of lime application on microbial community in acidic tea orchard soils in comparison with those in wasteland and forest soils. *Journal of Environmental Sciences* 22, 1253-1260.

Yan, F., McBratney, A.B. & Copeland, L. (2000). Functional substrate biodiversity of cultivated and un cultivated A horizons of vertisols in NW New South Wales. *Geoderma* 96, 321-343.

Zak, J.C., Willig, M.R., Moorhead, D.L. & Wildman, H.G. (2003). Functional diversity of microbial communities: a quantitative approach. *Soil Biology and Biochemistry* 26, 1101-1108.

Zhang, C., Liu, G.B., Xue, S. & Song, Z. (2011). Rhizosphere soil microbial activity under different vegetation types on the loess plateau, China. *Geoderma* 161, 115-125.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar