SOIL MICROBIAL COMMUNITY COMPOSITION IN RECLAIMED SOIL UNDER DIFFERENT VEGETATION IN WYOMING MINELANDS[1]

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Abstract. Vegetation is known to be an important determinant of soil microbial community structure and although cool season grass communities dominate most reclaimed mine sites in Wyoming, a variety of other plant communities may be found. The primary objective of this paper is to examine and compare microbial community recovery in reclaimed soils vegetated by different types of plant communities. Phospholipid fatty acid (PLFA) analysis was used to characterize soil microbial community structure under three types of plant communities (cool season grass, warm season grass and sagebrush steppe). Results indicate that all major soil microbial groups are reestablishing in reclaimed mine soils regardless of vegetation. Further, microbial communities in reclaimed sites dominated by sagebrush appear to recover more rapidly than those in soils vegetated by warm season and cool season grass communities. Mechanisms responsible for these differences will also be discussed in this paper.

Additional Key Words: Soil Microbial Community, Wyoming, PLFA, sagebrush steppe.

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Introduction

The primary goal of mineland reclamation is to reestablish a productive and sustainable ecosystem after mining disturbance. Only aboveground ecosystem components which can be easily evaluated by visual inspection are used for judging reclamation successes (Mummey et. al., 2002). However, productive, sustainable ecosystems are highly dependent on belowground organisms and processes for long term function. For example, belowground organisms are crucial to decomposition of organic matter, nutrient cycling and plant nutrient availability (Paul and Clark, 1996). Failure to successfully reestablish belowground ecosystems on reclaimed lands could lead to serious problems and future site degradation.

Relatively little is known about recovery of belowground ecosystem components after disturbance associated with surface mining. Although a number of studies have been conducted examining recovery of soil microbial communities, this is an area that requires further research. Currently, the only way in which progress of reclaimed mine lands is evaluated is vegetation parameters. A better understanding of mineland reclamation could facilitate our understanding relationships between belowground and aboveground ecosystems and provide additional information which may help us understand and speed up reclamation processes. Microbial populations, however, have received less study on restoration or rehabilitation projects than aboveground communities despite the essential functions they provide (DeGrood et al., 2004).

The main objective of this study was to examine recovery of soil microbial community structure under different vegetation in surface coal mine reclamation sites. We used phospholipid fatty acid (PLFA) analysis to measure microbial community composition (Baath et al., 1998, Bossio et al., 1998, Song et al., 1999, Zelles et al., 1999, DeGrood et al., 2004). Phospholipids are an essential part of cell membranes and are metabolized rapidly after cell death (Allison et al., 2005). So, PLFAs are considered to be good indicator of microbial community structure (Allison et al., 2005). Additionally, specific signature PLFAs are associated with specific taxa of the microbial community, including gram-positive and gram-negative bacteria, actinomycetes, mycorrhizae, protists and saprophytic fungi (Olsson, 1999).

Materials and Methods

Site Details

This study was conducted using three surface coal mine reclamation sites in Powder River Basin, Wyoming, USA. Two of mines used in this study, Belle Ayr Mine, located just south of Gillette, and the Jacobs Ranch Mine, located about 60 miles south of Gillette are functional coal mines. The third mine, Dave Johnston Mine located to the north of Glenrock, closed down in 2002 and currently the whole mine is in the process of being reclaimed. Our research site at the Belle Ayr Mine (N 44° 05.696' W 105° 25.520') had been reclaimed for 14 years and was inhabited with cool season grass (CSG). The research site at the Dave Johnston Mine (N 43° 00.547' W 105° 43.339') was dominated by sagebrush grassland (S) had been reclaimed for 5 years. The site we used at the Jacobs Ranch Mine (N 43° 43.433' W 105° 14.470') was vegetated with warm season grass (WSG) which was reclaimed for 10 years. At each mine a native, undisturbed site which has not been impacted by mining was also sampled as a reference site in which to compare again the reclaimed sites. Vegetation at the native sites was same as for the reclaimed sites.

Soil Sampling

Soil samples at each site were collected in May-June 2005 from 0-5 cm, and 5-15 cm depths. The top 5 cm of soil was collected with a trowel and the soil with 5-15 depth was collected with 2.5 cm diameter step probe. Three 45 m transects were randomly selected and soil samples were collected from four points along each transect. Once sampled soils were placed in plastic bags and stored in dry ice. After returning back to the University of Wyoming they were stored at -20°C until PLFA analysis was carried.

Phospholipid Fatty Acid (PLFA) Analysis

Phospholipid fatty acids were extracted from 10 g soil samples using a modified Bligh-Dyer methodology (Frostegard and Baath, 1991). Briefly, fatty acids were directly extracted from soil samples using a mixture of chloroform: methanol: phosphate buffer. Phospholipid fatty acids were separated from neutral and glycolipid fatty acids in solid phase extraction column. After mild alkaline methanolysis, PLFA samples were qualitatively and quantitatively analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) and fatty acids were identified by retention time according to the MIDI eukaryotic method (MIDI Inc., Newark, NJ).

Individual PLFA signatures were used to quantify the abundances of specific microbial groups in soil samples. Gram positive bacteria was identified by the presence of Iso- and anteiso- branched fatty acids, gram negative bacteria with β-OH fatty acids, eubacteria with 15:0, 17:0 cyclo, 15:1 iso, 17:1 iso and anteiso, fungi with 18:2 ω6c, actinomycetes with ISO 17:1 G, 18:1 ω9t Alcohol, 19:1 ω11c and arbuscular mycorrhizal fungi (AMF) with 16:1 ω5c (Cavigelli et al., 1995, Frostegard et al., 1993, Zelles et al., 1994 and Zelles et al., 1995).

In fatty acid nomenclature, the basic form is 'A:BωC', where A is the total number of carbons, B is the number of double bonds, and C is the position of double bonds from the methyl end of the molecule. The suffixes 'c' and 't' stand for cis and trans, the prefixes 'i', 'a', and 'me' refer to iso, anteiso, and mid-chain methyl branching, and the prefix 'cy' refers to cyclopropyl rings (Navarrete et al., 2000).

Statistical Analysis

Statistical analysis was conducted using SAS for windows version 6.12 (SAS Institute, 2003). Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were conducted using general linear model. Means were compared using Student Newman-Keuls (S-N-K) multiple range test. A canonical variates analysis, generated by the MANOVA, was used to identify the linear combination of variables that best separated the mine soil-microbial community structure. This analysis was used to generate plots that summarize group differences (Seber, 1984). All statistical analyses were accomplished at $p<0.05$.

Results and Discussion

Microbial Biomass

Soils from all three plant communities were found to contain different amounts of microbial biomass. The total PLFA content of soil from each site shown in Fig. 1 is an estimate of viable microbial biomass at the time of sampling (White et al., 1996). In general there was a trend for total PLFA to increase in the order of CSG>SB>WSG. As evidenced in the Fig. 1, total PLFA content of CSG was significantly greater than other sites; soil from the CSG site may have had

the greatest microbial biomass because it had been reclaimed for four years more than the other two sites. WSN site was significantly different than other sites in Fig. 2.
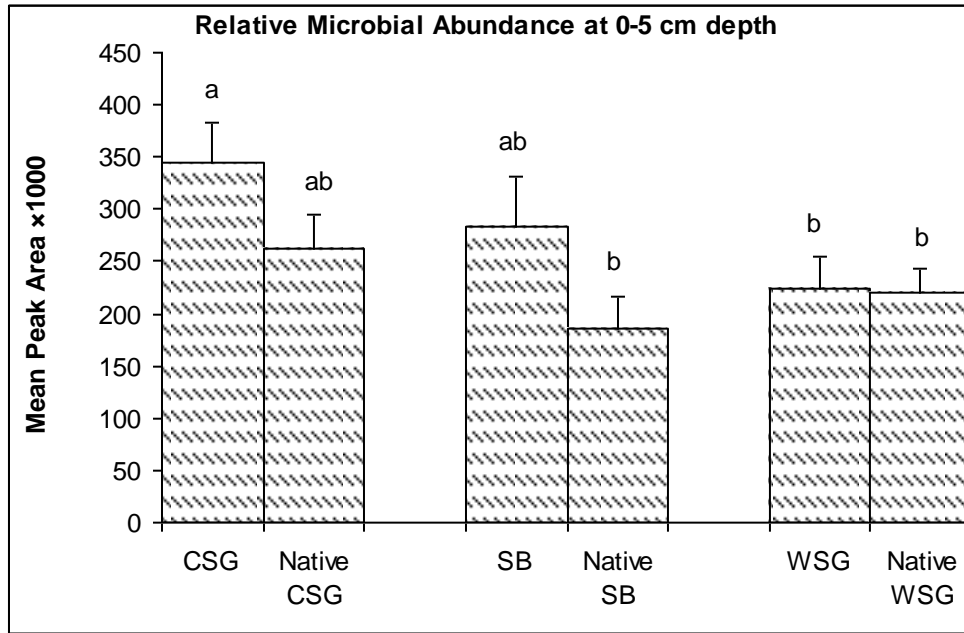
Relative Microbial Abundance at 0-5 cm depth

Figure 1. Relative abundance of microbial biomass at 0-5 cm depth. Different letters indicate significant differences ($p<0.05$). Error bars indicate standard error.

Relative Microbial Abundance at 5-15 cm depth

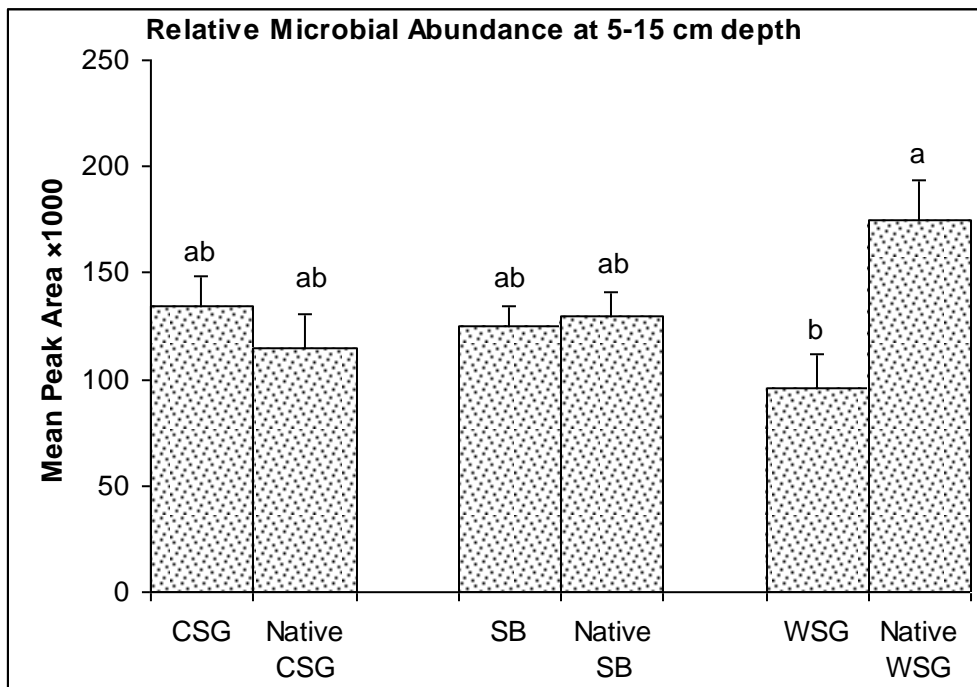Figure 2: Relative Microbial Abundance at 5-15 cm depth. Different letters indicate significant differences ($p<0.05$). Error bars indicate standard error.

Microbial community Structure

Results indicate soil microbial community structure is different among all three plant communities in terms of microbial community structure (Fig. 2). Fungal and AMF biomass was the greatest in soil from 5 year old shrub community in relation to 14 year old cool season grass and 10 year old warm season grass.
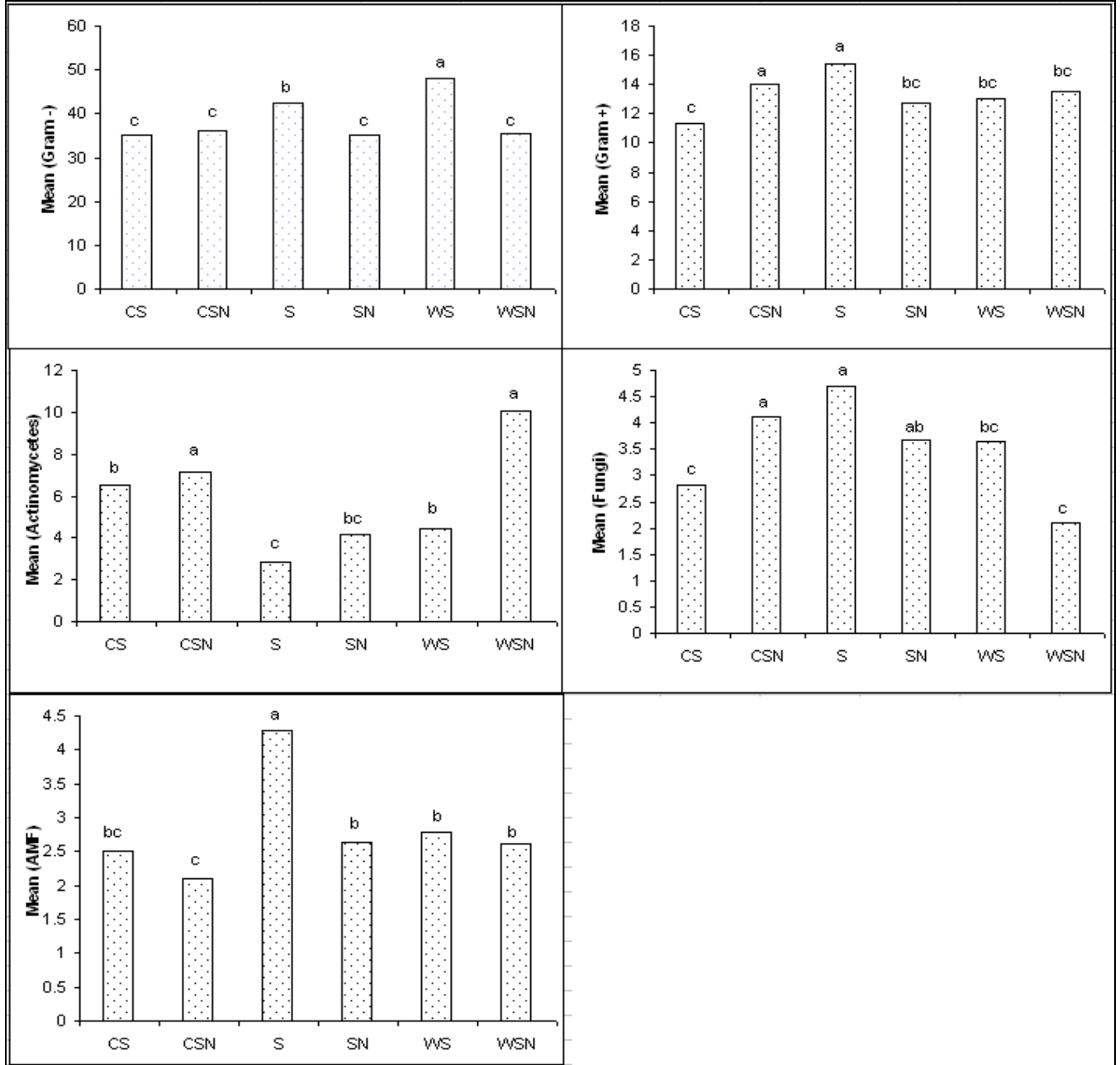

Figure 3. Means of various PLFA biomarkers from native as well as reclaimed sites of Cool season grass (CS), cool season grass native (CSN), shrub (S), shrub native (SN), warm season grass (WS), and warm season grass native (WSN). Letters above the bars indicate significant differences between each site.

The total fungal to bacterial ratio (F: B) for soil from the three ecosystem types, CSG, SB, and WSG, was 0.44, 0.53, and 0.66 respectively. The high biomass found in cool season grass soil was predominantly composed of bacterial community whereas the high fungal component was found shrub.

S-N-K tests of biomarker indices showed that biomarker fatty acids for actinomycetes (p<0.05) significantly greater in soil from native sites of warm season and cool season grass. The relative proportion of fungi and AMF was significantly higher in shrub than reclaimed warm season and cool season sites. The relative proportion of gram + bacteria was significantly higher in reclaimed shrub and cool season grass native sites. Biomarker for gram – bacteria was significantly higher in reclaimed warm season grass site (Fig. 3).
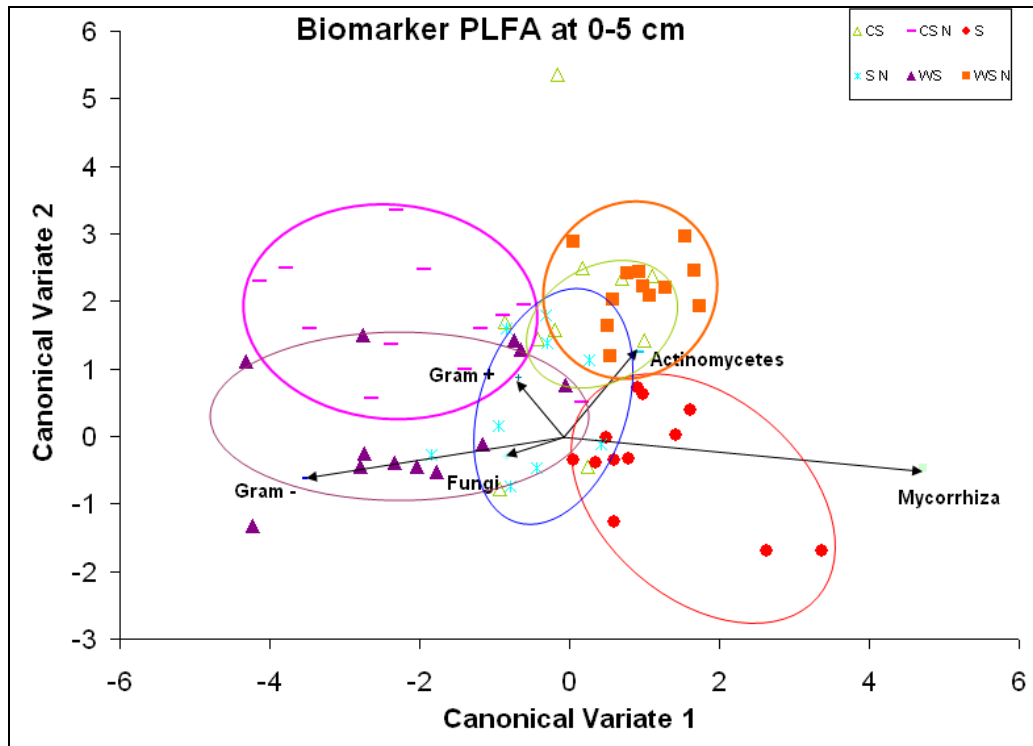

Figure 4. Canonical multivariate analysis of PLFA biomarkers for native and reclaimed sites at 0-5 cm depth
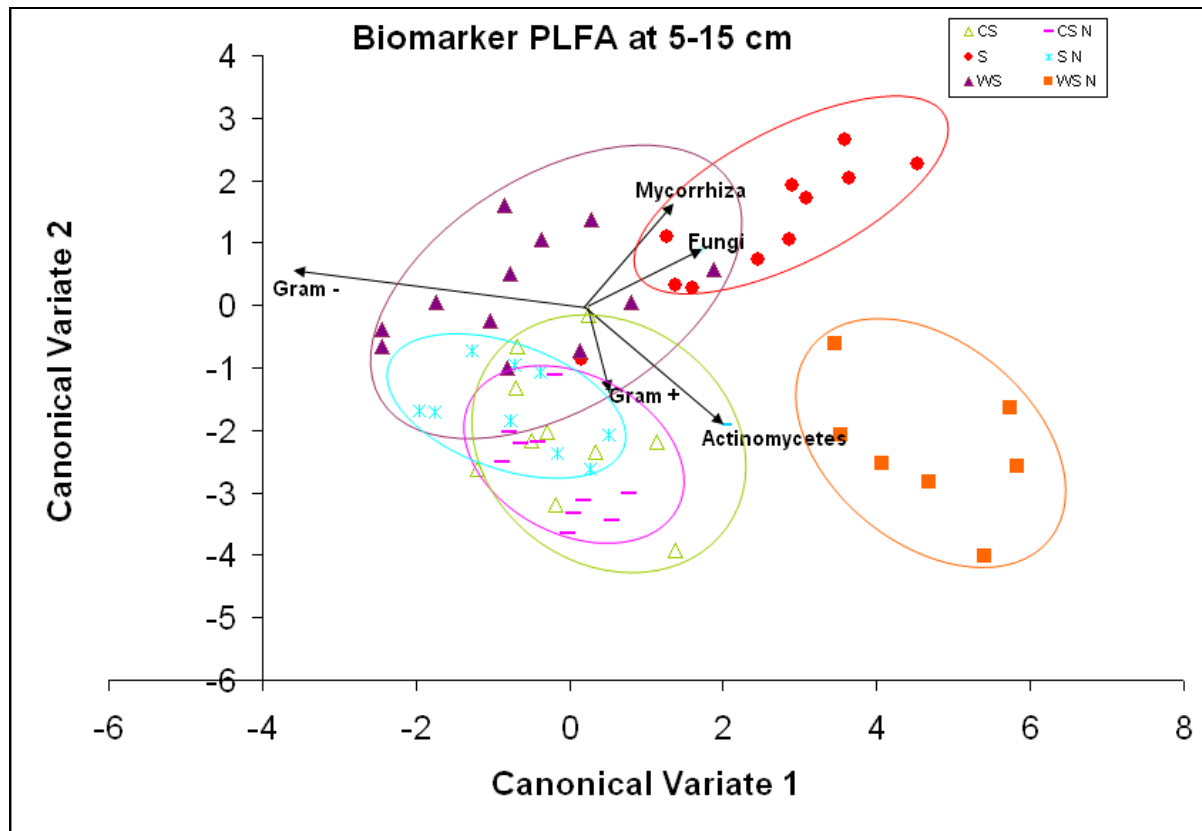
Figure 5. Canonical multivariate analysis of PLFA biomarkers for native and reclaimed sites at 5-15 cm depth.

Canonical multivariate analysis shows that soil under reclaimed warm season grass holds greater gram – bacteria, cool season grass had more of gram + bacteria and actinomycetes, the shrub site had proportionally diverse biomarker groups than the grass sites. The cool season native and shrub native sites had proportionally more gram + bacteria compared to other sites, warm season native had proportionally more gram + bacteria and actinomycetes, and proportionally less gram – bacteria and fungi (Fig. 4 & 5).

Conclusions

The use of PLFA analyses allowed us to differentiate between differences in both microbial biomass and community structure among three different vegetation communities. Microbial biomass discovered was sufficiently higher in sites with sagebrush in respect to the age of the site. Microbial community structure was found to be the least in quantity in warm season grass in comparison with shrub site of the same reclamation age. Bacterial biomass was among the highest in cool season grass and the fungal biomass was the greatest in the shrub site. With the canonical analysis we can conclude that the shrubland site seems to have recovered much more than the grassland sites

Our data indicates that the type of vegetation inhabiting a reclaimed site does have a significant influence on recovery of the soil microbial community both in terms of microbial

community productivity and structure. This study further recommends that for better comparison among the sites, any future study should be considered in sites with same age groups with similar environmental conditions.

Acknowledgements

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