ECOSYSTEM RECOVERY ON RECLAIMED SURFACE MINELANDS[1]

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Abstract: The ultimate goal of mineland reclamation is reestablishment of a productive, functional, and sustainable ecosystem suitable for postmining land use. Evaluation of reclamation success for bond release, however, is limited to examination of the reestablished plant community with emphasis also placed on soil erosion protection and landscape hydrologic function. Most ecosystem components and processes of the reclaimed site are not examined but are crucial to ecosystem function and sustainability. The objective of this paper is to present data from our work on recovery of ecosystem structure (e.g. organisms, soils, mycorrhiza) and function (e.g. biomass production, carbon cycling, nitrogen cycling) on reclaimed surface coal mines in Wyoming. Our studies of chronosequences of reclaimed sites indicate increasing productivity through time in all groups of organisms monitored (plants, bacteria, fungi, nematodes and arthropods) as well as increasing concentrations of soil organic matter, rapid incorporation of organic carbon into soil aggregates, redevelopment of mycorrhizae, and reformation of carbon and nitrogen pools. Although the precise trajectory of the restored ecosystems are very difficult to predict because of changing control variables such as potential biota (invasive species) and climate, our data indicates ecosystem structure and function is recovering on reclaimed surface minelands.

Additional Key Words: Reclamation, Restoration, Soil Organisms, Ecological Processes

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Introduction

The ultimate goal of mine land reclamation is reestablishment of a productive, functional, and sustainable ecosystem suitable for postmining land use (Munshower, 1993; Harris, Birch and Palmer, 1996). As stated in the Surface Mine Reclamation and Control Act of 1977 (SMCRA), to "restore the land affected to a condition capable of supporting the uses which it was capable of supporting prior to any mining, or higher or better uses of which there is reasonable likelihood". Postmining land uses include forest, pasture, rangelands, croplands, other agroecosystems, developed water resources, residential use, industrial use and recreation (hiking, hunting, bird watching, etc.). Most of these uses require recovery of ecosystem functions for success. For example, successful croplands require quality soils capable of capturing, storing and releasing water, cycling N, decomposing plant litter, producing humus, and providing habitat for the organisms contributing to these functions.

Evaluation of successful reclamation of surface mine lands as dictated by SMCRA, however, is not designed to appraise ecosystem recovery. Rather, successful reclamation as defined by SMCRA involves meeting reclamation performance standards including, in most states, replacement of topsoil, restoration of hydrologic function, effective erosion prevention, and reestablishment of a diverse, effective and permanent vegetative cover of the same seasonal variety native to the area. Reclamation of surface mined lands is proceeding at different rates in the coal producing states and is quite variable. The cumulative reclamation to disturbance ratio (acreage of reclaimed land divided by total acreage of disturbed land) is a good indicator of reclamation success and varies widely for different states. Some states, like West Virginia, report very low reclamation to disturbance ratios close to 0.02, while other states like Montana and Wyoming report have ratios above 0.40. Regardless of progress in reclaiming surface mined lands in coal producing states, large acreages of reclaimed surface mined land exist throughout the United States and the amount will increase as the demand for coal continues to grow.

When SMCRA was passed in 1977, analysis of ecosystem processes and components like nutrient cycling transformations and the soil microbial community were still uncommon and limited to basic ecological studies (Weigert, 1988; Golley, 1993). Management impacts on ecosystems, as generally evaluated by land managers working in the field, were based largely on visually distinguishable aboveground indicators, such as soil erosion and vegetation coverage and diversity (NRC, 1994). Today, however, many tools and methods are available for direct

assessment of ecosystem structure and function (e.g., arthropod, nematode, and microbial assemblages and nutrient cycling and energy flow rates) so the state of ecosystems as well as the impact of management practices and recovery of disturbed ecosystems can be assessed. The objective of this paper is to present data on the ecological structure and processes of different aged reclaimed surface coal mine lands to nearby relatively undisturbed land to assess ecosystem recovery in reclaimed minelands.

Methods

Two chronosequences of reclaimed surface coal mine sites were used to examine the recovery of reclaimed ecosystems over time. Chronosequences are useful in reclamation research for observation of site recovery over time, ecosystem change and evaluation of specific reclamation practices or techniques. Assemblages of plants, soil microorganisms, arthropods and nematodes as well as soil characteristics were examined as indicators of ecological structure. Biomass production, soil organic matter dynamics and soil development were monitored as indicators of ecological processes. We have examined chronosequences of reclaimed sites at two surface coal mines and have obtained generally similar results from both sites, but for the sake of brevity, data from just the Belle Ayr coal mine will be presented in this paper.

Reclaimed sites were sampled on two surface coal mines located in the Powder River Basin of northeastern Wyoming, USA. At the Dave Johnston Mine (N 43°03'/W 105°82') in Converse County, a chronosequence of four reclaimed shrub sites (<1, 5-6, 10-11, 16-17 years old) and an undisturbed site were sampled. Vegetation at sites in this chronosequence were dominated by Wyoming big sagebrush (*Artemesia tridentata* Nutt. spp. *wyomingensis* Beetle & Young). Average annual precipitation at this mine is 305 mm, mean air temperature 8.7°C and elevation is 1646 m (Western Region Climate Center 2006). Prior to mining, soils were classified as fine-loamy, mixed, mesic Ustic Haplargids (Westerman and Prink 2004; Munn and Arneson 1999).

At the Belle Ayr Mine (N 44° 10'/W 105°27') in Campbell County, the chronosequence included a topsoil stockpile (10-18 months) and two reclaimed sites (14-15 and 26-27 years old) at which vegetation was dominated by cool season grasses and an undisturbed site. The cool season grasses seeded on the reclaimed sites included prairie junegrass (*Koeleria macrantha* (Ledeb.) J.A. Schultes); western wheatgrass (*Pascopyrum smithii (Rydb.) A. Love)* and Idaho fescue (*Festuca idahoensis* Elmer). Average annual precipitation at this mine is 390 mm, mean air temperature 6.7°C and elevation is 1375 m (Western Region Climate Center 2006). Prior to

mining, soils were also classified as fine-loamy, mixed, mesic Ustic Haplargids (Westerman and Prink 2004; Munn and Arneson 1999). Each site sampled had similar soil type, topography (<1 percent slope) and was approximately 0.5 ha in size. Direct haul topsoil replacement (except at stockpile site) and similar seed mixes were used at each site.

Soil samples were collected from both chronosequences during May 2005 and 2006 when soils were moist and plants were initiating growth. At each different aged site, three 45-meter long randomly placed transects were laid out and soil was sampled at five evenly spaced points along each transect. The top 5 cm of soil was collected with a hand trowel and the 5-15 cm with a 2.5 cm diameter step probe. Five soil samples were collected for determination of bulk density (BD) at each site using a double-cylinder, hammer driven core sampler (Grossman and Reinsch 2002). Soil samples to be used for arthropod enumeration were placed in a plastic bag and stored on ice in an insulated cooler as were samples for determination of microbial community structure. Soil samples for physiochemical analyses were stored in plastic bags in large bins.

Plant Community Analysis

Vegetation was analyzed at our research sites in late June- early July 2005. Five aboveground biomass production plots were established using a stratified random method from a baseline (Chambers and Brown, 1983). Vegetation rooted within 1 m^2 plots was clipped and separated by species. A 50 m transect was randomly placed from each aboveground biomass production plot to determine cover at meter increments with an ocular device (ESCO Associates Inc, 0.05 mm accuracy). Cover was recorded for one point on either side of the transect at each meter for a total of 100 points. Diversity was determined along the same 50 meter transect, by recording each species within one meter of either side of the transect. Vegetation collected in the field was dried in a forced air oven at 55^{o}C for 24 hours, or until a constant weight was reached.

General Soil Properties

Samples were air dried and dry sieved to 2000 µm to remove large roots and break apart soil clods while leaving structure <2000 µm intact. Particle size distribution was determined on a subset of samples (4 per site) using the hydrometer method (Gee and Or, 2002). Electrical conductivity (EC) and pH were determined on all samples with a 1:1 soil:water mixture. An Oakton con 100 series EC probe (Vernon Hills, IL) and a Fisher Scientific Accument Basic pH meter with a glass electrode (Pittsburgh, PA) were used for analysis, EC and pH respectively. Total soil C and N values were obtained on all samples which were finely ground (<53 µm) prior

to analysis by dry combustion with a Carlo Erba NC 2100 Analyzer (Lakewood, NJ). Inorganic C (IC) content of soil was also determined on finely ground samples with the modified pressure calcimeter method (Sherrod et al., 2002). Organic C content was determined by subtracting IC from total C.

Aggregate Size Distribution

Water stable aggregate size distribution of soil was determined using a wet sieving protocol described by Six et al. (1998) on the 0-5 cm depth samples. In summary, 100 ± 0.02 g of air-dried soil were submerged in deionized water for 5 min at room temperature on a 250 μm sieve. Water stable macroaggregates (250-2000 μm) were separated from the whole soil by moving the sieve 3 cm up and down 50 times in 2 min. Material (water plus soil) that passed through the sieve was transferred to a 53 μm sieve and the above process repeated. Material collected from each sieve (250-2000 μm and 53-250 μm) was dried at 55˚C until a constant weight was achieved. Samples were then weighed and stored.

Sand the same size as macro- and microaggregates is not likely to be part of an aggregate and will vary across site ages (Elliott et al., 1991). Aggregate samples were corrected for sand content according to Denef et al. (2001); where 5 g of each aggregate sample was dispersed with 0.5% sodium hexametaphosphate on a shaker for 18 hrs. Following shaking, dispersed samples were sieved with 250 and 53 μm nested sieves for macroaggregates and a 53 μm sieve for microaggregates. Sand on the sieves was collected, dried and weighed. Sand corrected aggregate weights were determined according to Equation 1.

$$\text{Sand corrected weight} = \text{aggregate weight} - \left(\frac{\text{sand weight}}{5\text{ g}} * \text{aggregate weight}\right) \quad (1)$$

Microaggregates within macroaggregates (mM) were isolated according to a method described by Six et al. (2000) for the Belle Ayr choronsequence only. Macroaggregate samples (10 grams) from the 0-5 cm depth were slaked for 30 min and then transferred to a 250 μm sieve attached to a shaker. The sample was immersed in deionized water and shaken with 6 mm beads until all macroaggregates were disrupted and only coarse sand and coarse particulate organic matter (cPOM+sand) (250-2000 μm) remained on the sieve. Microaggregates were continuously washed through the 250 μm sieve onto a 53 μm sieve with deionized water. The microaggregates on the 53 μm sieve were wet sieved. All portions of the sample (cPOM +s and, micro- within macroaggregates, and silt+clay) were dried at 55˚C, weighed and stored. Each

micro- within macroaggregate proportion was also corrected for sand according to Denef et al. (2001).

Density Floatation

Particulate OM (POM) analysis (for both protected and free POM) was conducted according to methods described by Six et al. (1998) on the 0-5 cm depth samples. Samples of macro- and microaggregates (8 grams) were oven dried overnight at 105˚C. The samples were suspended in 35 mL of 1.85 g cm^{-3} density sodium polytungstate (SPT) in a 50 mL centrifuge tube and shaken gently by hand to bring the sample into suspension (approximately 10 strokes). Material on the lid was washed into the cylinder using 10 mL of SPT. Samples were then placed under vacuum (138 kPa) for 10 min to remove air trapped within aggregates. Samples were then centrifuged for 60 min at 2,500 rpm and floating material (Free LF) was aspirated through a 20 µm nylon filter and rinsed with deionized water. The material on the filter was transferred into a beaker and dried at 55˚C overnight. The material remaining in the centrifuge tube (iPOM, sand, silt and clay) was rinsed twice with deionized water, flocculated with 5 drops of 0.25 M $CaCl_2$ and 0.25 M $MgCl_2$ and centrifuged at 20˚C for 15 min at 2,500 rpm. Twelve 6 mm glass beads were added to each centrifuge tube, which were then placed on a reciprocal shaker for 18 h. Samples were removed from the shaker and sieved with nested 250 and 53 µm sieves for macroaggregate samples and a 53 µm sieve for microaggregate samples. Material remaining on the sieve (iPOM+Sand) and material washed through the sieve (Silt+Clay) were dried at 55˚C overnight (Fig. 1).

Aggregate Associated Carbon and Nitrogen

Samples (macro- and microaggregates and density floatation aggregate fractions) were analyzed for total C and N using the method described previously. Comparisons of C and N concentrations across sites are not valid unless corrected for sand (Elliott et al., 1991). The following formulas were used to calculate the sand free C content (Equation 2) and sand free N content (Equation 3) for each size class (Denef et al., 2001):

$$\text{Sand free } C_{fraction} = C_{fraction} * [\text{g aggregate}_{fraction}/(1 - \text{sand})] \quad (2)$$

$$\text{Sand free } N_{fraction} = N_{fraction} * [\text{g aggregate}_{fraction}/(1 - \text{sand})] \quad (3)$$

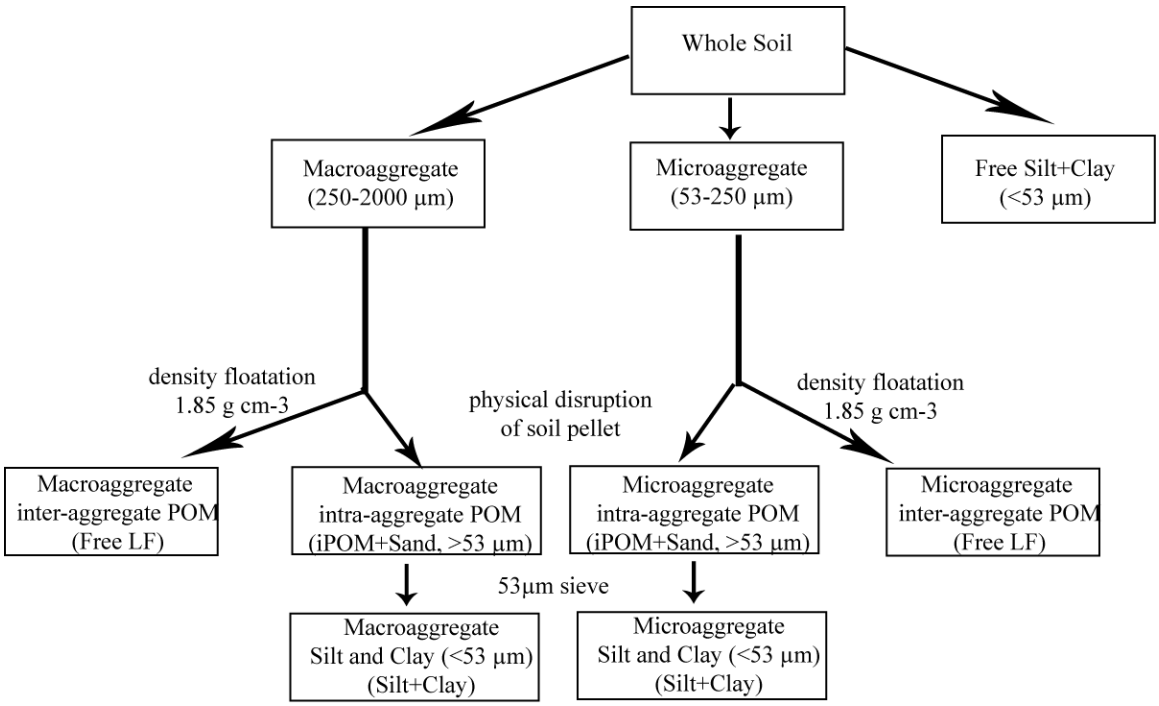

Figure 1. Flow chart of density floatations with acronyms used throughout text.

Microbial Community Analysis

Soil microbial community structure was conducted using phospholipids fatty acid methodology. Phospholipid fatty acids were extracted from 10 g soil samples using a modified Bligh-Dyer methodology (Bligh and Dyer, 1959; Frostegard and Baath, 1991; Buyer et al., 2002). 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; Buyer et al., 2002) and fatty acids were identified by retention time according to the MIDI eukaryotic method (MIDI Inc., Newark, NJ).

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).

PLFA signature biomarkers were used to quantify abundances of specific microbial groups in soil samples. Gram positive bacteria were 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). PLFAs were grouped into bacteria (gram positive & gram negative), fungi, mycorrhiza and actinomycetes.

It is important to note that only one PLFA biomarker (18:2 w6c) is used to indicate fungal biomass and just one (16:1 w5c) is used to indicate AM fungal biomass whereby a combination of 76 PLFA biomarkers is used to indicate gram negative bacterial biomass and 16 different PLFA biomarkers are summed to indicate gram positive bacterial biomass. Therefore, amounts of PLFA biomarkers indicated in the figures should not be used to compare biomass production for the different microbial groups discussed in this work.

Arthropod Analysis

Arthropods extracted from soil focused on four orders: Hymenoptera, Acari, Collembola, and Homoptera. Other arthropod orders were present (Diptera, Protura, Diplura, Aranae, Hemiptera, and Thysanoptera) in reclaimed soils examined but occurred irregularly and in low numbers. Arthropods captured in pitfall traps were separated into five orders: Coleoptera, Aranae, Diptera, Hymenoptera, and Acari. Other arthropod Orders were collected in pitfall traps (Orthoptera, Hemiptera, Homoptera, Collembola, Lepidoptera, Isopoda, and Chilopoda) but also occurred irregularly and in low numbers. No universal extraction method can be used for all arthropods due to the variation among organisms, biomes, and soil types (Walter et al. 1987; Macfadyen 1953). The two most commonly used are those separating arthropods from soil by physical methods (using flotation techniques) and those driving arthropods out as a behavior response to stimuli (such as heat, illumination and dessication). In this study, arthropods were extracted from 150 grams of soil using a modified kerosene flotation technique (Proctor 2001; Kethley 1991; Walter et al. 1987) using kerosene and ethanol to separate arthropods from soil by floating them to the liquid surface (Coleman et al. 1999). Once extracted, arthropods were enumerated and identified to order under a dissecting microscope. Total numbers of arthropods per sample were counted and expressed per 150 grams of soil.

Nematode Analysis

Taxonomic identification of nematodes to genus and species can be difficult. Therefore, ecologists interested in soil systems often identify nematodes according to their feeding habits (Yeates et al. 1993). This study categorized nematodes into four trophic groups which were distinguished by examination of mouthparts: (1) bacterivores: feed on bacteria only. Their mouth is a hollow tube for ingestion of bacteria. No stylet is present and the stoma is open; (2) fungivores: feed only on fungi. These nematodes use a stylet to puncture fungal hyphae; (3) herbivores: are plant parasites. Identification is based on the mouthpart having a needlelike stylet that is used to puncture cells; (4) omnivores: have an odontostylet, no bulb and possess a smooth cuticle.

Nematodes were extracted from 50 g soil, placed in kleenex and extracted in water, using the Baermann funnel technique for three days (Coleman et al. 1999). This methodology has several advantages and disadvantages. Advantages include being inexpensive, specialized equipment is not needed and it is easy to set up. Disadvantages include it is intended only for small soil samples and may not adequately represent the site, recovery of nematodes may be altered if the kleenex tissue becomes displaced or obstructs nematode movement and lack of aeration in the water of the funnel may reduce nematode movement and hinder recovery. Both a compound microscope and dissecting microscope (100 X) were used to identify and count different trophic groups. To prepare samples for counting, extracted nematodes were placed in a vial and positioned in a bath of warm water for 15 min. to slow movement of nematodes for easier identification. Nematode samples were transferred into a round 2 inch petri dish with lines engraved on the bottom for counting to ensure all nematodes within the sample were counted. If nematode numbers were greater than 100 for each sample a subsample was taken. Identification of nematode trophic groups was done within 4 days of extraction because specimens were stored in vials containing water and not preserved using formalin. Total number of nematodes per sample were counted and expressed as number per 50 grams of soil.

Results

Plant Community

Plant community composition differed throughout each chronosequence of sites due to vegetation succession. A shift in vegetation was apparent in the shrub chronosequence, where the newly reclaimed community consisted of mostly annual forbs (AF) with no shrub present

(Table 1). At the 5 yr old site, shrubs were present at high density and native cool season grasses also were present (NCS). At the 10 yr old site, perennial forbs (PF) and warm season grass (WS) were established and the shrub density had started to decline. The 16 yr old site was dominated by NCS grasses and had a lower shrub density than the 10 yr old site. Canopy closure, low shrub density and invasion of *Bromus tectorum* were the main characteristics of the native shrub community (data not shown).

The native site at the Belle Ayr mine had significantly greater species diversity ($p<0.001$) than the reclaimed sites (Table 1). The <1 yr old site of the cool season grass chronosequence was dominated by NCS grasses. As reclamation age increased, there was a decrease in cool season grass and annual forbs production and an increase in warm season grass and perennial forb production. The undisturbed, native site had a mixture of cool season and warm season grasses. Cover was greater in the 14 yr old reclaimed site and the native site compared to the other reclaimed sites.

Table 1. Plant community characteristics at the two reclaimed chronosequence sites.

	Shrub					**Cool Season Grass**			
	<1	**5**	**10**	**16**	**Undist.**	**<1**	**14**	**26**	**Undist.**
Production (g m^{-2})	14^{a}	39.6^{c}	46.5^{c}	75.7^{b}	33^{c}	12^{d}	199^{a}	142^{b}	93.2^{c}
Diversity	0.8^{d}	0.97^{c}	1.03^{b}	0.77^{d}	1.11^{a}	0.08^{c}	1.05^{a}	0.98^{ab}	0.91^{b}
Shrub density	0^{e}	232^{a}	58^{d}	200^{b}	80^{c}	0^{b}	0^{b}	0^{b}	73^{a}

General Soil Properties

Reclaimed soils present on the cool season grass chronosequence at the Belle Ayr Mine were primarily of sandy clay loam texture (Table 2). Bulk density was lower in the undisturbed site compared to the reclaimed sites. Soil organic C and N increased with reclamation age and soil depth. Soil pH was lower in the native site than the reclaimed sites and EC generally decreased with reclamation age.

Table 2. General soil properties of a reclaimed chonrosequence of sites at the Belle Ayr Mine, Gillette, WY. Data from Wick, 2007.

Site Age	Depth	Sand	Silt	Clay	Bulk Density	SOC[1]	N	EC	pH
(yrs)	(cm)	g 100 g^{-1} soil			g cm^{-3}	Mg ha^{-1}	Mg ha^{-1}	µS cm^{-1}	
<1	0-5	39	34	27	1.26± 0.07	5.54± 0.06	5.90± 0.00	516.90± 17.06	7.8± 0.1
	5-15	36	35	29	1.35± 0.06	9.85± 0.05	11.20 ± 0.00	555.90± 35.48	7.6± 0.1
14	0-5	38	28	34	1.20± 0.06	19.68± 0.24	6.85± 0.01	371.30± 12.81	7.6± 0.1
	5-15	38	26	36	1.33± 0.04	14.23± 0.08	18.20± 0.00	368.80± 11.11	8.1± 0.1
26	0-5	38	33	29	1.21± 0.04	13.43 ± 0.33	7.94± 0.02	413.90± 36.07	7.0± 0.1
	5-15	37	30	33	1.38± 0.02	13.39± 0.06	9.61± 0.00	305.00± 19.82	7.8± 0.1
Undisturbed/ Native	0-5	53	26	21	1.34± 0.04	9.92± 0.10	8.11± 0.01	156.50± 17.54	6.6 ±0.1
	5-15	54	21	26	1.44± 0.04	14.26± 0.11	12.00± 0.01	186.10± 17.04	6.9± 0.1

Aggregate Recovery Through Time

Soil macroaggregate (250-2000 µm) proportions under grasses at the Belle Ayr Mine were significantly greater in the reclaimed compared to the native soils (Fig. 2a). There was a significant increase in macroaggregate proportions between the <1 and 14 y old sites (Fig. 2a). Microaggregates within macroaggregates and macroaggregate associated silt and clay (<53 µm) followed the same trend, peaking in the 14 y old reclamation and were lower in the 26 y old reclamation and native site. (Fig. 2b). Between 50-60% of macroaggregate weight was attributable to the mass of mM for the 14 and 26 y old reclaimed sites and 35% for the native. Coarse POM + sand was lowest in the 14 y old reclaimed soils compared to the <1 and 26 y old reclamation and native site soil. No trends were observed for microaggregates or free silt and clay (<53 µm).

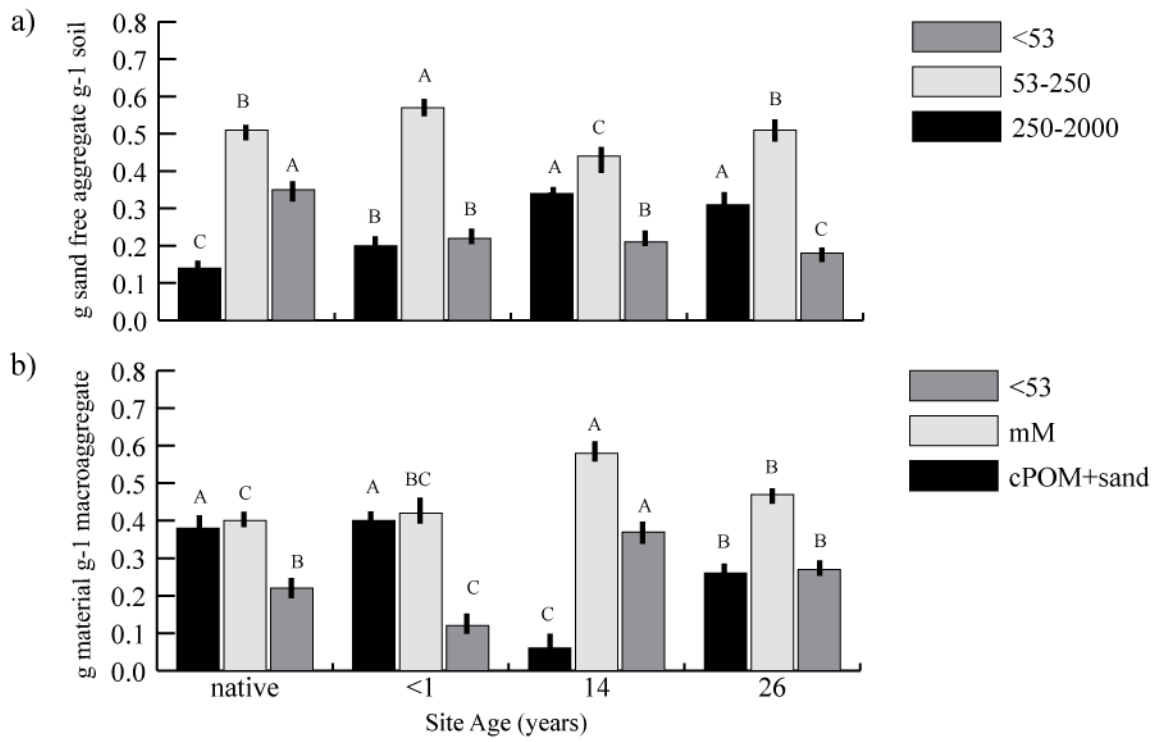

Figure 2. a) macroaggregates (250-2000 μm), microaggregates (53-250 μm) and silt and clay (<53 μm), and b) microaggregate within macroaggregate (mM) isolation for a grass chronosequence at the Belle Ayr Mine, Gillette, WY. For a given soil parameter, columns with a different letter above them are significantly different (P<0.05) among site ages. Bars represent one standard deviation, n=3.

Aggregate-associated Organic Carbon and Nitrogen

Carbon concentrations were reduced up to 50-70% and N concentrations by up to 70% for both macro- and microaggregates at the Belle Ayr Mine (Fig. 3a). Micro- within macroaggregate C and N values were close to 80% lower in <1 reclaimed sites than the native reference soil at Belle Ayr Mine (Fig. 3b). The greatest percentage loss (approximately 80%) of C and N was observed in the LF for macroaggregates (Fig. 3c). Macroaggregate iPOM+Sand C was reduced by 45-50%, while N decreased by 25-50%. Microaggregate iPOM+Sand C and N were reduced by 50-75% (Fig. 3d). Less change was observed in the macroaggregate Silt+Clay C and N concentrations with no losses in the macroaggregate fraction for soils located at Belle Ayr mine, but a substantial loss in the microaggregate Silt+Clay C and N between 40-60% was observed (Fig. 3e).

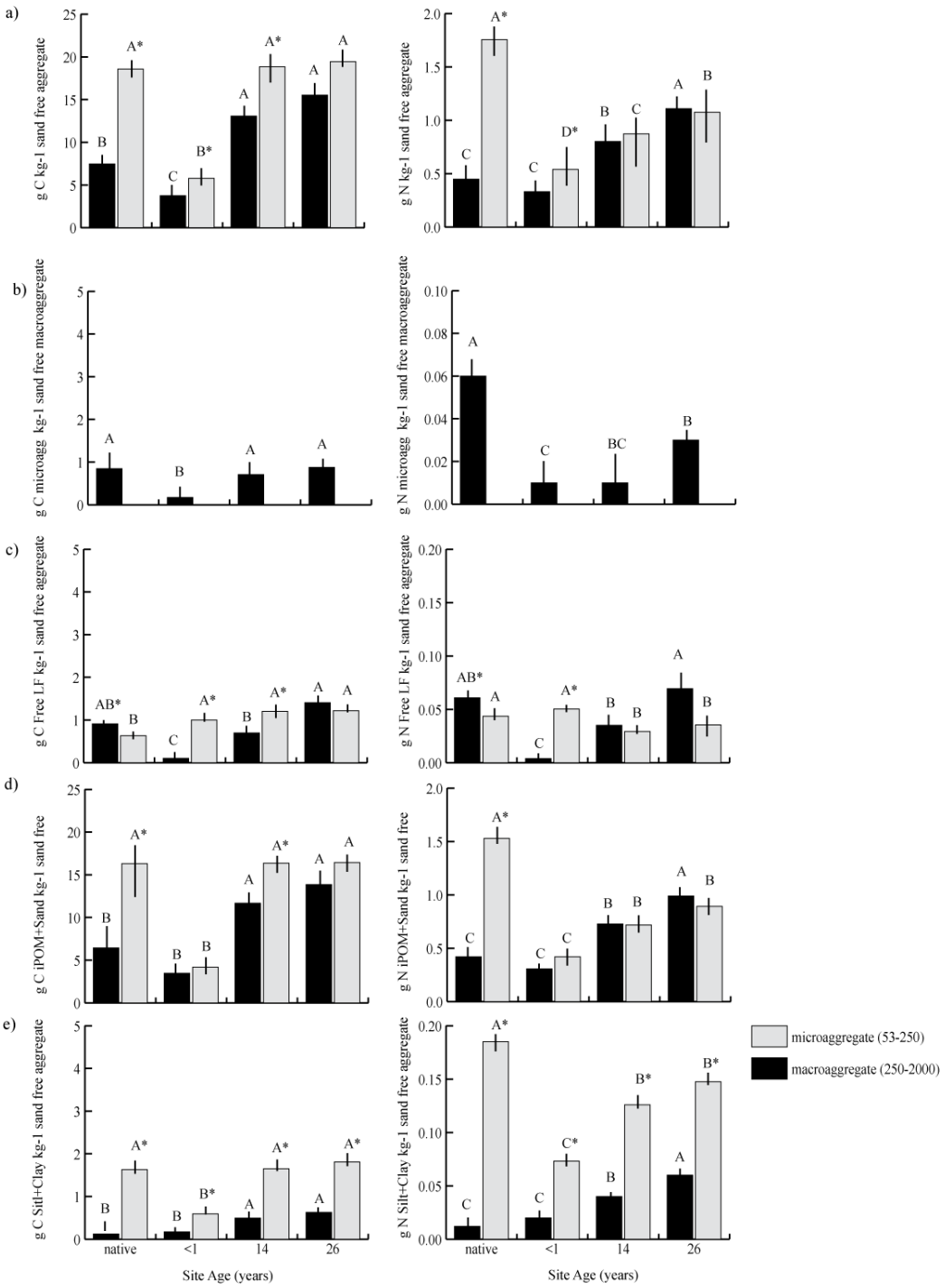

Figure 3. Carbon and Nitrogen concentrations in a) macro- and microaggregates b) micro-within macroaggregate (mM), c) aggregate associated light fraction (LF), d) aggregate associated heavy fraction (iPOM+Sand), and e) aggregate associated silt+clay fraction (Silt+Clay) a cool season grass chronosequence sites at Belle Ayr Mine, Gillette, WY. For a given soil parameter, columns with a different letter above them are significantly different ($P<0.05$) among site ages. Bars represent one standard deviation, n=3.

Within the reclaimed sites at Belle Ayr Mine, aggregate associated C was significantly lower in the <1 y old reclaimed soil than in the 26 y old reclaimed site for both macro- and microaggregate size fractions. Reclaimed soil macroaggregate C increased by 0.45 g C kg^{-1} sand free aggregate yr^{-1} and microaggregate C at are more rapid rate of 0.53 g C kg^{-1} sand free aggregate yr^{-1}. Microaggregate C was significantly higher than macroaggregate C for most fractions and site ages. Macro- and microaggregate N concentrations steadily increased with reclamation age. Macroaggregate N increased at a rate of 0.03 g N kg^{-1} sand free aggregate y^{-1} and microaggregate N increased at a slower rate (0.02 g N kg^{-1} sand free aggregate y^{-1}) in reclaimed soils.

Micro- within macroaggregate C increased significantly with reclamation age from 0.17 g C microaggregate kg^{-1} sand free macroaggregate in the <1 y old site to 0.88 g C microaggregate kg^{-1} sand free macroaggregate in the 26 y old reclaimed site. Micro- within macroaggregate N also increased significantly through time from 0.015 g N microaggregate kg^{-1} sand free macroaggregate in the <1 y old site to 0.029 g N microaggregate kg^{-1} sand free macroaggregate in the 26 y old reclaimed site. In newly reclaimed sites, C in the micro- within macroaggregate fraction comprised 5% of macroaggregate C concentrations. In the native reference site, micro-within macroaggregate C contributed 11% of macroaggregate C. Micro- within macroaggregate N contributed to <1% of macroaggregate N for all reclaimed sites and approximately 1% for the native reference site.

Macroaggregate Free LF C concentrations increased with reclamation age and reached concentrations observed in the native reference soil. Microaggregate Free LF C remained unchanged across reclamation age, but was higher than concentrations observed in the native reference soil. Again, a majority (90%) of aggregate C and N was found in the iPOM + Sand material; therefore, C and N in this fraction followed similar trends to aggregate C and N concentrations with reclamation age.

Microaggregate Silt+Clay C concentrations were the same or higher as that observed in the native soil for the 14 and 26 y old sites. Macroaggregate Silt+Clay N also increased with reclamation age. It is important to note that all C and N concentrations could be artificially inflated by coal dust contamination despite our best attempts to remove any coal material to analysis.

Microbial Community

Differences were observed in soil PLFA content at the different aged reclamation sites and the undisturbed site (Fig. 4). Total PLFA concentration in soil for 0-5 cm and 5-15 cm depth ranged from 6.4 to 24.9 nanomoles PLFA g^{-1} soil and 4.05 to 10.48 nanomoles PLFA g^{-1} soil, respectively, in the Belle Ayr Mine chronosequence. Phospholipid fatty acid content of soil from the undisturbed, 14, and 26-year-old reclaimed sites was significantly greater than in the 18-month-old soil (Fig. 4).

Biomarker PLFAs for both Gram positive and Gram negative bacteria at both depths were at their lowest concentration in soil in 18 month old reclaimed soil (Fig. 4). At both 0-5 and 5-15 cm depths, Tukey tests of biomarker indices showed no significant difference between the undisturbed site, the 14 year old and the 26-year-old reclaimed sites in terms of Gram positive and Gram-negative bacterial biomarkers.

The 18-month-old soil contained the lowest concentration of actinomycete biomarker. At 0-5 cm depth, the actinomycete biomarker was at its highest concentration in soil at the in 14 yr old site. This site contained more actinomycete biomass than 26 yr old, native and 18 month old soils. Unlike other PLFAs, mean soil concentration of actinomycete biomarkers at the 26 yr old site was generally slightly greater at the 5-15 cm depth than the 0-5 cm depth (Fig. 4).

The mean value for amount of fungal biomarker was lowest in soil from the 18-month-old reclaimed site. Concentration of fungal biomarker was greatest in soil from the undisturbed site and reclaimed 26 yr old site than any other reclaimed sites ($p<0.001$; Fig. 4) at the 0-5 cm depth whereas at the 5-15 cm depth, no significant ($p>0.67$) difference was found among the sites. For the 0-5 cm depth, AMF biomarker was lowest in soil from the 18-month-old site. The 26 year old site had a significantly ($p<0.001$) greater relative amount of AMF biomass. At the 5-15 cm depth, both 14 year old and 26 year old reclaimed site had greater relative proportion of AMF as shown by a Tukey test (Fig. 6). Bacterial to fungal biomass ratios as indicated by PLFA biomarkers in soil at the Belle Ayr Mine were 0.78, 0.74, 2.0 and 2.9 for undisturbed soil, 18 months old reclaimed soil, and 14 years and 26 years old reclaimed soils, respectively. The high biomass found in 26 years old reclaimed soil was primarily composed of bacteria whereas the fungal component in this soil was present with similar amount of biomass as in the native site. The PLFA analysis therefore, indicates that 14 and 26 yr old reclaimed sites have already approached fungal and bacterial abundances higher than those present in the undisturbed soil.

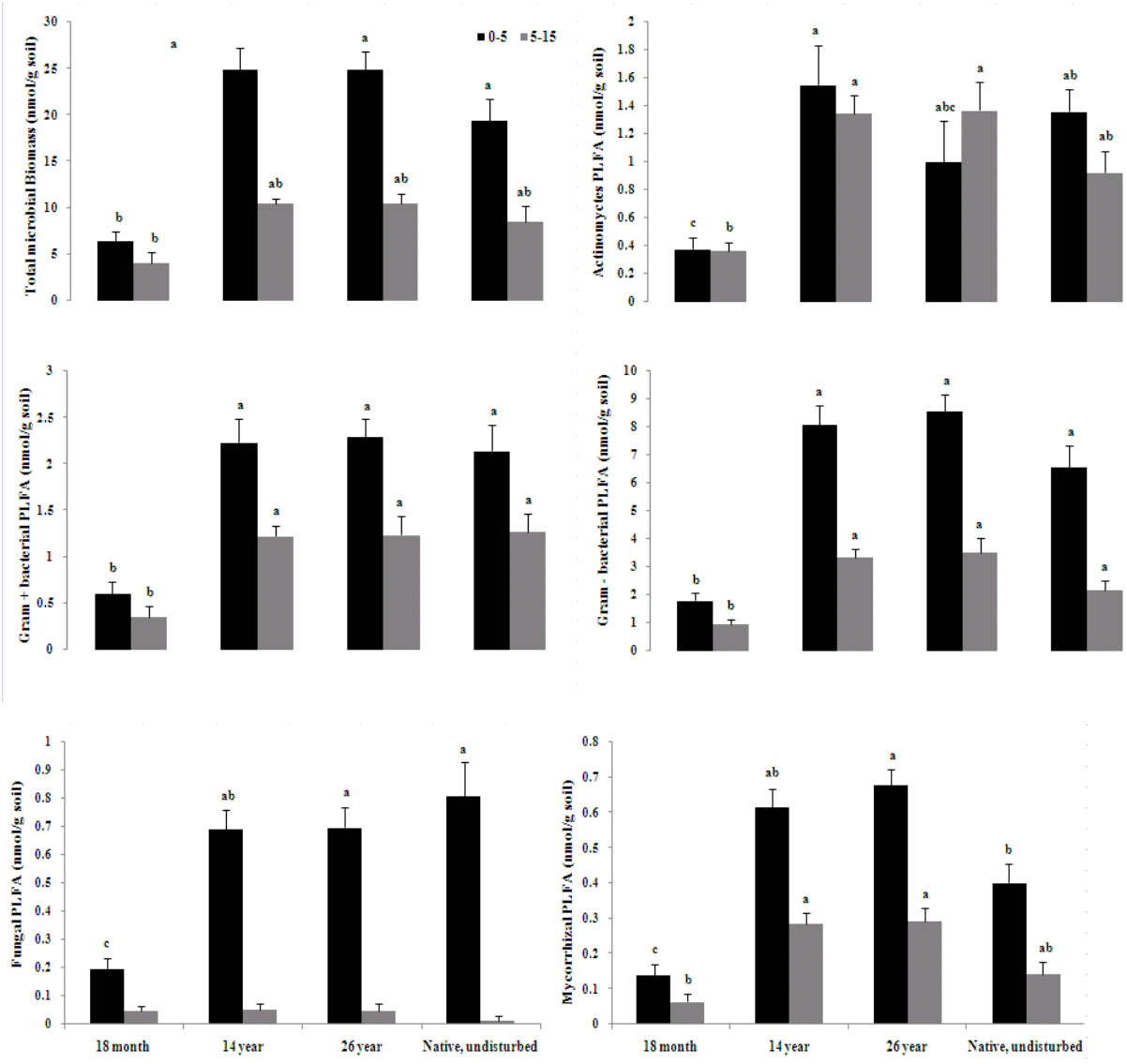

Figure 4. Phospholipid fatty acid biomarker content of chronosequence soils from the Belle Ayr Mine. Different letters within a given biomarker indicate significant differences at $P<0.05$.

Arthropods

Data on arthropod numbers from the different aged sites and depths for Belle Ayr Mine are shown in Fig. 5. Acari were the dominant order found in the reclaimed and undisturbed soil at both depths. Acari reached the same levels as the undisturbed soils within 10 months at the topsoil stockpile site at both depths. Hymenoptera recovered within 15 yr after reclamation at

both depths, however, a decrease in numbers were found in the 27 yr old reclaimed soil at both depths. Collembola had greater numbers in the 15 yr old and 27 yr old reclaimed soil than the undisturbed soil in the 0-5 cm depth and recovered within 10 months at the topsoil stockpile site in the 5-15 cm depth. Homoptera recovered within 15 years after reclamation in the 0-5 cm depth, however, never fully recovered in the 5-15 cm depth.

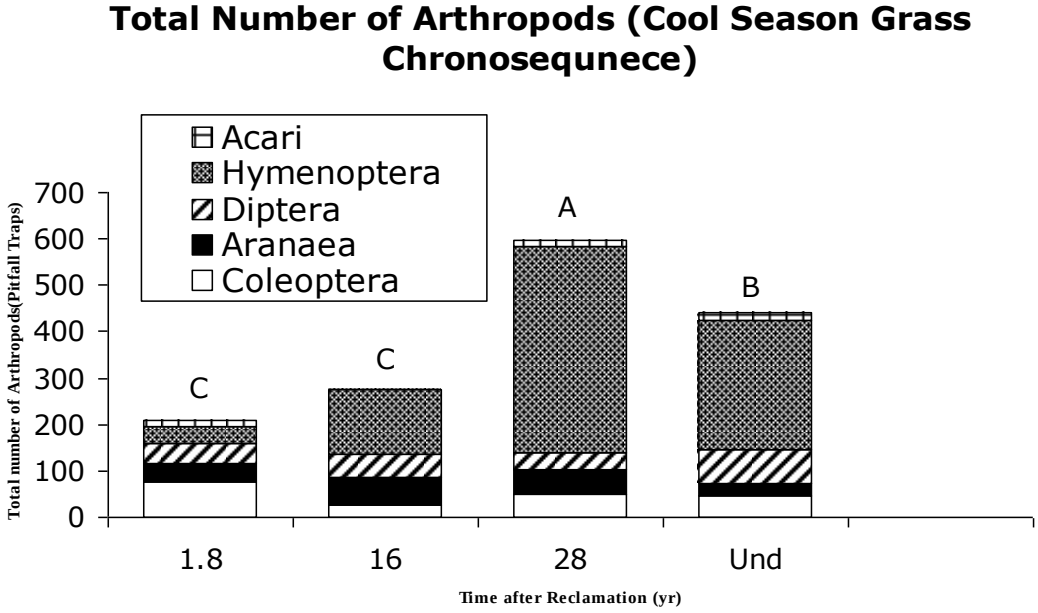

Figure 5. Total numbers of arthropods at the different aged reclaimed sites for taxa of arthropods from the chronosequence at the Belle Ayr Coal Mine. Statistical significance was determined at $P<0.05$.

Total arthropod numbers of arthropods appear to be recovering over time after reclamation (Fig. 5). Total numbers for all four orders at both depths were similar to those of the undisturbed site, except for the 10 month old stockpile site at 5-15 cm. Acari were the dominant arthropod order found in reclaimed and undisturbed soils at both depths. Arthropod assemblages in the 0-5 cm depth were comprised of Acari (52-78%), Collembola (9-28%), Hymenoptera (9-22%), and Homoptera (5-10%). At the 5-15 cm depth, arthropod assemblages consisted of Acari (41-69%), Collembola (22-32%), Hymenoptera (3-26%), and Homoptera (0-9%).

Nematodes

Data on nematode numbers from the different aged sites and depths at the Belle Ayr Mine are shown in Fig. 6. Bacteria feeding nematodes were the most common trophic group in the reclaimed soils and undisturbed soils at both depths. Bacteria feeding nematode numbers had not yet recovered after reclamation in the 0-5 cm depth but had within 27 yr after reclamation in the 5-15 cm depth. Fungivores recovered within 27 yr post-reclamation in the 0-5 and 5-15 cm depth similar to those of the undisturbed soils. Herbivores recovered within 11 yr after reclamation at both depths. Ominvores had not yet recovered to the same levels as the undisturbed soils at both depths.

Bacteria feeding nematodes were the dominant trophic group found in reclaimed and undisturbed soils at both depths. Nematode assemblages in the 0-5 cm depth comprised of bacterivores (44-49 %), fungivores (38-47%), herbivores (0-4%), and omnivores (5-13%). At the 5-15 cm depth, nematode assemblages consisted of bacterivores (41-56%), fungivores (34-39%), herbivores (1-7%), and omnivores (7-16%).

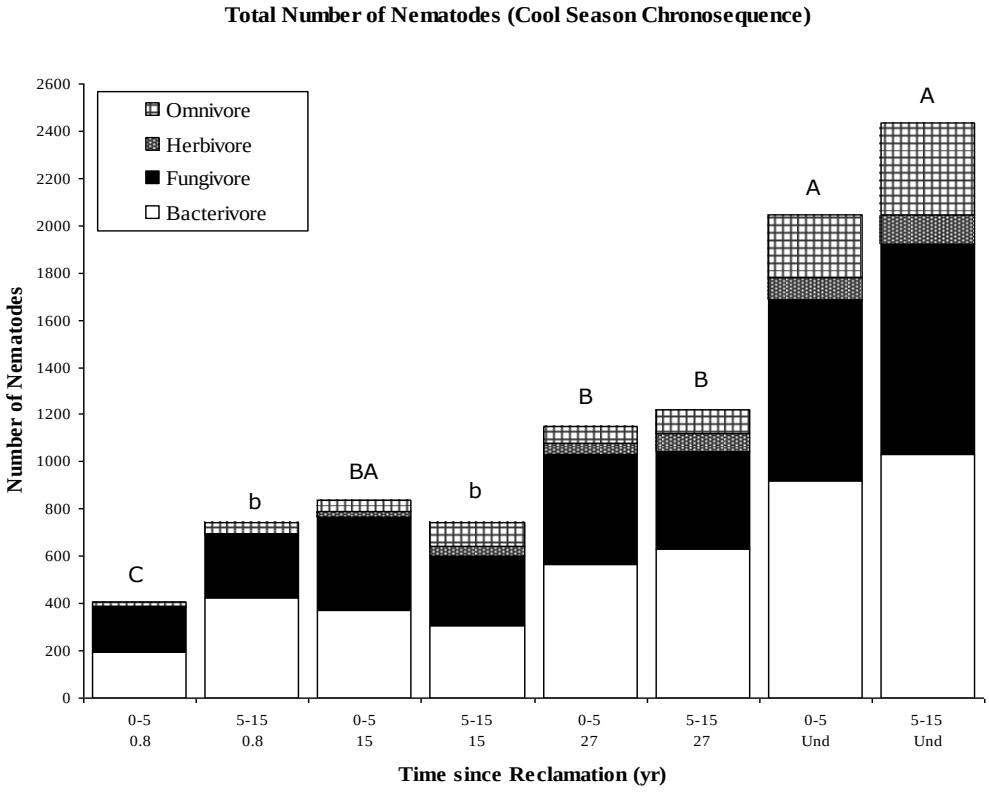

Figure 8. Total number of nematodes, by depth and time since reclamation was initiated, for trophic groups from the chronosequence at the Belle Ayr Mine.

Discussion

Ecosystems can be characterized in terms of their structure (the biota and the physical environment) and processes (transfer of energy and materials between organisms and the physical environment) (Chapin et al., 2002). At least 5 independent control variables, or state factors**: climate, parent material, topography, potential biota, and time** control the structure and processes in ecosystems (Jenny, 1941; Amundson and Jenny, 1997). The state factors driving recovery of reclaimed minelands examined in this study may not be the same as they have been historically. For example, problems with invasive species of plants in the Powder River Basin are altering the potential biota of the area. This could be extremely important in influencing the trajectory of this ecosystem.

Environmental conditions at the research sites we examined in this study appear to be returning to those present at nearby undisturbed sites and, presumably, to what they were before disturbance. Certainly this is true of climatic conditions, which are not affected by mining, although they may have been affected by climate change over the past 25 years. The soil physical and chemical characteristics we measured, with two exceptions, are ameliorating over time to conditions similar to those in undisturbed soil. This includes the redevelopment of soil structure as indicated by soil aggregate data. The exceptions are soil texture and pH. Texture is altered by mixing of soil horizons during topsoil salvage, transport and reapplication. This may also be true of soil pH.

Data presented in this study indicate the populations, assemblages, and communities of organisms examined are recovering from disturbance in that almost all are increasing in numbers or biomass through time towards amounts found in nearby undisturbed soil. Indeed, most groups of organisms examined in this study, appear to be more productive at the oldest reclaimed sites than they are in nearby undisturbed soil. Exceptions to this observation include nematode assemblages which, 27 years after reclamation was initiated, are not present in the numbers found in undisturbed soil. A very typical pattern of recovery observed in this study was low levels of production in early stages of reclamation increasing to levels greater than or equal to those found in undisturbed soil within 10 or 20 years.

Methods employed in this study, with the exception of plant community analysis, are not primarily designed to assess diversity. Diversity of the reestablished plant communities was either lower or similar to those of nearby undisturbed sites. Reestablished plant communities,

however, included more non-native and invasive species than did undisturbed plant communities. Diversity of soil microbial communities (data not shown) was calculated based on diversity of fatty acids extracted from soils (Yao et al., 2006; Zak et al., 1994) and indicated similar levels of diversity were found in the oldest reclaimed sites as in the undisturbed soils examined. Microbial communities in reclaimed soils may also include more weedy species than in undisturbed soil, but this is impossible to determine using the methods we chose.

Ecosystem processes examined in this study (soil organic matter dynamics, biomass production, and soil development) also appear to be returning to rates and outcomes similar to those monitored in undisturbed sites. Possibly because they are in early stages, processes such as soil development, biomass production and carbon storage may be occurring at rates greater than in undisturbed soil. Certainly, measured biomass production and carbon accumulation rates are greater in reclaimed soil than in undisturbed soil.

In conclusion, because our data indicates the large majority of ecosystem components and processes we examined are returning to levels and rates similar to or greater than those of adjacent undisturbed sites, we conclude that reclaimed surface mine ecosystems we studied in the Powder River Basin are recovering. As mentioned above, our methods did not allow us to fully address the question of biodiversity, or for that matter environmental diversity/spatial heterogeneity.

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