An application of space-for-time substitution in two post-mining chronosequences under rehabilitation

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Long-term monitoring of post-mining rehabilitation is inherently difficult and cannot be replicated, making the application of traditional experimental and statistical approaches for quantitative assessment of rehabilitation progress unattainable. An alternative approach to monitoring through time is space-for-time substitution; a technique used to infer a temporal trend from a study of different aged sites. In this investigation, space-for-time substitution was applied to two chronosequences of coal discard sites to determine the applicability of this approach to show the long-term effect of rehabilitation age on microbial communities. Sites at different stages of rehabilitation at separate locations (space) were identified to obtain a chronosequence of ages (time). Two chronosequences of rehabilitation ages from 1-11 and 6-17 years, respectively, were included, each with its own management regime. The long-term effect of the management regimes on soil microbial communities was investigated in terms of community function (enzymatic assays) and structure (phospholipid fatty acid profiles). Results showed no trends consistent with the rehabilitation ages of the respective sites for any of the investigated parameters. However, multivariate statistical analysis indicated a clear distinction between the chronosequences based on management regimes. This study shows the value of alternative statistical approaches in monitoring to elucidate long-term effects of management that might otherwise not be apparent.

Keywords: Coal discard, enzymatic activity, microbial community, PLFA, revegetation

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Introduction

Vegetative stabilisation is usually considered an effective means to achieve rehabilitation of mine discard sites. However, assessing the progress of rehabilitation on post-mining sites based merely on aboveground indicators is problematic and the necessity to consider the interaction between aboveground and belowground communities has been realised (Harris & Birch, 1989; Bartelt-Ryser et al., 2005). Furthermore, assessments in this context are complicated by the question of how to measure the success or failure of management practices applied to a particular site (Harris, 2003). It is widely accepted that the most reliable manner to measure change in an ecosystem and to gain an understanding of the basic structure and function of that ecosystem, is by long-term monitoring employing appropriate spatial and temporal scales. With reference to obtaining a realistic ecological assessment of a rehabilitation project, this implies monitoring the same site through time (Michener, 1997; Sparling et al., 2003). The inherent difficulties associated with the monitoring of rehabilitation processes combined with the fact that these investigations cannot be replicated or studied using traditional experimental approaches and statistical analyses (Michener, 1997), have necessitated the use of alternative investigative approaches to monitoring through time in order to quantitatively assess the success of rehabilitation.

One such approach is space-for-time (SFT) substitution (Michener, 1997; Sparling et al., 2003) – a technique used to infer a temporal trend from a study of different aged sites (Pickett, 1989). The SFT approach assumes that sites in different locations were initially similar, and that simultaneous sampling of different sites of increasing age is equivalent to resampling the same site through time. Therefore, when applying this approach, sites of different ages and stages of development at separate locations (space) are identified to obtain a chronosequence of ages (time) (Sparling et al., 2003). Applications of SFT substitution include studies on ecosystem dynamics (Purtauf et al., 2004; Dauber & Wolters, 2005; Martínez-Ruiz & Fernández-Santos, 2005) and investigations of recovery of ecosystems from various forms of disturbance (Pickett, 1989; Sparling et al., 2003).

The objective of this investigation was to apply SFT substitution to two chronosequences of coal discard sites to determine the applicability of this approach to show the long-term effect of rehabilitation age on microbial communities. This included sites aged 1 to 17 years constituting two chronosequences of rehabilitation ages from 1 to 11 years and 6 to 17 years, respectively. The long-term effect of the different management regimes on the soil microbial communities was investigated in terms of microbial community function (enzymatic activities) and structure (phospholipid fatty acid (PLFA) analysis).

Material and methods

Site description and sampling procedure

The two coal mines chosen for this study offered an opportunity to apply SFT substitution and in this way to compare the management regimes of the coal discard sites. Considering prior mining activities and prevailing environmental conditions (Claassens et al., 2008), the sites included in each of these chronosequences were considered to be comparable rehabilitations of different ages. The differences in manage-

ment regimes applied by the two mining companies included the application of soil cover layers of different depths. For rehabilitation of coal discard dumps, both mines applied a soil cover layer to discard material before revegetating the sites with a grass seed mixture. However, the soil cover applied at Mine B was deeper (30-60 cm) than that applied at Mine A (10-15 cm). Management practices applied after the initial revegetation of the discard sites also differed. Mining Company A applied management in the form of cutting and baling, as well as annual amelioration of soil cover layers. From 2003, the cutting and baling practice was replaced by a system of controlled grazing by livestock at all sites. In contrast, Mining Company B followed a less intensive management regime and only applied grazing to all sites with no application of fertilisers or organic material after initial revegetation.

At Mine A, seven discard dumps that varied between 1 and 8 years old, were sampled in the first year. After one year, the sites were resampled annually for two consecutive years to obtain a chronosequence of ages ranging from 1 to 11 years (ChrA). At Mine B, seven discard dumps between the ages of 6 and 17 years old, were sampled once-off (ChrB) (Table 1). For those ages with data from several sites (replicates), mean values and standard errors were calculated (Martínez-Ruiz & Fernández-Santos, 2005).

A random sampling design was used to obtain three composite samples per site (five cores per composite sample) of the soil cover layers from all coal discard sites. Although sites from Mine B had deeper soil cover layers than sites from Mine A, only the top 0-10 cm of the soil cover layer was sampled to facilitate comparison between the two chronosequences.

Table 1 Coal discard sites sampled to derive the two chronosequences of rehabilitation ages ranging from 1 to 11 years and 6 to 17 years, respectively

Rehabilitation Age (years)																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Mine A																	
Site 1	x		x	x													
Site 2			x		x	x											
Site 3				x		x	x										
Site 4				x		x	x										
Site 5				x		x	x										
Site 6				x		x	x										
Site 7								x		x	x						
Mine B																	
Site 1						x											
Site 2							x										
Site 3										x							
Site 4											x						
Site 5															x		
Site 6																x	
Site 7																	x

Estimation of vegetation cover

The ground and crown vegetation cover of all the sites were estimated in three 1 m^2 quadrats randomly placed over a 50 m transect. All plants rooted in a 1 m^2 quadrat were included in the estimation per plot. The ground cover included all living and non-living organic material on the ground surface per area and the crown cover was regarded as the canopy cover spread of all grass species over a fixed area. A quantitative value (percentage) was attributed to every plot and both values (ground and crown cover) were expressed as a percentage per m^2 surface area (Van Rensburg et al., 2004).

Physical and chemical soil analysis

Physical and chemical analyses of soil samples were conducted according to standard procedures. A 1:2 (v/v) water extraction procedure was conducted for the determination of the water-soluble basic cation fraction (Ca, Mg, K, and Na) (Peech, 1965). Quantification was done by means of atomic absorption spectrophotometry with a Spectr. AA-250 (Varian, Australia) using acetylene-air (Ramiriz-Munoz, 1968). The exchangeable cation concentration was measured by replacement of the exchangeable cations with ammonium by adding excess ammonium acetate solution to the soil samples (Simard, 1993) and analysed with a Spectr. AA-250 (Varian, Australia). The anions (Cl, NO_3, and SO_4) were quantified by means of ion chromatography (Metrohm 761 Compact IC, Switzerland). Concentrations of NH_4 were quantified by means of the ammonia-selective electrode method (Banwart et al., 1972). The pH and electrical conductivity (EC) of the soil was determined in the 1:2 extract with a calibrated pH/conductivity meter (Radiometer PHM 80, Copenhagen) at 25°C after a 12 h equilibration period with intermittent stirring. A P-Bray 1 analysis was also conducted to quantify the P concentration (Bray & Kurtz, 1945) and organic carbon was determined according to the Walkley-Black procedure (Walkley & Black, 1934). Particle-size distribution of all soil samples was conducted according to the procedures advocated by the American Society for Testing and Materials (ASTM, 1961).

Enzymatic activities

Before analyses, soil samples were passed through a 2 mm sieve. For the determination of dehydrogenase activity, soil was kept at field water content, while air-dried samples were used for determination of ß-glucosidase (EC 3.2.1.21), urease (urea amidohydrolase, EC 3.5.1.5), and acid (orthophosphoric monoester phosphohydrolase, (EC 3.1.3.2, pH 6.5) and alkaline (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1, pH 11.0) phosphatase activities (Dick et al., 1996). Soil water content was determined gravimetrically after drying soil samples at 105°C. Dehydrogenase and urease activities were assayed according to the procedures described in Alef and Nannipieri (1995). ß-glucosidase as well as acid and alkaline phosphatase activities were based on p-nitrophenol release after cleavage of a synthetic substrate (p-nitrophenyl glucoside and p-nitrophenyl phosphate, respectively) (Alef & Nannipieri 1995; Dick et al., 1996). Modified universal buffers, pH 6.5 and pH 11.0, were used for acid and alkaline phosphomonoesterase, respectively.

Total lipid extraction, fraction, and analysis

Total lipids were extracted from 5 g lyophilised soil according to a modified Bligh and Dyer procedure (Peacock et al., 2001) using a single-phase chloroform-methanol-aqueous buffer system in a ratio of 1:2:0.8 (v/v/v). Silicic acid column chromatography was used to fractionate the total lipid extract into neutral lipids, glycolipids, and polar lipids. The polar lipid fraction was transesterified to fatty acid methyl esters (FAMEs) by mild alkaline methanolysis (Guckert et al., 1985). FAMEs were analysed by capillary gas chromatography with flame ionisation detection on an Agilent 6890 series II chromatograph, using a 60 m SPB-1 column (0.250 mm I.D., 0.250 m film thickness). Methyl nonadecanonate (19:0) was used as a quantitative internal standard and definitive peak identification was made for representative samples by gas chromatography/mass spectrometry using an Agilent 6890 series II gas chromatograph interphased with an Agilent 5973 mass selective detector (McKinley et al., 2005). Microbial biomass was estimated as the total extractable phospholipid fatty acids (PLFAs) and microbial community composition was analysed on relative concentrations (mole percentages) of individual fatty acids. Standard fatty acid nomenclature was used (Guckert et al., 1985; Allison et al., 2005).

Statistical analysis

Statistical analyses were performed and graphs generated using Statistica 7.1 (Statsoft Inc., Tulsa, Oklahoma, USA), SigmaPlot 10.0 (Systat Software Inc., San Jose, California, USA), GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, California, USA), and Canoco for Windows 4.5 (Biometris – Plant Research International, Wageningen, The Netherlands; Ter Braak & Šmilauer, 1998).

Data of physical and chemical characteristics, enzymatic activities, and PLFA composition of samples were subjected to analysis of variance (ANOVA) followed by Tukey's test for mean separation where significant differences ($p = 0.05$) were indicated. Chronosequence data for Mine A and B were analysed using the 'Time Series Analysis' module in Statistica 7.1. Missing data (missing ages) embedded in either time series was replaced by using the 'predicted values from linear trend regression' option in Statistica. With this option, Statistica fits a least-squares regression line to the time series. The missing data are then replaced by the values predicted by this regression line.

Curve fitting was performed in GraphPad Prism 4.0 by using nonlinear regression. Due to the method chosen for predicting missing data points in the chronosequences, curve fits were also performed with linear regression. The p values obtained from both linear and nonlinear regressions after applying the F-test were then compared to determine whether there was consistency in the conclusions regarding the significant differences between the curve fits. Similarity or dissimilarity of fitted curves based on the F-test is expressed in terms of an F-ratio and corresponding p value. If $p < 0.05$, there is a statistically significant difference between the curves (Motulsky, 2007).

A canonical correspondence analysis (CCA) was performed to investigate the relationship between the sites from both chronosequences, based on microbial community function (enzymatic activities) and structure (PLFA profiles).

Results and discussion

Results from the physical and chemical characterisation of the soil cover layers, as well as percentage vegetation cover for all sites from Mine A and B, are summarised in Tables 2 and 3, respectively. In both cases (Mine A and Mine B), few of the physical and chemical properties showed significant differences between sites ($p < 0.05$, Tukey's test) and no trends consistent with the rehabilitation ages of the respective sites were apparent from this investigation.

The similarity in physical and chemical properties between sites was important in the application of SFT substitution to obtain chronosequences of rehabilitation ages. The 17-year old site from Mine B showed the highest percentage organic C of all sites sampled at this mine. It was also the oldest site sampled at either of the two mines during the investigation. However, the highest percentage organic C for all sites sampled from both mines was observed at the 11-year old site from Mine A. Although sites from both mines were grazed, sites from Mine A generally had higher organic C contents than sites from Mine B and this could be attributed to the addition of manure to sites managed by Mining Company A. Another observation that could be attributed to the different management regimes was that concentrations of P and NO_3-N varied less between sites from Mine B (Table 3) compared to the large variations between sites from Mine A (Table 2). Overall, sites managed by Mining Company B with a less intensive management regime (only grazing applied), had higher percentages of vegetation cover (Table 3) than sites managed by Mining Company A, which applied annual amelioration and grazing (Table 2). This was particularly obvious with regard to ground cover.

Table 2 Physical and chemical properties and vegetation cover of soil cover layers obtained from the coal discard sites managed by Mining Company A (Chr A) (Claassens et al., 2008)

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
First sampling							
Rehabilitation age (years)	1	3	4	4	4	4	8
Ca (mg kg^{-1})	25.10 (9.72)a	7.36 (0.27)a	3.73 (1.68)a	2.92 (0.53)a	3.13 (0.27)a	5.65 (1.29)a	49.30 (13.04)a
Mg (mg kg^{-1})	7.89 (4.41)a	2.08 (0.22)a	1.47 (0.64)a	0.92 (0.18)a	0.98 (0.16)a	1.22 (0.49)a	15.77 (3.40)a
K (mg kg^{-1})	5.90 (1.96)a	5.31 (0.78)a	25.08 (8.32)a	4.92 (1.26)a	12.59 (6.51)a	24.59 (9.31)a	6.29 (1.71)a
Na (mg kg^{-1})	1.56 (0.44)a	1.85 (0.12)a	1.21 (0.44)a	0.93 (0.12)a	0.87 (0.27)a	1.21 (0.17)a	2.08 (0.30)a
SO_4 (mg kg^{-1})	122.04 (71.69)a	28.27 (2.17)a	13.77 (4.72)a	25.62 (12.21)a	26.34 (6.06)a	32.62 (15.90)a	239.00 (72.57)a
NO_3-N (mg kg^{-1})	6.38 (5.15)a	6.38 (1.30)a	16.19 (6.59)a	1.31 (0.16)a	6.05 (3.50)a	11.45 (7.31)a	2.45 (1.23)a
NH_4-N (mg kg^{-1})	0.30 (0.04)a	0.26 (0.07)a	0.73 (0.23)a	0.43 (0.09)a	0.64 (0.07)a	0.47 (0.21)a	0.39 (0.07)a
Cl (mg kg^{-1})	5.35 (0.71)a	12.75 (5.20)a	5.71 (1.96)a	1.69 (0.32)a	4.01 (1.35)a	3.03 (1.03)a	2.59 (0.18)a
P (P-Bray1) (mg kg^{-1})	18.23 (9.75)a	5.60 (0.84)a	146.43 (65.07)a	27.37 (7.27)a	38.13 (16.77)a	96.77 (17.27)a	18.97 (7.53)a
Organic carbon (%)	0.31 (0.16)a	0.52 (0.10)a	2.20 (0.74)a	0.32 (0.08)a	0.67 (0.01)a	2.72 (1.36)a	0.69 (0.14)a
pH	7.41 (0.44)a	6.20 (0.26)a	7.03 (0.03)a	7.15 (0.50)a	6.24 (0.41)a	6.90 (0.37)a	5.51 (0.27)a
EC (mS m^{-1})	37.00 (4.36)a	21.67 (4.10)a	29.00 (6.27)a	26.33 (2.03)a	18.67 (6.23)a	35.00 (8.02)a	117.33 (46.25)a
CEC (cmol(+) kg^{-1})	7.06 (1.77)a	17.55 (1.92)a	9.34 (1.56)a	4.19 (0.61)a	4.90 (1.01)a	9.92 (3.95)a	24.34 (1.60)a
Base saturation (%)	112.29 (17.54)a	70.13 (12.70)a	98.94 (15.35)a	96.49 (13.94)a	61.28 (13.34)a	6.90 (0.37)a	54.33 (3.07)a
Sand (%)	58.70 (1.00)a	39.80 (2.62)a	68.18 (2.53)a	61.23 (1.62)a	68.12 (2.61)a	67.46 (0.91)a	79.22 (5.53)a
Silt (%)	34.27 (1.54)a	42.66 (1.76)a	23.42 (2.84)a	26.27 (2.61)a	20.93 (2.92)a	25.25 (2.83)a	8.46 (1.96)a
Clay (%)	7.02 (1.63)a	17.55 (0.86)a	8.39 (0.51)a	12.51 (1.10)a	10.95 (1.75)a	7.29 (2.26)a	12.31 (3.81)a
Ground Cover (%)	41.57 (15.67)ab	63.21 (23.72)a	71.25 (19.10)cd	28.92 (12.30)ab	86.75 (16.59)d	60.91 (14.97)bcd	77.42 (17.71)abc
Crown Cover (%)	45.71 (15.97)a	38.75 (12.36)b	80.13 (12.95)b	42.50 (22.11)a	84.83 (14.27)b	65.91 (19.98)ab	56.29 (19.85)b
Second sampling							
Rehabilitation age (years)	3	5	6	6	6	6	10
Ca (mg kg^{-1})	18.95 (2.91)a	25.00 (5.62)a	5.49 (1.22)a	13.51 (3.64)a	9.17 (6.96)a	7.56 (1.82)a	82.39 (19.82)ab
Mg (mg kg^{-1})	3.67 (0.57)a	7.52 (1.58)a	1.96 (0.51)a	1.83 (0.39)a	3.52 (2.95)a	2.69 (0.99)a	9.68 (3.13)a
K (mg kg^{-1})	8.25 (0.81)a	5.92 (2.41)a	18.98 (6.31)a	9.78 (2.61)a	12.34 (7.96)a	25.57 (13.55)a	12.47 (5.57)a
Na (mg kg^{-1})	0.81 (0.19)a	3.76 (2.69)a	1.68 (0.37)a	1.04 (0.43)a	1.39 (0.67)a	2.37 (0.93)a	5.27 (5.76)a
SO_4 (mg kg^{-1})	42.29 (2.53)a	27.52 (11.54)a	10.75 (4.87)a	50.51 (11.47)a	45.31 (39.51)a	22.47 (10.97)a	28.17 (7.01)a
NO_3-N (mg kg^{-1})	1.72 (0.19)a	9.83 (8.70)a	10.28 (3.39)a	2.81 (1.44)a	6.30 (3.26)a	28.11 (11.35)a	10.14 (3.00)a
NH_4-N (mg kg^{-1})	1.13 (0.45)a	0.95 (0.83)a	1.02 (0.28)a	0.82 (0.33)a	0.57 (0.09)a	0.55 (0.13)a	3.04 (1.02)b
Cl (mg kg^{-1})	9.99 (0.93)a	17.3 (8.19)b	4.86 (1.64)a	4.91 (1.37)a	2.90 (0.96)a	2.59 (0.89)a	16.95 (9.19)b
P (P-Bray1) (mg kg^{-1})	15.37 (4.97)a	9.47 (1.06)a	149.34 (37.46)c	18.20 (5.06)a	53.61 (20.5)b	118.45 (15.12)c	30.07 (19.45)a

Organic carbon (%)	0.58 (0.05)a	0.67 (0.05)a	2.42 (0.24)a	0.78 (0.08)a	1.08 (0.27)a	2.33 (1.86)a	1.54 (0.36)a
pH	7.81 (0.15)a	7.32 (0.13)a	7.12 (0.31)a	6.57 (0.22)a	6.58 (0.36)a	6.86 (0.37)a	5.85 (0.28)a
EC (mS m^{-1})	47.33 (1.78)a	60.67 (25.26)a	36.33 (5.07)a	46.67 (6.68)a	43.67 (26.40)a	50.83 (18.00)a	60.00 (28.20)a
CEC (cmol(+) kg^{-1})	5.06 (0.59)a	5.86 (1.04)a	9.55 (1.37)a	3.48 (1.07)a	4.86 (0.54)a	8.50 (2.94)a	9.90 (0.97)a
Base saturation (%)	99.18 (8.56)ab	97.04 (5.29)ab	99.21 (10.56)a	75.61 (3.19)ab	76.59 (10.70)a	50.86 (11.37)b	75.25 (5.38)ab
Sand (%)	73.20 (0.32)a	47.53 (2.74)a	74.23 (1.57)a	88.17 (1.10)a	74.69 (3.80)a	74.90 (2.81)a	56.03 (0.55)a
Silt (%)	8.27 (0.50)a	23.30 (3.20)a	16.68 (1.56)a	11.67 (1.04)a	14.33 (3.74)a	16.21 (2.96)a	11.43 (1.99)a
Clay (%)	18.53 (0.52)ab	29.17 (1.04)b	9.11 (0.40)a	10.17 (1.06)a	10.99 (1.63)a	8.91 (1.92)a	32.53 (1.55)b
Ground Cover (%)	19.99 (1.75)ab	27.54 (2.71)ab	39.28 (1.03)b	15.11 (3.57)a	24.06 (6.40)ab	39.50 (12.50)c	15.92 (2.34)a
Crown Cover (%)	61.36 (3.21)a	63.09 (3.07)a	61.82 (3.99)a	41.72 (6.97)a	62.28 (9.62)a	64.02 (3.50)a	49.34 (6.55)a
Third sampling							
Rehabilitation age (years)	4	6	7	7	7	7	11
Ca (mg kg^{-1})	54.14 (18.70)ab	15.12 (6.53)a	7.62 (0.77)a	5.14 (0.57)a	15.22 (13.52)a	9.48 (2.35)a	12.23 (5.87)a
Mg (mg kg^{-1})	19.14 (7.01)ab	8.01 (4.65)a	2.45 (0.37)a	2.93 (0.47)a	6.05 (5.37)a	4.16 (1.49)a	14.17 (3.40)ab
K (mg kg^{-1})	40.42 (21.73)a	18.98 (10.19)a	12.88 (4.13)a	8.16 (1.94)a	12.10 (9.41)a	26.56 (17.80)a	35.11 (10.93)a
Na (mg kg^{-1})	11.68 (4.61)c	4.11 (1.84)a	2.14 (0.31)a	2.37 (0.19)a	2.19 (1.07)a	3.53 (1.69)a	10.01 (1.74)bc
SO_4 (mg kg^{-1})	18.50 (6.26)a	33.31 (17.84)a	7.73 (5.03)a	34.28 (15.70)a	12.32 (6.04)a	14.66 (2.03)a	26.5 (7.31)a
NO_3-N (mg kg^{-1})	135.55 (23.60)c	13.57 (8.13)a	4.37 (0.19)a	15.29 (5.79)a	6.55 (3.03)a	44.77 (15.39)b	160.19 (93.33)c
NH_4-N (mg kg^{-1})	0.73 (0.09)ab	0.73 (0.06)ab	1.32 (0.34)b	0.59 (0.11)ab	0.50 (0.06)a	0.64 (0.04)ab	1.00 (0.34)ab
Cl (mg kg^{-1})	30.41 (14.58)a	5.08 (4.75)a	4.01 (1.32)a	0.80 (0.19)a	1.78 (0.58)a	2.14 (0.76)a	29.70 (9.99)a
P (P-Bray1) (mg kg^{-1})	21.39 (3.66)a	15.15 (4.34)a	152.25 (9.58)b	67.16 (19.68)a	69.08 (24.24)a	140.13 (12.97)b	44.85 (5.24)a
Organic carbon (%)	31.35 (1.11)b	11.56 (0.02)a	6.18 (1.04)a	4.65 (0.09)a	8.82 (0.03)a	10.93 (0.06)a	46.88 (1.03)c
pH	7.52 (0.07)a	7.49 (0.34)a	7.21 (0.32)a	7.19 (0.18)a	6.89 (0.31)a	6.96 (0.38)a	7.52 (0.40)a
EC (mS m^{-1})	53.00 (25.52)a	56.00 (27.16)a	43.67 (3.89)a	42.33 (4.14)a	48.97 (20.07)a	56.67 (27.99)a	74.67 (44.72)a
CEC (cmol(+) kg^{-1})	4.91 (0.22)a	14.17 (2.05)a	9.77 (1.18)bc	5.45 (0.57)a	4.81 (0.07)a	7.07 (1.93)a	17.25 (0.87)c
Base saturation (%)	99.92 (6.68)a	99.01 (5.18)a	99.48 (5.78)a	99.88 (18.10)a	91.90 (8.05)a	94.82 (22.37)a	99.68 (15.76)a
Sand (%)	67.20 (0.56)a	47.67 (4.78)a	80.27 (0.61)a	84.47 (0.76)a	81.27 (5.00)a	82.33 (4.72)a	54.13 (3.97)a
Silt (%)	13.4 (1.00)a	24.43 (3.27)a	9.93 (0.29)a	6.67 (1.77)a	7.73 (4.56)a	7.17 (3.09)a	26.57 (2.61)a
Clay (%)	19.40 (0.88)b	27.90 (2.92)c	9.83 (0.29)a	8.87 (2.49)a	11.03 (1.50)a	10.53 (1.59)a	19.30 (1.42)b
Ground Cover (%)	19.11 (3.08)ab	26.33 (2.95)abc	37.56 (3.83)bc	14.44 (6.71)a	23.00 (9.25)abc	76.00 (3.37)d	15.22 (6.30)a
Crown Cover (%)	59.00 (1.67)ab	60.67 (2.59)a	59.44 (0.98)a	40.11 (3.41)b	59.89 (6.11)a	61.56 (11.95)a	47.44 (2.24)ab

Standard error values are indicated in brackets. Data in rows with the same letters indicate no significant difference, while those with different letters indicate significant difference at $p < 0.05$ (Tukey's HSD). EC: Electrical conductivity; CEC: Cation exchange capacity.

Table 3 Physical and chemical properties and vegetation cover of soil cover layers obtained from the coal discard sites managed by Mining Company B (Chr B)

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 5	Site 5
Rehabilitation age (years)	6	7	10	11	15	16	17
Ca (mg kg^{-1})	9.15 (5.00)b	3.43 (1.61)a	2.02 (1.40)a	10.58 (4.17)ab	2.21 (0.12)a	2.67 (0.71)a	2.22 (0.75)a
Mg (mg kg^{-1})	7.64 (2.36)b	3.18 (1.91)ab	4.65 (2.38)ab	5.14 (1.74)ab	2.38 (0.57)ab	2.01 (0.12)a	2.28 (0.22)a
K (mg kg^{-1})	4.03 (1.94)a	3.64 (1.14)a	6.39 (4.52)ab	17.11 (8.08)a	3.44 (0.53)b	4.62 (0.13)ab	3.04 (0.73)a
Na (mg kg^{-1})	4.63 (2.06)b	1.09 (0.18)a	1.39 (0.98)ab	0.29 (0.07)a	0.46 (0.14)a	1.74 (0.56)ab	2.08 (0.24)b
SO_4 ($mgkg^{-1}$)	0.72 (0.01)a	2.42 (0.01)a	0.72 (0.25)a	0.73 (0.01)a	4.34 (0.49)ab	3.04 (0.66)c	10.63 (1.07)bc
NO_3-N($mgkg^{-1}$)	0.46 (0.01)a	0.47 (0.02)a	1.72 (0.53)a	1.71 (0.51)b	0.38 (0.11)a	1.55 (0.19)a	1.25 (0.18)a
NH_4-N($mgkg^{-1}$)	0.32 (0.05)a	0.33 (0.06)a	0.32 (0.22)a	0.31 (0.05)a	0.27 (0.00)a	0.32 (0.06)a	0.41 (0.02)a
Cl($mgkg^{-1}$)	5.71 (1.53)a	1.87 (0.33)a	3.03 (1.14)a	2.49 (0.47)a	1.15 (0.28)a	2.31 (0.39)a	3.39 (0.22)a
P(P-Bray1)($mgkg^{-1}$)	7.32 (0.34)a	7.30 (0.44)b	7.06 (2.99)a	5.95 (0.57)a	7.44 (0.54)a	10.83 (3.22)a	9.89 (0.49)a
Organic carbon (%)	0.38 (0.14)a	0.33 (0.03)a	0.71 (0.24)a	0.93 (0.15)ab	0.35 (0.06)a	0.81 (0.27)a	1.58 (0.11)b
pH	6.17 (0.93)a	5.28 (0.53)a	5.94 (0.49)a	5.63 (0.12)a	5.20 (0.57)a	5.28 (0.13)a	5.38 (0.03)a
EC (mS m^{-1})	0.26 (0.09)b	0.08 (0.02)ab	0.10 (0.04)ab	0.19 (0.07)ab	0.06 (0.01)a	0.07 (0.00)a	0.06 (0.02)a
Sand (%)	80.10 (3.11)ab	81.13 (2.04)a	82.87 (8.59)a	71.30 (1.46)bc	76.26 (2.73)ab	85.23 (1.14)a	66.63 (3.54)c
Silt (%)	6.87 (1.20)a	3.97 (1.02)a	6.20 (3.48)a	13.94 (1.98)b	9.83 (2.02)ab	6.23 (1.06)a	8.90 (1.11)ab
Clay (%)	13.03 (2.05)a	14.90 (1.81)a	10.97 (2.75)a	14.77 (1.23)a	13.9 (1.27)a	8.53 (0.94)a	24.50 (3.80)b
Ground Cover (%)	45.00 (4.04)a	48.00 (7.60)a	66.67 (5.76)b	78.30 (2.88)b	43.30 (4.08)a	50.00 (0.00)a	43.33 (4.08)a
Crown Cover (%)	58.00 (2.80)bc	63.00 (5.70)ab	78.33 (2.87)a	97.30 (2.51)d	45.00 (8.66)c	65.00 (5.00)ab	76.67 (8.16)a

Standard error values are indicated in brackets. Data in rows with the same letters indicate no significant difference, while those with different letters indicate significant difference at $p < 0.05$ (Tukey's HSD). EC: Electrical conductivity.

An investigation into the microbial community function and structure of post-mining sites of different ages at Mine A indicated no relationship based on rehabilitation ages of the sites. Rather, the effect of management practices in the form of induced changes to the soil cover layers of the sites caused similar changes in microbial communities in all sites, irrespective of rehabilitation age (Claassens et al., 2008). The minimum and maximum values for enzymatic activities, PLFA composition, and PLFA ratios obtained for individual sites of ChrA and ChrB over the study period are shown in Table 4.

Table 4 Minimum and maximum values for enzymatic activities, phospholipid fatty acid (PLFA) composition, and PLFA ratios obtained for individual sites of ChrA and ChrB over the study period

	ChrA	ChrB
Enzymatic activities		
Dehydrogenase (g INF g^{-1} $2h^{-1}$)	24 – 340	47 – 122
ß-Glucosidase (g PNP g^{-1} h^{-1})	85 – 295	85 – 320
Alkaline Phosphatase (g PNP g^{-1} h^{-1})	212 – 1309	242 – 1168
Acid Phosphatase (g PNP g^{-1} h^{-1})	343 – 1260	773 – 1278
Urease (g NH_4-N g^{-1} $2h^{-1}$)	14 – 73	15 – 61
PLFA analyses		
Normal saturated fatty acids	20.5 – 49.2	33.1 – 41.8
Mid-chain branched saturated fatty acids	2.1 – 5.0	2.4 – 4.1
Terminally branched saturated fatty acids	18.1 – 30.3	16.0 – 31.4
Monounsaturated fatty acids	18.3 – 41.8	22.5 – 40.2
Polyunsaturated fatty acids	5.2 – 17.1	0.8 – 8.0
Viable microbial biomass (pmol g^{-1} dry weight)	1656 – 16277	4247 – 16318
Fungal / Bacterial ratio	0.07 – 0.32	0.02 – 0.23

For a comparison of trends in ChrA and ChrB, the most pertinent microbial properties, as determined from previous investigations (Claassens et al., 2008) were selected (Figures 1.1 to 1.7). The Y-axes were scaled according to the highest activities or values observed for individual samples during the study period. The embedded line graphs represent fitted curves for the relevant data indicated on the graph for each chronosequence. A comparison of curves was performed between chronosequences to determine the best fit for each data set. In those cases where the same fit was indicated as the preferred model for both curves, the F-test was performed to compare the curves. Models fitted to the data by linear regression are not shown on the graphs and are only discussed in those instances where opposing conclusions pertaining to significant differences ($p = 0.05$) occurred. In the majority of the cases, however, models fitted by linear and nonlinear regression gave the same result regarding the similarity of the fitted curves.

In order to compare curves derived from different data sets, it is necessary to compare them as models (Motulsky & Christopoulos, 2005). The first model was based on two distinct curves - separate curves were fitted and the overall sum-of-squares (SS) was defined as the total SS for each separate curve. The second model used global fitting – all the data were fitted at once, finding one shared value of the measured parameter. In this way, the F-test could be applied to evaluate different curves and a comparison was possible between entire curves instead of only between single parameters. The F-test is an adaptation of ANOVA and is based on the difference between the SS of two models (in this case, the two chronosequences). The question is whether the difference between SS values is greater than would be expected by chance. It also takes into account the number of data points and the number of parameters of each model. The result is expressed as the F-ratio from which a p value is calculated. The p value tests the null hypothesis that there is no difference between the two curves overall and any observed difference is due to chance. If all variability was random – in other words, if the two chronosequences really were the same and differences between them were due to chance, F would be near 1.0. In this case, the resulting p value would not be significant ($p > 0.05$) and a conclusion can be made that the curves did not differ in a statistically significant manner (Motulsky & Christopoulos, 2005; Motulsky, 2007).

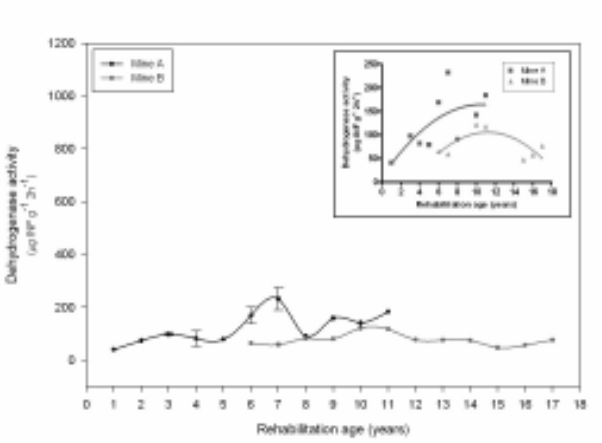

Figure 1.1

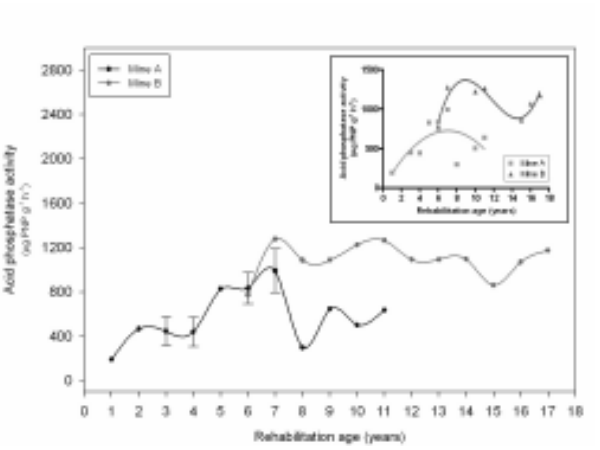

Figure 1.2

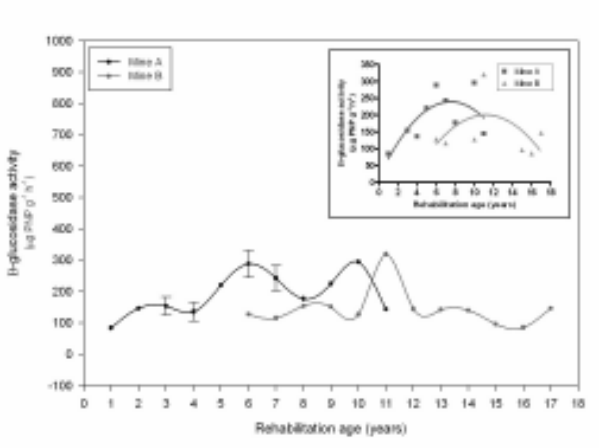

Figure 1.3

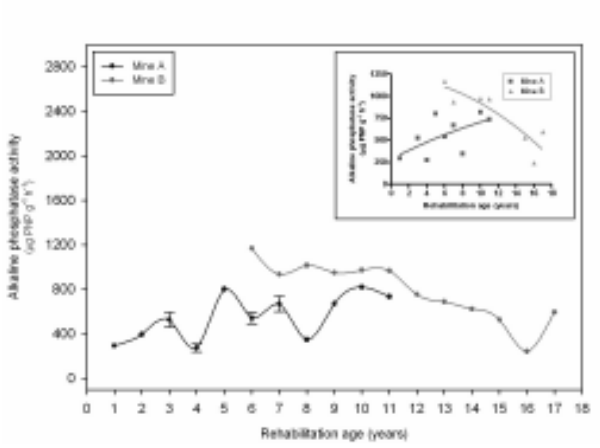

Figure 1.4

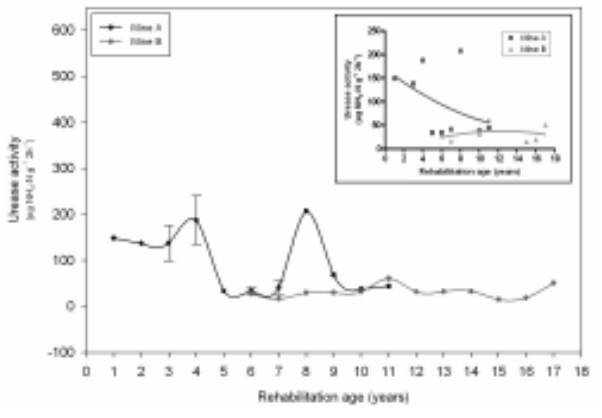

Figure 1.5

Figure 1.1-1.5 Changes in enzymatic activities during rehabilitation in the chronosequences from Mine A and B, respectively, with (1.1) – dehydrogenase, (1.2) -β-glucosidase, (1.3) – alkaline phosphatase, (1.4) – acid phosphatase, (1.5) – urease. The embedded graphs indicate the curve fits for each chronosequence

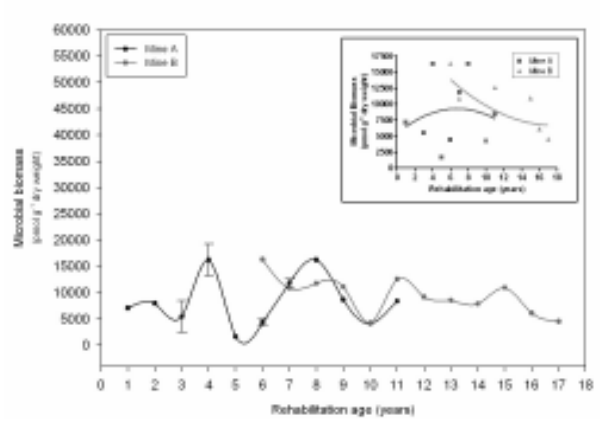

Figure 1.6 Changes in microbial biomass during rehabilitation in the chronosequences from Mine A and B, respectively. The embedded graph indicates the curve fits for each chronosequence

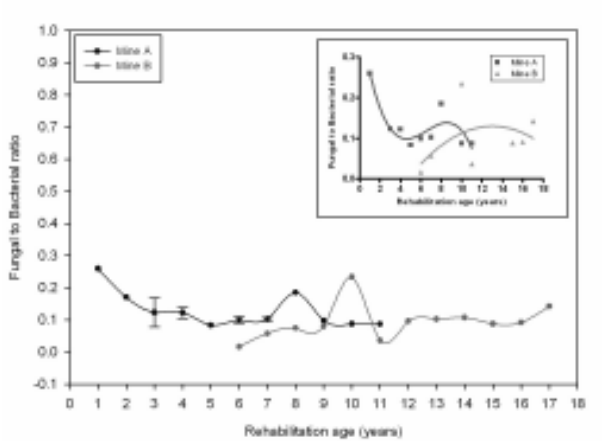

Figure 1.7 Changes in the fungal to bacterial ratio during rehabilitation in the chronosequences from Mine A and B, respectively. The embedded graph indicates the curve fits for each chronosequence

Dehydrogenase activity (Figure 1.1), which is an indicator of overall microbial activity (Taylor et al., 2002), fluctuated in both chronosequences with higher activities observed in older sites from Mine A (ChrA) when compared to younger sites within the same chronosequence. Mine B (ChrB) sites had higher activities during the first half of the rehabilitation period. ChrA had higher dehydrogenase activities and more pronounced fluctuations than ChrB, which may be related to the input of fertilisers and lime that is characteristic of the management regime applied at Mine A. A comparison of curve fits indicated a second order polynomial ($y = a + bx + cx^2$) as the preferred model for data sets from both chronosequences. The F-test applied to the two fitted curves indicated no significant differences between these fits ($F = 1.98$; $p = 0.18$). It could therefore be concluded that the temporal trends in these two data sets were essentially similar.

The same conclusion regarding similarity of the two data sets was reached for β-glucosidase activity based on the F-test ($F = 1.20$ and $p = 0.36$) (Figure 1.2). This enzyme was assayed for its relevance to the C cycle (Turner et al., 2002). Activities for ChrA and ChrB were in the same range (84.45 – 295.33 µg PNP g^{-1} h^{-1} and 84.53 – 319.54 µg PNP g^{-1} h^{-1}, respectively. Fluctuations in this chronosequence were less pronounced than those in ChrA. As in the case of dehydrogenase activity, the temporal variations in β-glucosidase activity can be ascribed to management inputs.

The changes in alkaline and acid phosphatase activities during rehabilitation in ChrA and ChrB are shown in Figure 1.3 and 1.4, respectively. Phosphatases are involved in organic phosphorus transformations in soil and their activities are sensitive to management practices and soil pH (Dick et al., 2000; Aon et al., 2001). Alkaline phosphatases are produced by microorganisms, while acid phosphatases are mainly attributed to plant roots (Criquet et al., 2004). Alkaline phosphatase activity (Figure 1.3) showed fluctuations over the rehabilitation period for ChrA, with higher activities observed in the older sites compared to younger sites. Conversely, the activities for ChrB decreased with increasing age of the rehabilitation and only showed an upward tendency again in the 17-year old site. When related to the microbial origin of alkaline phosphatase, these trends may indicate the sensitivity of the microbial community to management practices. As in the case of the other enzymatic activities, a second order polynomial was indicated as the preferred curve fit for data from both chronosequences. However, the p value calculated from the F-ratio for these data was close to 0.05 and while strictly no significant differences existed between the two fits based on the F-test ($F = 3.49$ and $p = 0.06$) the data were also fitted with linear regression models. These indicated that the difference between the slopes was significant ($p = 0.001$). ChrA showed clear changes in acid phosphatase activity (Figure 1.4) over the rehabilitation period, whereas activities for ChrB varied to a lesser extent and was higher over the rehabilitation period. Curve fit comparisons for acid phosphatase activity indicated different models as the preferred fits for each chronosequence. Chronosequence data from Mine A was fitted to a second order polynomial, while a third order polynomial ($y = a + bx + cx^2 + dx^3$) was fitted to data from Mine B. Accordingly, the temporal trends that existed in these data were different for the two chronosequences. The higher vegetation cover observed at Mine B (Table 3, 4) and the association of acid phosphatase activity with plant roots, may be the reason for these dissimilar trends. This clearly shows the sensitivity of acid phosphatase activity to management practices. Two distinct management regimes result in different percentages in vegetation cover and are reflected in two sets of data with markedly different trends over the long-term.

Urease activity (Figure 1.5) in soil has been correlated with microbial biomass (Klose & Tabatabai, 2000) and is measured for its relationship to the N cycle (Gil-Sotres et al., 2005). The changes in urease activity were greater for ChrA than for ChrB, which had relatively stable urease activities over the rehabilitation period. This could be attributed to the application of fertilisers, specifically nitrates, to sites at Mine A compared to Mine B where no fertiliser inputs occurred. Even though sites from Mine B had lower urease activities, the minimum activities for these sites were similar to the minimum activities for sites from Mine A. Despite the dissimilarity in urease activities between the two chronosequences, a comparison of curve fits indicated no significant differences between these fits ($F = 0.61$; $p = 0.63$). This shows similar tendencies in urease activity for the different chronosequences.

The estimation of microbial biomass by means of PLFA analysis is a reliable manner in which the viable microbial community can be quantified (Caldern et al., 2000; Rütters et al., 2002). In addition, PLFA profiles can signify changes in the bacterial and fungal composition of a soil (Ibekwe & Kennedy, 1998; Hill et al., 2000) and the ratio of fungal to bacterial PLFA (F:B ratio) has been applied to measure soil recovery (Bardgett & McAlister, 1999). Estimations of viable microbial biomass varied markedly between sites in individual chronosequences over the rehabilitation period – especially in ChrA (Figure 1.6). The biomass abundance in both chronosequences varied within a comparable range (1656.22 – 16277.30 pmol g^{-1} dry weight for Mine A and 4247.11 – 16318.51 pmol g^{-1} dry weight for Mine B) and curves fitted to biomass data from the respective chronosequences indicated no statistically significant differences between the data

sets based on the F-test (F = 0.39; p = 0.77). It may therefore be concluded that the temporal trends observed for microbial biomass in the two chronosequences were also similar. The changes in the ratio of fungal to bacterial PLFA in ChrA and ChrB over the rehabilitation period is shown in Figure 1.7. ChrA showed a decreasing trend in the F:B ratio, while the same ratio was higher in the older sites than in the younger sites from ChrB. A comparison of curve fits indicated a second order polynomial as the preferred model for ChrA and a third order polynomial as the preferred model for ChrB. This translates to a significant difference between the temporal trends for the two chronosequences. On the contrary, models fitted by linear regression indicated no significant difference between the slopes (F = 3.05; p = 0.11) of the two data sets. In the context of the investigation and considering that those values in the data that cause the difference in temporal trends are real data and not predicted missing values, the curve fit comparison obtained by the nonlinear regression model was considered valid.

The first inclusive observation that can be made from the graphs in Figures 1.1 to 1.7, is that fluctuations in enzymatic activities and abundances of microbial communities occur in both chronosequences, despite different management regimes. It seems that the initial input of fertilisers, organic material (manure), and lime at the onset of rehabilitation brings about an increase in microbial activity and biomass. After approximately three years, activities and abundances of microorganisms decrease and without additional management inputs should reach an equilibrium level characteristic of the specific environment (in this case a mining disturbed area under rehabilitation). Fluctuations for ChrA were more pronounced than those for ChrB and can be related to the more intensive management regime applied at Mine A. Although the enzymatic activities and microbial abundance measurements indicated lower overall values for ChrB, the trends over time of rehabilitation are similar to those observed for ChrA. There was more fluctuation in ChrA, which is related to the regular management inputs that occurred; in other words, the microbial communities in the less intensively managed sites (ChrB) maintain their functional and structural integrity within bounds in the absence of management inputs or disturbance, without evidence of an upward or downward trend.

The three parameters that showed statistically significant differences between the fitted curves were alkaline and acid phosphatase and the F:B ratio. These parameters are strongly influenced by soil pH (Dick et al., 2000; Bååth & Anderson, 2003). Furthermore, this study indicated a difference in the trends for F:B ratios but not for microbial biomass over time in the two chronosequences. This correlates with the conclusions of Rousk et al. (2010), that soil pH has a profound influence on the microbial PLFA composition, while not having a significant influence on the total concentration of PLFAs. Curve fit comparisons for pH data from the two chronosequences also indicated significant differences in trends over time. The corresponding differences between these parameters over time demonstrate the validity of the space-for-time hypothesis to accurately indicate the effect of management over the long-term.

While similar trends existed in both chronosequences for other individual microbial community measurements, the suggestion is not that the effect of the management practices on the microbial communities was exactly the same. It simply means that the common denominator in both these chronosequences is fluctuation. Similarity in temporal trends does not signify that the prevailing conditions in the two ecosystems are the same. What does differ in these chronosequences is the amount of fluctuation – ChrB showed less variability in microbial community function and structure. While ChrB contained some older sites than ChrA, statistical analyses did not indicate any relationships between microbial properties and rehabilitation ages of sites. This translates into fluctuation being a reflection of management, which is why sites managed in the same way group together for the respective chronosequences when subjected to canonical correspondence analysis (CCA) (Figure 2), despite the similarity in trends observed for the individual microbial properties. Eigenvalues for the first two axes of the CCA were 0.068 and 0.014, respectively. Total observed variance of the first two axes was 77.1%. The cumulative percentage variance of the species-environment relation was 71.6, 86.8, 99.8, and 100.0%, respectively for the four axes. The first axis was significant in explaining the variation in enzymatic activities (p = 0.0020), as were all four axes together (p = 0.0020). The first canonical axis correlated most strongly with normal saturated fatty acids (r^2 = -0.7678) and the second axis with terminally branched saturated fatty acids (r^2 = -0.6897).

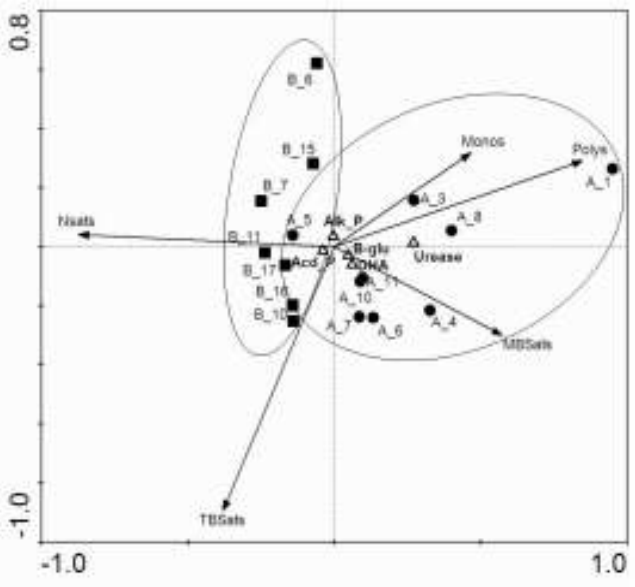

Figure 2 Canonical correspondence analysis diagram illustrating the relationship between the coal discard sites based on enzymatic activities and phospholipid fatty acid groups. Each site is indicated according to the chronosequence to which it belongs (A or B) followed by the rehabilitation age of the site. Key to abbreviations: DHA: dehydrogenase; β-glu: β-glucosidase; Acid-P: acid phosphatase; Alk-P: alkaline phosphatase; Nsats: normal saturated fatty acids; TBSats: terminally branched saturated fatty acids; MBSats: mid-chain branched saturated fatty acids; Polys: polyunsaturated fatty acids; Monos: monounsaturated fatty acids

Conclusions

In the context of this investigation it is important to keep in mind that conclusions drawn from curves fitted by a computer programme must be considered with respect to the relevant research (Motulsky & Christopoulos, 2005). It would be inappropriate to base conclusions on curve fits while ignoring the scientific principles and/or known features of the context in which the investigation is taking place.

The fluctuations in microbial properties observed for sites from Mine A and Mine B, indicate that a final hypothetical equilibrium stage exists for each chronosequence and that functional and structural aspects of microbial populations will tend towards these equilibrium stages unless this propensity is altered by management inputs. A comparison of the two chronosequences based on minimum and maximum values and temporal trends for individual microbial community measurements indicated no bias towards management regime over the long-term. This situation might change only if management practices affected vegetation to such an extent that the feedback mechanisms that exist between aboveground and belowground communities resulted in long-term effects on microbial community function and structure.

What is important to consider in terms of rehabilitation of mining disturbed sites and the management thereof, is that rehabilitation emphasises the reparation of ecosystem processes, productivity and services, not necessarily including the re-establishment of the pre-existing biotic integrity (SERI Science & Policy Working Group, 2004), in other words obtaining a self-sustaining ecosystem. Thus, while distinct management regimes may lead to similar long-term trends in rehabilitated discard sites, the question of self-sustainability remains. On a human time-scale, post-mining sites such as these cannot reach the same status as natural soils in terms of species composition and community structure. Therefore, the goal of a self-sustaining ecosystem defined in the context of the particular environment in question, is a more realistic approach to defining rehabilitation status. However, the first step in determining whether the ecosystem under management is a stable one, would be to stop interfering in that ecosystem by means of management inputs in the short term. Only in the absence of management inputs would it become obvious whether a specific ecosystem has reached a state of equilibrium where biological parameters do not fluctuate beyond certain minimum and maximum limits. To facilitate such a step without the risk of detriment to the managed ecosystems, monitoring of appropriate environmental parameters is of the essence in management decisions. The possibility of achieving a stable ecosystem while lessening the amount of management inputs will translate into significant economic implications for mining companies, which is another critical aspect of rehabilitation in the mining context.

Acknowledgements

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