THE USE OF DIFFERENT ECOSYSTEM COMPONENTS AS INDICATORS OF ECOSYSTEM DEVELOPMENT DURING PLATINUM MINE TAILINGS REHABILITATION

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"...only rarely have we stood back and celebrated our soils as something beautiful and perhaps even mysterious. For what other natural body, worldwide in its distribution, have so many interesting secrets to reveal to the patient observer"

- Les Molloy -

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This dissertation represents a compilation of manuscripts; each chapter is an individual entity and therefore repetition between chapters may occur

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SUMMARY

Platinum mining activities contribute substantially to South Africa's economy since it exceeded gold as economical contributor in 2001. Mining activities contribute to large amounts of waste production in the form of tailings and rock waste, deposited in the surrounding environment of the mine premises. Mining companies are held responsible for damages caused to the surrounding environment. These companies are required to introduce the cost of ecological rehabilitation in their operation costs as well as compile an environmental management plan. Numerous attempts to rehabilitate mine waste have proven unsuccessful. New and improved rehabilitation techniques are required to facilitate in the rehabilitation of these mine spoils. Woodchip-vermicompost produced from platinum mining wastes (woodchips and sewage sludge) was used as an alternative amendment to inorganic fertilisers during the rehabilitation of platinum mine tailings. The effectiveness of the woodchip-vermicompost as an alternative amendment during the platinum mine tailings rehabilitation were monitored using different ecosystem components. A natural veldt in the vicinity of the mine area was randomly selected to serve as a reference site. These ecosystem components selected have previously been shown to be effective as indicators of ecosystem quality. The components selected for this study includes the use of microbial enzymatic activity, microbial community structure, nematode trophic structures, and other mesofaunal groups such as micro-arthropods. The physical and chemical properties of the platinum mine tailings and reference area as well as the vegetation cover of the platinum mine tailings were determined. Statistical and multivariate analyses were use to determine the correlation between the dependent microbial components and dominate independent chemical properties. Nematode trophic structure, Maturity Index, and Plant-Parasitic nematode Index were used to compare the two rehabilitation techniques in terms of nematodes as indicators. Micro-arthropods family structures were used to compare the two amendments in terms of diversity and abundance. Enzymatic activity was positively affected by the addition of woodchip-vermicompost, than in the sites treated with inorganic fertilisers. The microbial community structure showed no statistically significant ($p < 0.05$) differences between the two amendments. A higher abundance of nematodes especially plant-parasitic nematodes and bacterivorous nematodes were observed in

the woodchip-vermicompost sites than in the inorganic fertilised sites. According to the Maturity Index, both amendments became more enriched during the study period, while the Plant-Parasitic nematode Index showed that the carrying capacity for plant-parasitic nematodes on the woodchip-vermicompost sites increased while it decreased in the inorganic fertilised sites, which can be related to the decrease in vegetation cover on the inorganic fertilised sites. Both coloniser (Prostigmata) and persister (Cryptostigmata and Mesostigmata) groups of the micro-arthropods, as well as a higher diversity of micro-arthropods, were present on the woodchip-vermicompost sites whereas the inorganic fertilised sites showed only the presence of colonisers, with a decrease in diversity and abundance of micro-arthropods over the study. The colonisation of micro-arthropods may have been affected by the addition of woodchip-vermicompost and vegetation cover, which contribute to the establishment of suitable microhabitats for these soil biota. By intercorrelating the results, it may be concluded that the addition of woodchip-vermicompost may be an essential part of the rehabilitation process, by contributing to soil organic material to the ecosystem system, which may improve the recolonisation of soil biota and ecosystem processes. However further studies need to be conducted in order to determine the long-term sustainability of the woodchip-vermicompost in providing organic material and sustaining the ecosystem processes. The study also showed the necessity to integrate various ecosystem components when evaluating ecosystem development due to the unique role each component plays and the impact it may have on other components.

Keywords: *Inorganic fertilisers, Mesofauna, Microbial community structure, Microbial enzymatic activity, Nematode trophic structure, Platinum mine tailings, Woodchip-vermicompost*

OPSOMMING

Platinum mynaktiwiteite het goud as bydraer van die ekonomie gedurende 2001 oorskry, en dra tans grootliks by tot die Suid-Afrikaanse ekonomie. Mynslikdamme en rotshope wat in die omliggende mynomgewing gedeponeer word, word geassosieer met die mynaktiwiteite. Die mynindustrieë word verantwoordelik gehou vir enige skade wat deur mynaktiwiteite aan die omgewing aangerig word. Dus moet mynindustrieë die koste van ekologiese rehabilitasie by hul produksiekoste insluit. Die opstel van 'n omgewingsbestuursplan word ook vereis. Verskeie pogings is al aangewend om mynafval te rehabiliteer, maar dit was onsuksesvol tot dusver. Nuwe en verbeterde rehabilitasietegnieke word benodig om rehabilitasie te vergemaklik. "Woodchip"-vermikompos word geproduseer uit afvalprodukte (houtsplinter en rioolslyk) afkomstig van die platinummyn en kan gebruik word as alternatiewe toevoeging teenoor die byvoeging van anorganiese kunsmis gedurende die rehabilitasieproses van mynslikdamme. Die doeltreffendheid van "woochip"-vermikompos as alternatiewe toevoeging teenoor die gebruik van anorganiese kunsmis is gemoniteer deur die gebruik van verskeie ekosisteemkomponente. 'n Natuurlike gebied in die omliggende myngebied is ewekansig gekies om as verwysingsarea te dien. Die ekosisteemkomponente wat gedurende die studie gebruik is, is al voorheen aangedui as effektiewe indikatore van omgewingskwaliteit. Die komponente wat gebruik is sluit in die gebruik van mikrobiese ensiematiese aktiwiteit, mikrobiese gemeenskapstruktuur, nematood trofiese strukture, en ander mesofauna groepe soos mikro-artropode. Die fisiese en chemiese eienskappe van die mynslik- en verwysingsarea, asook die plantegroeibedekking van die damme is bepaal. Statistiese- en meervoudige variansie analises is uitgevoer om die korrelasie tussen die afhanklike mikrobiese komponente en dominate chemiese eienskappe te bepaal. Nematood trofiese strukture, "Maturity" Indeks en Plantparasietiese aalwurmindeks, is gebruik om die twee rehabilitasie-tegnieke in terme van nematode as indikatore te vergelyk. Mikro-artropood familiestrukture is gebruik om die twee toevoegings in terme van diversiteit en bevolkingsdigtheid van die mikro-artropode te vergelyk. Ensiematiese aktiwiteite is positief beinvloed deur die byvoeging van die "woodchip"-vermikompos. Die mikrobiese gemeenskapstruktuur het geen statistiese verskille ($p < 0.05$) tussen die twee toevoegings getoon nie. Die eksperimentele

gebiede, behandel met die "woodchip"-vermikompos, het 'n hoër bevolkingdigtheid van nematode, veral plantparasitiese en bakteriovoriese nematode getoon teenoor die eksperimentele gebiede behandel met anorganiese kunsmis. Die "Maturity" Indeks het getoon dat toevoeging van albei produkte ("woodchip-vermikompost en anorganiese kunsmis) aanleiding gegee het tot die verryking van die ekosisteem gedurende die studie, terwyl die Plantparasitiese aalwurmindeks getoon het dat die drakapasiteit van die eksperimentele gebiede, behandel met "woodchip"-vermikompos verhoog, terwyl die van die anorganiese kunsmis gebiede verlaag het. Die afname in drakapasiteit in die eksperimentele gebiede, behandel met anorganiese kunsmis, kan die gevolg wees van die afname in plantegroeibedekking wat waargeneem is. Beide die koloniseerders (Prostigmata) en persisteerders (Cryptostigmata en Mesostigmata) groepe van die mikro-artropode, asook 'n hoër diversiteit van mikro-artropode, was teenwoording in die "woodchip"-vermikompos gebiede. Die eksperimentele gebiede behandel met anorganiese kunsmis het 'n afname in diversiteit van mikro-arthropode getoon. Die "woodchip"-vermikompos en plantegroeibedekking kon bygedra het tot die kolonisering van mikro-artropode deur 'n ideale mikro-habitat te bewerkstellig.

Deur interkorrelasie van die resultate, kan afgelei word dat toevoeging van die "woodchip"-vermikompos 'n essensiële deel van die rehabilitasieproses kan wees, as gevolg van die bydrae tot grond organiese materiaal in die ekosisteem, wat resulteer in die vinnige kolonisering van grond fauna en ekosisteem prosesse. Langtermyn studies word egter benodig om die doeltreffenheid van die "woodchip"-vermikompos as deurlopende bron van organiese materiaal, asook die bydrae to volhoubaarheid van ekosisteemprosesse, te ondersoek. Die huidige studie toon egter ook die noodsaaklikheid om verskeie ekosisteemkomponente te gebruik tydens die evaluering van ekosisteemontwikkeling, as gevolg van die unieke rol wat elke komponent speel, en die impak wat dit mag hê op die ander komponente in die eksosisteem.

Sleutelwoorde: *Anorganiese kunsmis, Mesofauna, Mikrobiese ensiematiese aktiwiteit, Mikrobiese gemeenskapstruktuur, Nematood trofiese struktuur, Platinummynsluk, "Woodchip"-vermikompos*

CHAPTER 1

INTRODUCTION

1.1 The soil ecosystem

Soil is a dynamic component of the ecosystem, of which living resources and biologically mediated processes play an important role in its ecological functioning (Nsabimana et al., 2004), such as the production of sufficient biomass for a growing human population (Filip, 2002). An understanding of the functioning of these biologically mediated processes in the ecosystem may aid in monitoring of ecosystem health and may be used as early indicators of ecosystem degradation (Dick, 1994). An ecosystem can be defined as healthy when the soil functions within ecosystem boundaries (to sustain biological productivity) maintain environmental quality and promote plant and animal health (Doran & Zeiss, 2000). Soil scientists have recently discovered the importance of the use of bio-indicators to assist in the assessment of soil quality. Factors such as the presence of certain species and genetic diversity within the soil may be used as easy measurements of soil quality (Ashman & Puri, 2003).

Soil fauna play an important role in the ecosystem and constantly contribute to the changes which occur in an ecosystem (Wolters, 2001). These changes include the decomposition of organic matter, the cycling of essential elements such as carbon (C), nitrogen (N), phosphorous (P), as well as the contribution to soil structure and soil porosity (Brussaard, 1998; Altieri, 1999). Of specific importance are the micro- and mesofauna populations, which are the main contributors of decomposition of organic matter. The density and diversity of this soil biota are dependent on the quality of the natural ecosystem, which is often influenced by anthropogenic activities (Beare et al., 1997; Laiho et al, 2001).

Studying of microbial biomass, activity and diversity may provide an accurate measure of the condition of an ecosystem (Hill et al., 2000). The measurement of microbial activity provides insight into temporal changes in soil due to different management practices and may provide an indication of the long-term effect of these management practices on the ecosystem (Dick, 1994). Microbial population and biomass has been found to change constantly throughout the restoration process of degraded ecosystems (Turco et al., 1994), providing insight in the success or failure of the restoration process (Harris et al., 1991). Microbial biomass that represents the fraction of the soil, which is responsible for nutrient cycling and energy, contributes to the regulation of organic matter transformation within the soil ecosystem (Turco et al., 1994). Soil mesofauna evaluation and interpretation of their abundance and function, which includes the presence of specific organisms or populations (functional groups) and biological processes within an ecosystem, offers an assessment of the condition of the ecosystem (Laiho et al., 2001). Several studies used the presence of certain nematode trophic groups within various habitats and the level of disturbance to evaluate the condition of a site (Ferris et al., 2001).

1.2 Problem statement

Since the discovery of diamonds and gold at the end of the 19th century, mining has been the backbone of South Africa's economy. South Africa produces 55 different minerals from 713 mines and quarries, which have been exported to 83 countries in 2000. Gold was South Africa's major economical contributor before Platinum Group Metals (PGM) exceeded it in 2001 (Chamber of Mines, 2003a). South Africa contributes to 62% of the world's PGM production and satisfies 75% of the global platinum demand, making it the largest producer of platinum (Chamber of Mines, 2003b). Although mining contributes largely to South Africa's economy, it has been devastating to the biological diversity of ecosystems (Milton, 2001) and contributed 25% to natural ecosystem loss in South Africa (DEAT, 1999).

Mining activity generates two categories of by-products: mine tailings (generated during the processing of excavated material) and waste rock (product removed to obtain the desired material). (Ledin & Pedersen, 1996). The large amount of waste

rock and tailings, may damage the land surface where it is deposited and may become a source of pollution (Freitas et al., 2004). The deposited mine spoils may have a direct or an indirect effect on the environment: direct effect entitles the loss of cultivated land, forests and grazing land, whereas air and water pollution may be the indirect effect of the deposited mine spoils (Wong, 2003).

The impact that mine spoils will have on the surrounding environment will largely be determine by the physical and chemical characteristics of the spoil material (Ledin & Pedersen, 1996). Due to the lack of organic matter and the fine texture of tailings material, it may compact easily. The fine tailings material is subjected to erosion by wind and water (Bradshaw, 1997), leading to dust pollution and siltation of rivers (Wong, 2003). In order to reduce the impact mine spoils will have on the environment, mines are held liable by the South African Environment and Conservation Act (Act 73 of 1989) and Minerals and Petroleum Resource Development Act (MPRDA) (Act 28 of 2002), to reduce the impact it may have on the environment and to restore the damages caused to the environment. The law also requires developers to introduce the cost of ecological rehabilitation within their operational costs and to compile an Environmental Management Plan (EMP) (Milton, 2001).

The establishment of vegetation on mine spoils contributes to the stabilisation, pollution control and visual improvement of these sites and so remove the threats it may hold for human beings (Wong, 2003). Plants have been shown to protect the soil surface against erosion and allow the accumulation of fine particles (Tordoff et al., 2000), whereas root growth, accumulation of organic material and associated microbial activity prevents the compaction of the soil and so lower the bulk density (Bradshaw, 1997). Vegetation assist in the problem of leaching toxic minerals by returning a large proportion of water to the atmosphere through transpiration and so reducing the concentration of soluble heavy metals entering the watercourse (Tordoff et al., 2000).

Due to the fact that mining causes soil damage, vegetation will grow with difficulty if the soils are not remediated beforehand (Bradshaw, 1997). Platinum mine tailings are a nearly biologically sterile medium with a low water holding capacity and high base saturation (Van Rensburg & Morgenthal, 2004). The large amount of manganese, iron and sulphur gives the platinum mine tailing a saline characteristic, which may be phototoxic in high concentrations (Walmsley, 1987). In order to establish a sustainable plant population on these tailings, the growth medium needs to be remediated. Woodchip-vermicompost was added to the platinum mine tailings rather than the inorganic fertilisers in order to create a suitable ecosystem for the establishment of vegetation cover. In order to ensure the sustainability of the established vegetation, the rehabilitation process should be monitored. In this study, different ecosystem components, which include microbial enzymatic activities, microbial community structure and activity, nematode trophic structure and mesofauna family structure, were used to monitor the sustainability of the platinum mine tailings in providing a suitable growth medium. This will facilitate the mine in meeting the terms require to obtain a closure certificate at the end of the mining operation, by providing baseline information on soil characteristics, vegetation conditions and soil faunal assessments.

1.3 Specific objectives

The aim of this study was to use different soil ecosystem components to compare the effectiveness of two methods of rehabilitation implemented on mine tailings of two different ages on the property of Impala Platinum mine. The mine attempts to establish a self-sustaining plant community on the platinum tailings material on these sites.

Specific objectives of this study included:

1. Characterising the biochemical properties of the sites by determination of the potential enzymatic activities. Focus is placed on carbon cycling (β-glucosidase), phosphorous cycling (Alkaline- and acid phosphatase), the overall microbial activity (Dehydrogenase) and nitrogen cycling (Urease) within the soil environment.

2. Characterising the microbial community structure by using different phospholipid fatty acid biomarkers.
3. Determining nematode trophic structures, Maturity and Plant-Parasitic nematode Indices.
4. Determining mesofaunal communities focusing on micro-arthropods family structures and abundance.

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CHAPTER 2

LITERATURE REVIEW

2.1 The soil ecosystem

Soil has long been seen as a store and supplier for basic nutrients necessary for plant growth (Theocharopoulos et al., 2004). Recently, soil has been recognised as a natural object of properties, which are the product of the nature of its physical, biological and chemical components. Soil properties are also modified by the interaction of these latter components (Tate, 2000). In order to study soil, knowledge of its basic definition and the properties which makes it unique must be clearly understood. Soil consists of a solid phase, which includes minerals and organic components (plants, living organisms, organic matter and non-decomposed plant and animal debris), water and air (Agnelli et al., 2004).

2.1.1 Abiotic composition of the soil ecosystem

Soil consists of different sized mineral particles and can be classified as indicated in Table 1 (USDA, 2001). The flow of water through and into soil is critical for soil life and is controlled by the type, size and arrangement of the soil particles (Agnelli et al., 2004). The arrangement of the soil particles determines the size of the pores between soil particles. These pores control aeration, water infiltration, as well as storage and drainage (Singer & Munns, 1992).

Table 1. Size distribution of soil particles. (Tate, 2000).

Particle Class	Subclass	Mean Diameter (mm)
Sand	Very coarse	2.00 – 1.00
	Coarse	1.00 – 0.50
	Medium	0.50 - 0.25
	Fine	0.25 – 0.10
	Very fine	0.10 – 0.05
Silt		0.05 – 0.002
Clay		< 0.002

Due to the negative charge distribution on soil particles, water tends to aggregate around it and this water film is of critical importance to soil organisms. Clay is the most important mineral in soil due to its relationship to biological processes. Soil microorganisms are often associated with the surface of soil minerals (Tate, 2000).

Clay minerals contribute to the cation exchange capacity of soil and are involved in the interaction of soil organic components and microbes by providing a surface for interaction (Tate, 2000). Carbon dioxide, which evolves from respiration of soil organisms, dissolves in the water film around the soil particles to form carbonic acid, which dissociates into hydrogen ions. The hydrogen ions may displace cations on the soil particles, making it available for plant use (Richards, 1994).

Soil structure is a key factor in soil due to its ability to support life, moderate soil quality, (with emphasis on soil carbon sequestration) and water quality (Bronick & Lal, 2005). Aggregation of soil particles which results from rearrangement, flocculation and cementation (Duiker et al., 2003) have been used as an indicator of soil structure (Six et al., 2000) and is one of the important factors controlling microbial activity and soil organic matter turnover.

Soil organisms can be found on the outside of these aggregates and in the small pores between them. The pore size may limit the ability of soil organisms to move and thereby limit their ability to obtain nutrients. Macroaggregates (>2 mm) are formed by the aggregation of microaggregates (50 – 250 μm). Components of the latter form macropores, which provide refuge for microfauna against predators (Paul & Clark, 1990). Soil organic carbon (SOC), clay, ionic bridging, soil biota and carbonates all contribute to the aggregation of soil particles. Soil organic matter contributes to the development of soil structure, which in turn controls the dynamics of soil organic carbon (Six et al., 2004). The decomposition rate of soil organic matter influences its effectiveness as an aggregant (Martens, 2000). In turn, the clay content acts as an aggregant, binding particles and influencing the decomposition rate of soil organic matter (Bronick & Lal, 2005). Soil organic matter is a reservoir of carbon and nitrogen that is subject to rapid biological decomposition (Knops & Tilman, 2000).

2.1.2 Biotic components of the soil ecosystem

The soil organic matter (SOM) consists of both living and dead biomass, which is an integral component of organic matter transformation (Ekschmitt et al., 2005). Soil organic matter consists of 1- 5% of living microbial biomass (Nsabimana et al., 2004) and can be defined as organisms smaller than 10 μm which includes both dormant and metabolically active organisms (Schloter et al., 2003). Soil microorganisms are one of the few fractions of soil organic matter which is sensitive to management strategies applied to soil ecosystems (Nielsen & Winding, 2002). Subsequently, it may serve as an indicator of microbial significance in soil (Schloter et al, 2003). Non-living soil organic matter consists of carbon, hydrogen, oxygen, phosphorous, nitrogen and sulphur and is the major source of nitrogen and sulphur for plants in an unfertilised ecosystem (Singer & Munns, 1992). Organic matter accumulates in soil as a result of the activity of soil biota: plants supply organic matter while microorganisms are responsible for its transformation (Fontaine et al., 2003). The soil ecosystem hosts a vast diversity of organisms due to its spatial and temporal heterogeneity (Bongers & Ferris, 1999). Table 2 contains the three groups into which soil organisms can be classified according to their size differences.

Table 2. Three groups of soil biota based upon their different sizes (Richards, 1994).

Microfauna	Algae, protozoa, fungi and bacteria
Mesofauna	Nematodes, springtails, small arthropods and Enchytraeidae worms.
Macrofauna	Earthworms, molluscs, large enchytraeid worms and arthropods

Table 3 summarises the activities of these organisms, which contribute to the constant alteration of properties within the soil. Soil organisms aid in the decomposition and accumulation of organic matter, thereby contributing to the flow of energy through the soil ecosystem, with microfauna being the largest group of contributors (Richards, 1994).

Table 3. Summary of the biotic components and their influence on soil processes in the ecosystem (Hendrix et al., 1990, Liiri et al., 2002).

	Nutrient cycle	**Soil structure**
Microfauna	Mineralise and immobilise nutrients, catabolise organic matter	Produce substance that bind aggregates, hyphae bind aggregates
Mesofauna	Regulate microfauna populations, decompose plant material, alter nutrient turnover	Affect aggregate structure, create biopores and promote humification
Macrofauna	Stimulate microbial activity through fragmentation of plant materials	Distribute microorganisms, mix organic matter and soil, create biopores, promote humification

2.1.2.1 Microfauna

2.1.2.1.1 Soil bacteria

Soil bacteria are found on the surface of soil particles and require water and nutrients in their immediate vicinity for survival (Prescott et al., 2002). Of the soil microorganisms, bacteria are the most abundant in the soil ecosystem (containing 10^4 - 10^9 bacterial cells per gram of soil) (Kennedy, 1999). Soil bacteria, which occur in the soil ecosystem, perform vital services related to water dynamics and nutrient cycling (SWCS, 2000).

The majority of bacteria found in soil are heterotrophs or chemoorganotrophs. Bacteria can physiologically be grouped as autochthonous and/or zymogenous based on their energy and carbon source (Paul & Clark, 1990). Zymogenous bacteria can be classified as r-strategists, which are dependent on availability of substrates, demonstrate rapid growth and may become dormant when substrates are exhausted. Late decomposition are characterised by the appearance of K-strategists (autochthonous bacteria) which are generally slow in growth and decomposition processes (Ekschmitt et al., 2005). K-strategists contribute to soil organic matter and nitrogen assimilation. Therefore, an increase in K-strategists is of importance for soil fertility (Fontaine et al., 2003).

Table 4, summarises the main groups of bacteria found within soil. Arthrobacters are r-strategists, which have a predominantly oxidative metabolism and are numerous in the soil ecosystem. The Streptomycetes group of bacteria can be classified into three genera, Streptomycetes, Pseudomycetes and Bacillus, which occur commonly in soil and can be classified as Gram-positive, oxidative organotrophs. These bacteria are intolerant to waterlogging and acidity. Pseudomonas, on the other hand is Gram-negative flagellated bacteria. Most are aerobic, except the denitrifying species (Paul & Clark, 1990). Three families of Gram-negative cocci and rods occur widely in the soil environment. These three families include the free-living N_2-fixers (Azotobacteriaceae), the Pseudomonadaceae and Rhizobiaceae (the symbiotic N_2-fixers) (Richards, 1994). Biodiversity of the microbial community is important with relation to the maintenance of soil ecosystem function (Nsabimana et al., 2004).

Table 4. Main groups of bacteria that occur in soil (Richards, 1994).

Group	Genera	
Arthrobacters	*Arthobacter*	Gram-positive, numerous in soil, oxidative metabolism
Streptomycetes	*Streptomycetes* *Pseudomycetes* *Bacillus*	Gram-positive, oxidative organotrophs, intolerant to waterlogging and acidity
Pseudomonads	*Pseudomonas*	Gram-negative, aerobic except denitrifying species, organotrophs
Bacilli	*Bacillus* *Clostridium* *Azotobacter* *Lactobacillus*	Gram-positive, motile, organotrophic, endospore forming bacteria

Bacteria occupy an important position in the nutrient web as primary decomposers, since 90 – 95% of nutrients pass through these organisms to higher trophic levels. Bacteria help in the decomposition of complex molecules to smaller and simpler molecules providing substrate for other microbes. The contributions that bacteria make to the soil ecosystem can be listed as:

- Decomposition of organic residues and nutrient cycling
- Formation of beneficial soil humus by decomposing organic residues and through synthesis of new compounds
- Release of plant nutrients from insoluble inorganic forms
- Transformation of atmospheric N_2 to N available for absorption by plants
- Improvement of soil aggregation, aeration and water infiltration (Kennedy, 1999).

2.1.2.1.2 Soil fungi

Fungi depend on chemical sources for energy and are thus known as chemotrophic organisms (Richards, 1994) Fungi are primarily responsible for decomposition of

organic materials (Paul & Clark, 1990) and form an important part of the microbial community influencing plant growth and nutrient uptake (Johansson et al., 2004). Fungi, besides bacteria and actinomycetes, are the third most abundant organisms in the soil environment and occur as 10^5 – 10^6 organisms per gram soil (Chen et al., 2003). An increased area for microbial interaction is provided in soil by the presence of fungi due to the binding of aggregates. Fungi also provide an increased absorption area for plants through the extension of its hyphae (Johansson et al., 2004).

The contribution of fungi with regard to the stabilisation of soil aggregates may regulate the biotical mechanism of soil organic matter, accounting for the storage of soil organic carbon in soils (Beare et al., 1997). Myccorhizal fungi form a symbiotic relationship with plants and contribute to the improvement of their nutrient uptake and supply, stress tolerance and productivity (Carpenter-Boggs et al., 2003). Arbuscular myccorhizal fungi may enhance the mobilisation of organically bound nitrogen in plant litter (Hodge et al., 2001). The colonisation of arbuscular myccorhizal fungi in plant roots may have both a direct and indirect effect on soil bacteria. A direct effect includes the provision of energy-rich carbon compounds via fungal hyphae, while an indirect effect includes the myccorhiza-mediated effects on root exudation and soil structure (Johansson et al., 2004).

2.1.2.2 Mesofauna

2.1.2.2.1 Micro-arthropods

Mesofauna includes all living soil organisms with a size ranging from 200 µm to 1 cm and includes most nematodes, rotifers, springtails, mites, small enchytraeids and arthropods. Arthropods comprises of the greatest diversity of the mesofauna (Richards, 1994). Mesofauna is a key indicator of soil biota and includes the order Acari, which play an important role in soil formation and transformation (Parisi et al., 2005). The order Acari can be divided into the following two suborders: (1) Acariformes, which consists of the Acaridida (Astigmata), Actinedida (Prostigmata) and (2) Oribatida (Cryptostigmata) and Parasitiformes, which consists of the Gamasina (Mesostigmata) (Koehler, 1997).

Mites contribute little to the chemical decomposition of organic matter but play an important role in the later stages of organic decay (Richards, 1994). The Cryptostigmata (Oribatida) are particulate feeders, influence decomposition and soil structure by comminuting organic matter. The faecal pellets of Oribatida mites are an integrated component of soil structure and provide a large surface area for decomposition. Oribatida mites contribute to the distribution of bacteria and fungi by carrying it on their body surface or by digesting dormant spores, which survive the passage through the alimentary tracts (Behan-Pelletier, 1999). Hülsmann & Wolter (1998) and Behan-Pelletier (1999) distinguished three feeding types of Oribatida mites: (1) macrophytophages, which feed on higher plant material, (2) phanphytophages, which feed on both microbial and higher plant material during different life stages and (3) microphytophages, which feed primarily on soil microflora. Actinedida (Prostigmata) mites are commonly found in soils with a low organic content (Osler et al., 2000). They are a large, diverse group of mainly predatory mites. However, some of the Actinedida mite species are phytophagous (Richards, 1994). The phytophagous group of Prostigmata mites are general opportunists, which are able to reproduce rapidly after disturbance or a sudden shift in resources. Acaridida or Astigmata are less common occurring mites. Astigmata mites is typically found in areas with high moisture and organic materials (Coleman et al., 2004).

Mesostigmata are free-living mites, living in the litter layer of soil or on plants and are typically predators. Uropodina and Gamasina mites belong to the Parasitiformes group of the Mesostigmata (Koehler, 1999). Uropodina mites mainly prey on nematodes, larvae of insects and decaying organisms (Willis & Axtell, 1968). These mites generally prefer habitats with abundant decaying organic material and nematodes. After decomposition of organic material has occurred, Uropodina mites have to disperse to new habitats containing a fresh nutrient source. This is accomplished by adhering to insects (Koehler, 1999). Gamasina mites are mobile predators, which hunt in the litter layer on the soil surface for small arthropods (Koehler, 1997) which are digested pre-orally (Koehler, 1999). According to the reproductive biology of Gamasina, they may be classified into r and K-strategists,

which are often used as bioindicators (Ruf, 1998). Enchytraeids are microarthropods, which contribute to litter decomposition, exploit the biochemical decomposition of microorganisms and contribute to the external rumen type of digestion (Ekschmitt et al., 2005). Two feeding types exist for Enchytraeidae mites, namely: microbivorous, which is the feeding preference of 80% of the Enchytraeidae mites and saprophagous, which is typical for 20% of these mites (Didden, 1993). The faecal material of Enchytraeidae mites, like that of Cryptostigmata counterparts, may serve to stimulate microbial activity (Richards, 1994).

2.1.2.2.2 Nematodes

Nematodes are primarily aquatic organisms, which live in the water film between soil particles (Bongers & Ferris, 1999). Nematodes feed on a wide variety of soil organisms and are therefore dependent on the continuous availability of the water film between the soil particles for movement. Their activity is, therefore, controlled by soil biological and physiological conditions (Neher, 2001). Soil nematodes contribute to the release of nutrients from bacterial biomass, making it available for plant growth (Bongers & Bongers, 1998) and thus contributing to the cycling of nutrients (Bulluck et al., 2002). As a result of this contribution, there is a possibility of using nematodes as indicators of overall soil conditions (Yeates & Bongers, 1999).

Several life strategies have been developed within nematodes (Bongers & Bongers, 1998). Nematodes are considered r-strategists when they have a large nutrient requirement, short life cycle and a relatively high reproduction tempo. Nematodes which have a slow reproduction tempo, low nutrient requirement and relatively long life cycle are, however, considered as K-strategists (Bulluck et al., 2002). Opportunistic nematodes colonise habitats with low nutrient resources and these species of r-strategists contribute to community development in degraded soils (Paşca et al., 1998), whereas the K-strategists colonise areas, containing a high nutrient content, more rapidly (Bongers & Bongers, 1998). Even though nematodes only contribute to a small amount of the biomass found in soil, their presence in the soil ecosystem are of critical importance for the functioning of soil processes (Barker & Koenning, 1998).

2.2 The importance of maintaining a healthy ecosystem through good soil quality

2.2.1 Ecosystem health

The soil ecosystem functions as a buffer, filter and transport system regulating the biogeochemical flow of substances into and out of the ecosystem, leading to the production of biomass (Snakin et al, 1996). Only a healthy soil ecosystem can produce sufficient biomass necessary for the survival of the increasing human population (Filip, 2002). This has lead to the awareness that soil health is of critical importance. Three functions of soil namely, (1) productivity, (2) environmental quality of natural resources and (3) health of humans, plants and animals (which relates to environmental aspects and sustainable land management) are affected by soil quality and/or soil health (Theocharopoulos et al., 2004).

Soil health and *soil quality* will be used as synonymous in this dissertation. Soil health can be seen as the soil capacity to be used for a certain purposes for example agricultural uses (Doran & Zeiss, 2000). Soil quality can be defined as "The capacity of the soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health." Soil quality can be classified in two different groups:

- as an inherent characteristic of soils or
- as a capacity to perform certain productivity, environmental and health functions (Doran et al., 1996).

Both abiotic and biotic properties of the soil ecosystem should be considered when studying soil quality (Schloter et al, 2003). An ecologically based approach in studying soil quality is to focus on an individual population of soil organisms, which has been recognised as an important agent of soil processes or sustainable biodiversity (Filip, 2002). Several microbial activities have been recognised as potential rapid indicators of changes in soil quality (Bending et al., 2004). There has been a specific interest in studying soil enzymes as indicators of soil quality since enzymes provide an integrated insight on microbial, as well as the physical and chemical conditions of

a site (Aon et al., 2001). Phospholipid fatty acid biomarkers assigned to specific microbial taxa may be used to study how different management practices affect microbial community structure (Jackson et al., 2003) and give a measurement of the total estimated viable microbial biomass (Bassio & Scow, 1995). During cell death, phospholipid fatty acids are rapidly degraded and used by other microorganisms as substrate. These phospholipids are subsequently metabolised to diglyceride and PO_4^{3-} (White et al., 1979). Due to the rapid turnover of the phospholipid fatty acids, it can serve as a measure of viable biomass (Zak et al., 1996). Phospholipid fatty acid (PLFA) biomarkers can therefore be seen as an indicator of soil quality (Schloter et al., 2003).

Abiotic properties, which include soil structure and chemical composition, are important to consider since they may directly influence microbial processes. The capacity of soil to hold and supply nutrients and to transport available water is determined by the physical and chemical properties of the soil. Soil pH in particular is one of the chemical properties that influence the availability of nutrients in soil. Therefore, it is significant to include pH when quality of soils is determined (Schoenholtz et al., 2000).

Soil organic matter is commonly known to be an important indicator of soil quality due to its contribution to specific soil functions. These include the regulation of water flow, air and nutrients through the soil, its role in aggregate stability and serving as a source of atmospheric carbon (Herrick & Wander, 1998). Soil organic matter mediates changes in soil properties and processes, which relates to (1) soil fertility and productivity, (2) soil physical integrity and (3) environmental quality.

2.2.2 Biotic components and ecosystem health

2.2.2.1 Microorganisms as indicators of soil quality

Microbial activity reflects the microbiological processes in soil for which mainly fungi and bacteria are responsible (Chen et al., 2003). Microorganisms are sensitive to changes, which occur in the environment. These organisms are continuously adapting

to these changes, making it a useful indicator of soil quality (Lewandoswki & Zumwinkle, 1999; Schloter et al., 2003). However, due to the vast variety of biological and biochemical properties of microorganisms involved in maintaining soil function (Gil-Sotres et al., 2005), it may be classified into groups containing similar specific properties (Visser & Parkinson, 1992). The biotic community exhibiting properties such as the structure, composition and distribution of the different functional groups of microorganisms forms the first group. The second group consists of population studies and considers the dynamics of organisms or communities of organisms that serve as biological indicators. The third group focuses on ecosystem level and uses properties involved in the transformation of organic material in the soil (Gil-Sotres et al., 2005). Microorganisms play an important role in biochemical transformations and nutrient cycling of carbon, nitrogen and phosphorous in soil (Verchot & Borelli, 2005), thus making nutrients available for plant use.

2.2.2.1.1 Nitrogen cycle

The cycling of nitrogen can take place through different processes in the soil ecosystem, namely nitrogen fixation, nitrification and denitrification. Nitrogen fixation involves the transformation of soil atmospheric nitrogen gas into accessible nitrogenous compounds, which can be utilised by plants (Chen et al., 2003). Nitrogen fixation takes place through enzymatic reduction activities that convert nitrogen to ammonia, which can be utilised for the growth and maintenance of plant cells (Delgado & Follett, 2002). Ammonia is released during the decomposition of soil organic matter and rapidly transformed to NO_3^- (Nielsen & Winding, 2002). Once ammonia is converted to NO_3^-, it is subjected to several changes; namely (1) it may be transformed to a gaseous oxide of nitrogen and to nitrogen by denitrifying microorganisms, (2) it may be used by microorganisms in the synthesis of amino acids, (3) it may be used by microorganisms as an electron acceptor and be reduced to NH_4 in the absence of oxygen, (4) it may accumulate in the soil, or (5) it may be leached from the soil and transported from the site by runoff (Paul & Clark, 1990).

Nitrification, conducted by two bacterial species (Chen et al., 2003), is a chemolithoautotrophic oxidation process (Schloter et al., 2003), which involves the

conversion of ammonia into nitrate. Firstly, the ammonia-oxidising bacteria (*Nitrosomonas* spp.) convert ammonia to nitrite (Ibekwe et al, 2002) and secondly, *Nitrobacter* spp., convert the formed nitrite to nitrate (Delgado & Follett, 2002). Nitrification of ammonia to nitrate results in the acidification of the soil ecosystem and the increase in mobility of nitrate, which is an essential nutrient for plants (Tate, 2000). Denitrification is the process during which nitrate is converted to nitrogen gas in the absence of oxygen. During anaerobic conditions the main process of nitrogen cycling occurs by denitrification (Richards, 1994). Nitrate is converted to nitrite and then to nitric oxide gas, nitrous oxide gas and finally to nitrogen gas. This conversion process is regulated by denitrifying organisms, including *Thiobacillus denitrificans, Micrococcus denitrificans* and species of *Serratia* and *Pseudomonas* (Delgado & Follett, 2002). Nitrogen availability in soil is an important indicator of soil quality. The mineralisation of nitrogen in soil is influenced by the soil organic matter, microbial biomass and soil moisture content (Knoepp et al., 2000).

2.2.2.1.2 Carbon cycle

The carbon cycle involves the cycling of carbon between the inorganic phase carbon dioxide (CO_2) and various organic compounds of which living components consist of (Atlas, 1997). Chemolithotrophic organisms oxidise organic compounds to obtain energy-yield and carbon for nutrition (Richards, 1994). Once carbon is fixed into organic compounds, it can be utilised by aerobic heterotrophic microbial soil communities through decomposition, as carbon and energy source to increase their biomass (Waldrop et al, 2000). Soil carbon is the driving force of microbial soil respiration and nitrogen mineralisation (Fontaine et al., 2003). Carbon is either available from water, air, or decomposed soil organic matter (Chen et al., 2003). The rate of soil carbon compound degradation may be influenced by the composition of the microbial community (Degens, 1999), as only a small number of microbial populations has the enzymatic capacity of initiating the degradation of macromolecular carbon compounds (Hu & van Bruggen, 1997). Knowledge of the relationship between microbial community composition and physiological capacity is of great importance in agroecosystems (Waldrop et al, 2000), as carbon and nutrient alteration may occur frequently within these ecosystems (Bassio et al., 1998).

2.2.2.1.3 Phosphorous cycle

Phosphorous (P) exists in a variety of organic and inorganic forms in a natural ecosystem, but primarily occur as either insoluble or partially soluble inorganic phosphorous (Paul & Clark, 1990). Phosphorous does not enter to a gaseous phase or undergo any valence changes within the phosphorous cycle. Microorganisms assimilate inorganic phosphorous and mineralise organic phosphorous compounds under both aerobic and anaerobic conditions, with the maximum activity under aerobic conditions (Tate, 2000). The mineralisation of organic phosphorous involves the hydrolysis of esters and the release of orthophosphates facilitated by the extracellular enzyme phosphatase (Šantrůčková et al., 2004). Fungi are considered the greater contributor to the total phosphatase activity in soil due to its production of external phosphatase (Krämer & Green, 2000). Organic phosphorous is derived from soil organisms and plants and may be stabilised in soil organic matter or recycled by soil microbial biomass (Oehl et al., 2004). Soil organisms contribute to the solubilising of phosphorous through the production of CO_2 and organic acids, which contribute to the mobilisation of phosphorous (Atlas, 1997; Paul & Clark, 1990).

2.2.2.2 Mesofauna as indicators of soil quality

Due to the abundance of mesofauna, their role in soil transformation and their life cycle duration, several species have been recognised as indicators of soil quality (Parisi et al., 2005). Oribatida and Astigmata mites are useful as bioindicators of terrestrial ecosystems due to their abundance through all seasons, easy sampling method, high diversity and their representation of all heterogeneous groups. Oribatida mites cannot adapt rapidly to short-term environmental alterations, therefore, their population is likely to decline - a characteristic, which may be used as an indicator of environmental disturbance (Behan-Pelletier, 1999). In addition, Oribatid mites stimulate the respiration of microorganisms by feeding on fungal hyphae and spores and stimulate microbial growth via mobilisation of nutrients. This way nutrient leaching is minimised and the nutrient pool in the soil stabilised (Maraun et al., 1998). Prostigmata mites show an increase in population in the presence of drainage, irrigation and addition of fertilisers within an agroecosystem. Therefore, the abundant

presence of Prostigmata mites may be indicative of a disturbed system (Behan-Pelletier, 1999).

Collembola has been used to evaluate the progression of rehabilitation by monitoring their recolonisation rate in soil (Greenslade & Majer, 1993). Collembola belongs to the fungal feeding functional group (De Ruiter et al., 1993) and can be used as indicators due to their ability to enhance microbial activity and nutrient cycling (Lee, 1994). Enchytraeidae has been found to be bioindicators of abiotic factors, which affects the soil ecosystem (Röhrig et al., 1998). The borrowing activities of Enchytraeidae which leads to superficial deposited casts (Langmaack et al., 2001), have shown to improve air permeability and hydraulic conductivity of soil (Didden, 1990). Inorganic nitrogen and phosphorous mineralisation (Briones et al., 1998), as well as nutrient leaching and soil respiration (Setälä et al., 1991) may be improved or affected by Enchytraeidae.

2.2.2.3 Nematodes as indicators of soil quality

Of the micro-invertebrates, nematodes have been preferred as bioindicators due to their successful sampling methodology, feeding preference and interpretation of data (Neher et al., 1998). Soil nematodes make useful bioindicators of soil status and processes within an ecosystem due to several characteristics (Porazinka et al., 1999). These include their abundance in nearly any environment and their vast diversity of feeding habits and life strategies (Schloter et al., 2003). Nematodes also have a short response time, to disturbance and are relatively simple to identify.

Nematode species groups, which have the same effect on ecosystem processes, can be divided into functional groups. These functional groups are a practical necessity in identifying soil quality, since it is difficult to determine how a single species affects ecosystem processes (Bongers & Bongers, 1998). An integrated interaction of all soil factors, including management and pollutants, can be obtained by studying nematodes on community level rather than single species level (Schloter et al., 2003).

2.3. Assessing the soil quality of an ecosystem

2.3.1 Methods of soil quality assessment

2.3.1.1 Enzymatic activities

Microorganisms play an important role in biochemical transformations and nutrient cycling in soil (Verchot & Borelli, 2005), making nutrients available for plant use. Soil microbial activity can be measured through catalysing substrate-specific transformations through enzyme assays and are frequently used to study functional groups in relation to soil quality. (Nielsen & Winding, 2002). In laboratory assays conditions are optimised for enzyme production. Determination of soil enzymatic activity thus only gives an indication of the potential activity which would have been expressed under optimum veldt condition. This can be said to be the "soil genotype", but will barely ever be expressed in natural soil systems (Schloter et al., 2003). Two kinds of enzymes occur in soil; extracellular enzymes that are responsible for the breakdown of organic macromolecules and intracellular enzymes that are involved in the breakdown of smaller molecules (Insam, 2001). Enzymatic activity, rather than quantity of enzymes in the soil is measured, since it is difficult to extract enzymes that cohere to soil particles (Knight & Dick, 2004). Soil enzymes representative of the main biogeochemical nutrient cycles, including β-glucosidase (C-cycle), phosphatase (P-cycle), urease (N-cycle) and dehydrogenase (microbial biomass). Basic reactions catalysed by these enzymes are given in Table 5.

Table 5. Soil enzymes of concern in measuring soil quality and their ecosystem function, modified from Dick (1997).

Enzyme	**Reaction catalysed**
Hydrolases	
β – Glucosidase	Glucosidase + $H_2O \rightarrow$ ROH + glucose
Phosphatase	Phosphate ester + $H_2O \rightarrow$ ROH + phosphate
Urease	Urea $\rightarrow$ 2 NH + CO_2
Oxidoreductases	
Dehydrogenase	$XH_2 + A \rightarrow X + AH_2$

β-glucosidase (EC 3.2.1.21) plays an important role in the cycling of carbon and acts as the rate-limiting enzyme in the degradation of cellulose to glucose (De Mora et al., 2005). Its activity contributes to the release of important energy sources for microbial use (Bandick & Dick, 1999) and is typically correlated with biomarkers of Gram-positive and Grams-negative bacteria (Waldrop et al., 2000). Sastre et al. (1996) also reported correlations between β-glucosidase and fungal activity. Changes with management practices can be detected with β-glucosidase activity in a relative short period of 1 to 3 years (Ndiaye et al., 2000). β-glucosidase does not vary much between seasons and can therefore be useful in monitoring soil quality (Turner et al, 2002).

Phosphatases (EC 3.1.3) are enzymes, which play an important role in the soil ecosystem and originate from bacteria, fungi and plants. Plant roots are an important source of acid phosphatase (EC 3.1.3.2) in soil, whereas bacteria and fungi are the main source of alkaline phosphatase (EC 3.1.3.1) (Criquet et al., 2004). Hydrolyses of organic phosphorous to inorganic phosphorous is catalysed by the enzyme phosphatases (Verchot & Borelli, 2005). Inorganic phosphorous can be utilised by plants, making phosphatase activity of great agronomic value (Pascual et al., 2002). Phosphatases can be classified according to their specific pH optimum into acid (orthophosphoric monoester phosphohydrolase, pH 6.5) and alkaline (orthophosphoric monoester phosphohydrolase, pH 11) phosphatase (Verchot & Borelli, 2005). Phosphomonesterase is the phosphatase enzyme that has been the most extensively studied in terrestrial ecosystems (Criquet et al., 2004). The quantity and quality of the phosphorous within the soil ecosystem can be determined through the activity of phosphatases and can therefore be a useful indicator of soil quality (Roa & Tarafadar, 1992).

The effect of different management practices on soil quality and the degree of recovery of degraded soils may be evaluated by using dehydrogenase activity (Gil-Sotres et al., 2005). The main localisation of dehydrogenase is in the plasma membrane of bacteria and in the mitochondrial membranes of fungi (Aon & Colaneri, 2001). Dehydrogenase is part of the intracellular group of enzymes and will therefore

not be found as a free enzyme in the soil and can consequently be used to determine the overall microbial activity (Dick, 1997, Fernández et al., 2005). The transformation of soil organic matter within the ecosystem has been found to be directly affected by dehydrogenase activity (Sicardi et al., 2004).

The capacity of soil to transform organic nitrogen compounds into ammonium/nitrate under optimal conditions is referred to as the nitrogen mineralisation capacity of soil, which is catalysed by the enzyme urease (Gil-Sotres et al., 2005). Urease (EC 3.5.1.5) is an enzyme that is widely distributed throughout the soil ecosystem, being present in plants, microorganisms and animal cells (Samborska et al., 2004). Within the soil, urease may be present as both an extracellular and intracellular enzyme. Extracellular urease is derived from the lyses of dead cells (Swensen & Bakken, 1997). Urease activity is typically inhibited by the presence of heavy metals and can therefore be used as indicator of the restoration of platinum mine tailings (Hinojosa et al., 2004).

2.3.1.2 Signature lipid biomarkers

Microbial community structure and viable biomass can be quantified using phospholipid fatty acids (PLFAs). However, this method does not have the capability to identify species of microorganisms, but rather give a description of the microbial community based upon functional groups (Ibekwe et al, 2002). Phospholipids are rapidly degraded following cell death and therefore only represent the active components of the microbial community (White et al., 1979). The fatty acids released during the hydrolysis process can easily be extracted from the soil and used for identification (Drenovsky et al., 2004). Phospholipids are a vital component of cell membranes, making it an essential component of life (Peacock et al., 2001). Phospholipids are polar lipids (Turco et al., 1994), which are separated from other lipids in the soil sample based on their polarity, using solid phase extraction. The extracted phospholipids are then converted to fatty acid methyl esters during methanolysis (Leckie, 2005). The latter can be used to characterise different subgroups of microorganisms within a community e.g. Gram-negative and Gram-positive bacteria, fungi and actinomycetes (Zelles, 1999). Phospholipid fatty acids indicative of the presence of bacteria are as follows: a15:0, 15:0, i16:0, 17:0, i17:0,

cy17:0, 18:1ω7c and cy19:0. Additionally 18:2ω6,9 are use to identify fungi (Frostegård et al., 1993). Polyunsaturated PLFAs, mostly 18:2ω6c, are characteristic of fungi (Ibekwe et al, 2002). Monosaturated fatty acids are, on the other hand, indicative of Gram-negative microorganisms (Waldrop et al., 2000). Shifts in the microbial community indicated by changes, which occur in the PLFA profiles, may reflect the effect of past and present management practices on the soil ecosystem (Ponder & Tadros, 2002).

The presence of mid-chain branched saturated PLFAs is an indicator of Actinomycetes. Due to the ability of these bacteria to grow hyphae and span through soil to locate water and nutrient sources, they are better able to survive harsh environmental conditions (Peacock et al., 2001, Urzi et al., 2001). Terminal branched PLFAs, which are found in Gram-positive bacteria typically, show an increase in an environment with lower organic carbon contents (Ponder & Tadros, 2002, Ibekwe et al, 2002). Above ground disturbance causes soil to become more exposed to natural elements such as sunlight, causing limited mixing of organic materials and thereby increasing nutrient availability through mineralisation (Ponder & Tadros, 2002). The increase in availability of organic substrates within the soil ecosystem typically correlates with an increase in monounsaturated PLFAs (Bassio et al., 1998, Peacock et al., 2001). Pennanen et al. (1998) found that the bacterial community was affected by pH, shown by an increase in the amount of branched PLFAs and a decrease in monosaturated PLFAs with a decrease in pH.

The total concentration of PLFAs may give an indication of viable microbial biomass present. Changes between the relative microbial biomass of a healthy ecosystem and that of a disturbed ecosystem over a period of time may be indicative of restoration (Mummey et al., 2002), since the change in microbial biomass is concurrent with the change in microbial community structure (Bardgett & McAlister, 1999). This is true due to the fact that the amount of phospholipids per unit biomass differ between the different organisms (Olssen, 1999).

Fungal-to-bacterial ratios can be determined by dividing the concentration of 18:2ω6,9 with the sum of the bacterial PLFAs (il15:0 + a15:0 + 15:0 + i16:0 + 16:1ω5c + i17:0 + a17:0, 17:0 cy + 17:0 + 18:1ω7c + 19:0cy) (Klamer & Bååth, 1998). An indication of a shift in the microbial community contribution to soil processes can be obtained by determining the fungal-to-bacterial activity ratio of a soil sample. A ratio of 1 indicates that the fungi and bacteria contribute equally to the microbial activity in the soil (Bailey et al., 2002). In an unimproved ecosystem, the fungal-to-bacterial ratio tends to lean towards a fungal dominating community, whereas a bacterial dominating community is more likely to be found in an improved ecosystem, such as agricultural lands (Bardgett & McAlister, 1999). A correlation between fungal-to-bacterial ratio and pH has also been observed and has showed to cause a decrease in the fungal-to-bacterial ratio (Bååth & Anderson, 2003). Changes in fungal-to-bacterial ratio have also been shown to be effected by the shift in trophic structures of soil fauna (Bardgett et al., 1998).

The ratios of cyclopropyl fatty acids to their monoenoic fatty acids can also give an indication of the nutrient status of the microbial community and may give an indication of microbial activity. Therefore, it may also be important in following ecological restoration processes (Harris, 2003). Cyclopropyl fatty acids tend to increase during prolonged stationary growth of some bacteria under conditions of low carbon and oxygen concentrations as well as low pH (Guckert et al., 1986). Fierer et al. (2003) found that a ratio of greater than 0.1 indicate that the microbial community is experiencing stress. Bassio & Scow (1995) also found that the ratio of saturated fatty acids to unsaturated fatty acids might give an indication of carbon present in the soil. The latter authors found a higher ratio of saturated fatty acids to unsaturated fatty acids in environments with low carbon content and high nutrient limitations.

2.3.1.3 Microbial biomass (Chloroform fumigation)

Microbial biomass is the catalyst of all microbial transformation (Bååth & Anderson, 2003) and can be seen as the quantity of microorganism expressed as the mass of carbon immobilised in microbial cells (Hofman et al., 2003). Chloroform-fumigation is the most commonly used technique for determining soil microbial biomass due to

its simplicity. The chloroform-fumigation procedure is based on the disruption of the cell membranes by the chloroform and the quantification of the carbon content of the cells killed by the chloroform vapours through the biological conversion of carbon to carbon dioxide (Tate, 2000). Biomass carbon is calculated by correcting the carbon dioxide value with that of the carbon dioxide evolved in the nonfumigated samples (Ladd et al., 2004). By measuring microbial biomass, an indication of the potentially available nutrients and the rate of cycling can be obtained (Sparling, 1997).

A comparison between different land management systems can be made by using microbial biomass. A typically degraded area will have a lower microbial content. Microbial biomass can therefore be used as an indicator of the recovery of a degraded land area (Sparling, 1997). An increase in microbial biomass is accompanied by an increase in soil aggregates, soil structure stability, improved plant litter decomposition and increased nutrient transformation and cycling (Carpenter-Boggs et al., 2003), all of which contribute to a healthy soil ecosystem. Microbial biomass can therefore be seen as both a source and sink of nutrients for the soil ecosystem, which supports soil fertility (Hofman et al., 2003).

2.3.1.4 Mesofauna and nematodes

2.3.1.4.1 Nematode feeding functional groups

Nematode species, which have similar effects on ecosystem processes, can be classified into functional groups (Bongers & Bongers, 1998). Nematodes can be grouped into five feeding functional groups, namely, bacterial feeders, fungal feeders, predators, plant feeders and omnivores (Lazarova et al., 2004). Through quantification these feeding functional groups can give an indication of changes, which can occur within the decomposition pathways (Bongers & Bongers, 1998). Plant-feeding nematodes are typical primary consumers whereas bacterial-feeding and fungal-feeding nematodes are typical secondary consumers (Bulluck et al., 2002) that feed on primary decomposers (fungal and bacterial). This leads to an increase in the decomposition of soil organic matter and subsequently an increase in the availability of nutrients for plant growth (Neher, 2001). Furthermore, it appears that the number of fungal feeding and bacterial feeding nematodes are closely related to the number of

fungi and bacteria present within the soil ecosystem (Bongers & Bongers, 1998). Predatory nematodes are tertiary consumers and feed on bacterial- and fungal-feeding nematodes, thereby affecting their abundance (Vestergård, 2004).

2.3.1.4.2 Nematode Maturity Index

The functional feeding types can also be reflected by an index of trophic diversity (Schloter et al., 2003). Bongers (1990) proposed a nematode Maturity Index (MI) which can be used to evaluate soil ecosystem conditions. Nematodes can be ordered on a coloniser-persister (cp) scale (Bongers & Ferris, 1999), based on their potential intrinsic rate of natural increase (Bongers et al., 1997). The cp scale ranges from one (enriched opportunists) to five (persisters) (Bongers & Ferris, 1999). Group cp 1 indicates nutrient-enriched conditions, cp 2 stress caused by heavy-metal pollution and the presence of groups 3, 4 and 5 an advanced succesional stage with the absence of stress (Bongers & Bongers, 1998). Organisms characterised by enriched-opportunists, which have a short life cycle, small eggs, high fecundity and forms *dauerlarvae* in low nutrient conditions can be grouped as cp 1 (Ferris & Matute, 2003). These nematodes include rhabditid, diplogastrid and panagrolaimid bacterial feeders (Bongers & Bongers, 1998). General-opportunists include fungal-feeding nematodes of Aphelenchidae, Aphelenchodidae and Anguindae (Ferris & Matute, 2003). Bacterial-feeding nematodes of the groups Cephalobids, Plectids and Monhysterids with a short generation time and rapid reproduction tempo may be grouped into cp 2 (Bongers & Bongers, 1998).

Organisms which are relatively sensitive to disturbance have a longer generation time (life cycle) (Ferris et al., 2001) and contain bacterial feeding teratocephalids, the Araeolaimida and chromadorida, tylenchid nematodes (large nematodes), the diphtherophorids (a fungi feeder) and tripylids which are assumed to be carnivores. These nematodes can all be grouped in cp 3 (Bongers & Bongers, 1998). Large bacterial feeding nematode of the groups Alaimidae and Barthyodontida and the smaller dorylaimid nematodes and plant feeding nematodes of trichodorids can be grouped into cp 4. This group is sensitive to pollutants due to a permeable cuticle and have a long generation time (Ferris et al., 2001). Groups with a long life span, low

reproduction rate and low metabolic rate can be grouped into cp 5. These nematodes have a permeable cuticle which is sensitive to disturbance and pollution. The large omnivores, predators and plant feeding doylaimids belong to this group (Bongers & Bongers, 1998). Families with their assigned cp values, used to calculate the Maturity Index are indicated in Table 6.

Table 6. Nematode families and their assigned cp-values, including changes proposed by Bongers et al. (1995).

Families	cp-value	Families	cp-value
Alaimidae	4	Longidoridae	5[a]
Aphelenchidae	2	Monhysteridae	2
Ahelenchoididae	2	Mononchidae	4
Anguinidae	2[a]	Nordiidae	4
Aporcelaimidae	5	Panagrolaimidae	1
Bastianiidae	3	Paratylenchidae	2[a]
Belondiridae	5	Plectidae	2
Bunonematidae	1	Pratylenchidae	3[a]
Cephalobidae	2	Prismatolaimidae	3
Chromadoridae	3	Qudsianematidae	4
Criconematidae	3[a]	Rhabditidae	1
Diphtherophoridae	3	Teratocephalidae	3
Diplogasteridae	1	Thornematidae	5
Dolichodoridae	3[a]	Tobrilidae	3
Hemicyclophoridae	3[a]	Trichodoridae	4[a]
Hoplolaimidae	3[a]	Tripylidae	3
Leptonchidae	4	Tylenchidae	2[a]

[a] Families which should be included into Plant Parasitic Index rather than Maturity Index.

The Maturity Index (MI) recognises which taxa of monophyletic families are physiologically and anatomically adapted to various environmental conditions and therefore reacts in a similar way to specific environmental conditions (Ferris et al., 2001). The weighted mean of the five cp values are indicated by the Maturity Index

(Bongers et al., 1997) and excludes the plant-parasitic nematodes which is incorporated in the Plant Parasitic Index (PPI). The level of disturbance correlates with the Maturity Index values, which range from <2, indicating nutrient-enriched environments to >4, indicating pristine environments with no disturbance (Bongers & Ferris, 1999). A high Maturity Index indicates an environment suitable for K-strategists (Porazinka et al., 1999).

2.3.2 Rehabilitation of degraded land and sustainability

One of the main objectives to obtain sustainability is to reverse the effect of land degradation through reclamation and rehabilitation of these ecosystems (Urbanska et al., 2000). The Society for Ecological Restoration (SER, 2002), defines ecological restoration as "a process to assist in the recovery of a damaged, degraded, or destroyed ecosystem, through alteration of its physical and biotical conditions which include reclamation, rehabilitation, mitigation and ecological engineering". The term restoration has been widely used to cover all sorts of activities, including those better described as rehabilitation (Bradshaw, 2000a). Rehabilitation can be defined as the reparation of ecosystem processes, productivity and service (SER, 2002) to an optimal useable condition within economic boundaries (Van Wyk, 1994).

Several anthropogenic activities may contribute to the degradation of viable land. These include intensive agriculture, industrial activities and mining. Agriculture has caused a dramatic impact on the earth's landscape, transforming it to yield an abundance of nutrient and fibre to meet the demand of a growing human population (Doran et al., 1996). Modern agriculture depends on the use of fertiliser and pesticides to increase crop yield to meet the nutritional demand of an increasing human population. This leads to nonpoint source of pollution, jeopardising surface and subsurface water through the imbalance of carbon, nitrogen and water cycles in soil (Altieri, 1999; Doran et al., 1996). Agricultural pesticides and herbicides contribute to the anthropogenic effect on the atmosphere as organohalogen and organometallic compounds. The loss of vegetation and the failure to care for barren agricultural land, may lead to erosion and a contribution to dust pollution (Brimblecombe, 2002).

Mining activities may also have a negative effect on ecosystems. Natural vegetation and soils must be removed in order to obtain the desired mineral, resulting in a post-mining environment of barren subsoil and mine spoils (Bradshaw, 2000b). Mine spoils commonly lack organic matter and nutrients (Wong, 2003) and may contain high levels of toxic metals which may lead to acid mine drainage (Ledin & Pedersen, 1996). Drainage from mine spoils with a low pH may contain high concentrations of metals. The low pH tends to improve the mobility of toxic metals from the mine spoils which is a major source of environmental pollution (Wong, 2003). The primary effect of mining is the damaging of soil (Bradshaw, 1997) by a reduction in porosity and an increase in bulk density, causing the acceleration of erosion. To achieve a sustainable ecosystem, a balance between the rate of damage and restoration of an ecosystem must be obtained. It must be realised that it is necessary to include all ecological levels of organisation if an effective ecological system is to be achieved (Perrow & Davy, 2002).

2.4 The state of ecosystems of South Africa

The increase in human population has caused a decrease in the availability of land and nutrient production, which has leads to a rapid increase in settlements, agricultural land use, mining industries and related industries. These activities have lead to the loss of 25% of South Africa's natural habitats to urbanisation. Of the unchanged terrestrial habitat of South Africa, 5% are threatened by soil degradation and overgrazing (DEAT, 1999). It has been estimated that 2,5 tonnes of soil per hectare are lost annually due to wind and water erosion which is eight times faster than it can be replaced by natural processes. Currently only 6% of natural terrestrial habitats and only 17% of South Africa's coastal areas are properly protected, which is under the prescribed 10% of the World Conservation Union (IUCN). South Africa's natural habitats are some of the most sensitive ecosystems due to the low rainfall, sensitive soils, high variation in climate and restricted area of existence (DEAT, 1999).

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CHAPTER 3

MICROBIAL ACTIVITIES AS INDICATOR OF ECOSYSTEM DEVELOPMENT DURING PLATINUM MINE TAILINGS REHABILITATION

ABSTRACT

Potential enzymatic activities (β-glucosidase, alkaline and acid phosphatase, urease and dehydrogenase) have been used in this study to evaluate the effect of two different rehabilitation processes in terms of establishing vegetation cover and sustainability. Enzymatic activities play an essential role in the cycling of phosphorous (P), nitrogen (N) and carbon (C) within the soil ecosystem and can be used as indicators of ecosystem recovery due to their sensitivity to management practices. The effect of inorganic fertiliser and woodchip-vermicompost as an amendment has been evaluated. Woodchip-vermicompost contributes to the improvement of nutrient supply by lowering the base saturation (BSat) and increasing the cation exchange capacity (CEC) and water holding capacity of platinum mine tailings. Potential enzymatic activity was found to be higher in sites treated with the woodchip-vermicompost, whereas the inorganic fertilised sites had lower overall potential enzymatic activity. Seasonal variation of potential enzymatic activity has been observed. The vegetation cover on woodchip-vermicompost sites was higher than sites treated with inorganic fertilisers. Multivariate statistical analyses were performed on all components in order to determine their importance to the contribution of the progress of the two rehabilitation methods. From this multivariate statistical analyses it was concluded that the addition of the woodchip-vermicompost plays an important role in the establishment of vegetation by increasing potential enzymatic activity and the improvement of the nutrient status of the platinum mine tailings.

Platinum mine tailings, Potential enzymatic activity, Vegetation cover, Woodchip-vermicompost

3.1 Introduction

The mining industry is of great economic and social importance to South Africa (Chamber of Mines, 2003a). Fifty-five different minerals were produced by mine industries, which were exported to 87 countries during 2002. Gold was the countries greatest earner of foreign currency until it was surpassed by the Platinum Group Metals (PGM) in 2001 (Chamber of Mines, 2003b). South Africa produced 239.6 tonnes of PGMs in 2002, which contributed to 62% of the world's production and 75% of its platinum demand. This makes South Africa the world's largest platinum producer (Chamber of Mines, 2003a). Due to the high demand for these minerals, the waste rock and tailings produced by mines have increased tremendously (Freitas et al., 2004). This leads to the damaging of the land surface and often these mine waste sites become a source of pollution (Wong, 2003). A growing need has developed to reclaim these degraded lands in order to improve the quality of the environment after mining operations have been completed (Tordoff et al., 2000). Mining industries in South Africa are obligated by the Mineral and Petroleum Resource Development Act (Act 28 of 2002) to reduce the impact on the environment through rehabilitation of mine waste (Van Rensburg & Morgenthal, 2004).

The establishment of vegetation cover can fulfil the need for stabilisation, visual improvement and reduction of pollution (Tordoff et al, 2000). However, physical and chemical properties of mine tailings tend to inhibit the establishment of vegetation and soil-forming processes (Wong, 2003). Ameliorants may aid in the improvement of the substrate and so improve the establishment of vegetation (Tordoff et al., 2000). Woodchip-vermicompost were added to the platinum mine tailings in order to contribute to the incorporation of organic material in these systems and so possibly contribute to the establishment of vegetation and biological activity. Soil organic matter accumulation and microbial activity plays an important role in the soil formation process, which is essential for restoring ecosystem function (Frouz & Nováková, 2005). Microbial enzymatic activity provides measurement of the total activity of microbes in soil, due to the stability of enzymes in the soil matrix (Knight & Dick, 2004). Furthermore, microbial enzymatic activity is sensitive to changes that occur in soil, thereby providing an indication of the current soil quality (Pascual et al.,

2000). Soil quality can be defined as "The capacity of the soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health." (Doran et al., 1996). Soil microbial processes are an essential part of soil quality (Turco et al., 1994), as they play a role in the mineralisation of organic nutrients to inorganic nutrients and provide plants with required nutrients for growth (Chen et al., 2003). The biogeochemical cycling of phosphorous (P), nitrogen (N) and carbon (C) (Taylor et al., 2002) by soil microorganisms are essential for the maintenance of a balanced ecosystem (Wittmann et al., 2004). β-glucosidase, urease and phosphatase are enzymes which contribute to the hydrolyses of the biogeochemical cycling of these nutrients (Taylor et al., 2002). Thus, by measuring soil microbial enzymatic activity, an indication of process diversity, which is important for resilience and manipulation of the soil system, as well as changes in important soil functions can be detected. These changes may provide information on the progress of ecosystem rehabilitation (Taylor et al., 2002).

Soil microbial activity is a widely used indicator of soil quality and is one method which can be used to monitor rehabilitation processes (Bending et al., 2004; Gil-Sores et al., 2005; Dick, 1997). Numerous researchers have emphasised the need to monitor biological properties of developing young mine soils over a period of time to achieve successful establishment of vegetation and other soil functions (Izquierdo et al., 2005; Frouz & Nováková, 2005). The aim of this study was to evaluate the influence of two different rehabilitation methods on the establishment of vegetation cover and potential enzymatic activity on platinum mine tailings dams in the semi-arid region of the North-West province of South Africa. Rehabilitation processes were monitored by changes in microbial activity using enzymatic activity as indicator.

3.2 Materials and Methods

3.2.1 Experimental design

The experimental sites were situated at Impala Platinum Mine, Rustenburg, South Africa. A total of five sites were surveyed in the study and these sites were situated on two tailings dams as follows:

- Three sites of 30m x 10m were located on an old tailings dam, which has been dormant since 1976. Woodchip-vermicompost was applied to two of the three sites and inorganic fertilisers to the other site.
- Two sites of 30m x 30m were located on a new tailings dam, which was still in production. Woodchip-vermicompost was applied to one of the two site and inorganic fertilisers to the other site.

Platinum mine tailings were previously analysed to determine the fertilisation rate for optimum growth conditions during the study conducted by Van Rensburg & Morgenthal (2004). The aspect of all experimental sites was in a northerly direction. A total of 50 tons per ha of woodchip-vermicompost was manually applied to the tailings and worked in to a depth of 30 cm. Woodchips were obtained from mine blasting and separated from the excavated rock after milling during the screening process (Van Rensburg & Morgenthal, 2004). Subsequently it was mixed with sewage sludge and water and underwent decomposing using the earthworm species *Eisenia fetida*. Sites were sown with 12 different premixed grass species, which contained status groups. No irrigation was applied to the treated sites. The experimental site received an average of 474.4 mm rainfall, during the duration of the experiment. A natural veldt in the vicinity of the study area was chosen as a reference area, since there was no suitable rehabilitated platinum mine tailing which could be used as benchmark in the rehabilitation of platinum mine tailings. The sampling was conducted during April 2004, November 2004 and April 2005.

3.2.2 Soil sampling

The top organic matter layer was removed from the soil surface before soil was sampled. Three samples for each site (n = 18) were obtained in triplicate from the first 10 to 15 cm of the soil surface and placed in plastic bags whereafter they were kept on ice. Samples for enzymatic activities were homogenised and incubated at 25°C for seven days. These samples where than passed through a 2 mm sieve and dehydrogenase activity analysis was preformed on the field-moist soil. Soil for β-glucosidase, urease and phosphatase activities was air dried before activity was determined.

3.2.3 Vegetation cover

The vegetation cover for each site was determined by placing twenty 1 m^2 quadrants out at random locations within the sites. Vegetation cover was then determined by determining the ratio between the vegetation cover and bare ground. The mean representative value of the twenty quadrants served as vegetation cover for the whole site (Kent & Coker, 1997).

3.2.4 Analyses

3.2.4.1 Soil physical and chemical analyses

The tailings were analysed for particle size distribution as indicated by the American Society for Testing and Materials (ASTM, 1961). Soil samples were analysed for the water soluble cation fractions (Ca, Mg, K and Na), trace elements (Fe, Mn, Cu and Zn) and heavy metals (As, Se, Al, Cr, Co, Ni, Pb and Cd) by means of a 1:2 (v/v) water extraction as described by Black (1965). Analyses were done using an Agilent 7500c ICP-MS. Soil pH and electrical conductivity (EC) were determined in the 1:2 (v/v) water extraction with a WTW LF92 conductive and pH meter. Percentage organic carbon was determined using the Walkey-Black method (Walkey, 1935).

3.2.4.2 Potential enzymatic activities

Soil enzymatic activities were based on the release and quantitative determination of the product produced in the reaction mixture when specific buffering solutions were added to the soil samples (Frankenberger & Dick, 1983, Verchot & Borelli, 2005). Dry weight was determined as indicated in Alef & Nannipieri (1995).

3.2.4.2.1 Dehydrogenase activity

Dehydrogenase activity was determined by weighing 1 g of field-moist soil into a 50 ml Erlenmeyer flask. Controls were prepared by autoclaving 1 g of field-moist soil in a 50 ml Erlenmeyer flask prior to the addition of 1.5 ml Tris (hydroxyl-methyl)-aminomethane buffer (THAM buffer) and 2 ml iodonitrotetrazolium chloride (INT) to both the autoclaved and non-autoclaved samples. Samples were incubated for 2h in the dark at 40°C. After incubation, 10 ml of the extractant solution was added to the

samples and shaken at 200 rpm at 20-minute intervals for 1h to stop the reaction. The soil suspension was filtered through Whatman no. 2 filter paper. Iodonitrotetrazolium chloride-formazan (INF) development was determined spectrophotometric at 464 nm and using soil dry weight and expressed as µg INF g^{-1} dry soil $2h^{-1}$ (Alef & Nannipieri, 1995).

3.2.4.2.2 Alkaline- and Acid phosphatase activity

Phosphomonesterase activity (EC 3.1.3) assays were based on the *p*-nitrophenol release after cleavage of *p*-nitrophenyl phosphate (PNP) (Taylor et al., 2002). Air dried soil (1 g) was placed into a 50 ml Erlenmeyer flask and treated with the respective modified universal buffers (MUB) [MUB pH 6,5 for acid phosphatase (EC 3.1.3.2) and MUB pH 11 for alkaline phosphatase (EC 3.1.3.1)] and PNP, whereafter the samples were incubated at 37°C for 1 hour. After incubation, PNP was added to the controls together with calcium chloride 0.5 M ($CaCl_2$) and sodium hydroxide 0.5 M (NaOH). Contents were mixed and filtered through Whatman no. 2 filter paper. The *p*-nitrophenyl release was determined spectrophotometric at 410 nm and expressed as mg PNP g^{-1}dry soil h^{-1} using soil dry weight. (Alef & Nannipieri, 1995).

3.2.4.2.3 β-glucosidase activity

β-glucosidase (EC 3.2.1.21) activity assay was based on *p*-nitrophenol release using *p*-nitrophenyl-β-D-glucoside (PNG) as substrate (Izquierdo et al., 2005). Air dried soil (1 g) was placed into a 50 ml Erlenmeyer flask and treated with a modified universal buffer (MUB pH 6) and PNG. Samples were incubated at 37°C for 1 hour, whereafter the PNG was added to the controls. The contents of the flasks were mixed and filtered through Whatman no. 2 filter paper. The *p*-nitrophenol release was determined spectrophotometric at 410 nm and expressed as mg PNP g^{-1} dry soil h^{-1} using soil dry weight (Alef & Nannipieri, 1995).

3.2.4.2.4 Urease activity

Urease activity (EC 3.5.1.5) was assayed by weighing 5 g of air dried soil into a 125 ml Erlenmeyer flask. Samples were treated with 2.5 ml urea solution and incubated

for 2 hours at 37°C. Urea solution was added to the controls after incubation, whereafter 50 ml 1.0 M potassium chloride (KCl) was added to both the samples and controls. Samples were shaken for 30 min, whereafter it was filtered through Whatman no. 2 filter paper. Filtrate (1 ml) was used for ammonium determination. Ammonium development was determined spectrophotometric at 600 nm. Urease activity was expressed as μg NH_4-N g^{-1} dry soil $2h^{-1}$ using soil dry weight (Alef & Nannipieri, 1995).

3.2.5 Statistical analyses

Statistical analyses was preformed on all data sets using STATISTICA 7 (StatSoft, Inc ©). Parametric and non-parametric statistical analyses were performed on all data sets. The Shapiro-Wilk's test was used to test for normality. In the case of data being parametric (normally distributed), a one-way breakdown ANOVA was performed, whereafter a Tukey's Honest significance test was performed to determine statistical significance between the various samples. If data were non-parametric, such data were analysed using the Kruskal-Wallis ANOVA and Median test was used to determine statistical significant differences. If no difference was observed between the parametric and non-parametric analyses, parametric analyses were used to discuss relevant statistics.

Correlations between soil chemical properties and microbial enzymatic activity was investigated using Principal Components Analysis (PCA) and Redundancy Analysis (RDA) multivariate ordination techniques using CANOCO (Canoco for Windows Version 4.5, GLW-CPRO ©). A Principal Components Analysis (PCA) based on the linear model of variance analysis (Badiane et al., 2001) was performed on soil chemical properties and potential enzymatic activity to determine how these variables were inter-correlated to assess for multicollinearity between the variables. A Redundancy Analysis (RDA) multivariate ordination techniques were preformed using the species dependent variables (potential enzymatic activity) and the dominant independent environmental variables (soil chemical properties) (Claassens et al., 2005).

3.3. Results and Discussion

3.3.1 Experimental sites

3.3.1.1 Vegetation cover

Vegetation contributes to the aesthetic value of mine tailings by reducing dust pollution and water erosion and by serving as a barrier on which small particles accumulate. The estimated vegetation cover as observed in the study is summarised in Table 3.1. Vegetation may contribute to the soil formation process by contributing to physical, chemical and biological properties of the growth medium (De Mora et al., 2005). The woodchip-vermicompost site (NR_N) had the highest initial crown cover in April 2004 with a ratio of 64%:37% (vegetation: bare ground) followed by NR_1 with a ratio of 72%:28% and NR_2 with a ratio of 66%:34%. Both the sites treated with inorganic fertilisers showed the lowest percentage of vegetation cover to bare ground. The vegetation cover to bare ground of the two inorganic fertilised sites was as follows: Old_N had a cover ratio of 34%:66% and Old_O showed a ratio of 38%:62%.

At the end of the experimental study in April 2005 the vegetation cover to bare ground ratio of NR_N showed no change, whereas the vegetation cover of NR_1 and NR_2 as well as the inorganic fertilised sites Old_O and Old_N showed a significant ($p < 0.05$) decrease in vegetation cover from April 2004 to April 2005 (Table 3.1). The woodchip-vermicompost sites NR_1 and NR_2 showed a decrease of 17% and 13% in vegetation cover respectively, while the inorganic fertilised site Old_O showed a decrease of 13% and Old_N showed a decrease of only 8% in vegetation cover.

Plant species, which were most likely to have contributed to the highest degree of crown cover on the sites treated with woodchip-vermicompost were the perennial species *Cenchrus ciliaris* (L.) var. Molopo and Gayndah, *Chloris gayana* Kunth, *Melinis repens* (Willd) Zizka and *Heteropogon contortus* (L.) Beauv and the stoloniferous species *Cynodon dactylon* (L.) Pers., *Digitaria eriantha* Steud. Most of these species were part of the sown grass seed and may therefore indicate a successful state of rehabilitation on the woodchip-vermicompost sites. The decrease in

vegetation cover may be attributed to the decrease in rainfall received on the sites, since no irrigation took place. Rainfall decreased from 719.1 mm rain received during the first growth season of November 2003 to February 2004 to 413.5 mm rain during the period of November 2004 to February 2005. The high percentage of crown cover lost on the old tailings dam may furthermore be attributed to a veldt fire prior to the second rainfall season of November 2004 to February 2005. The decrease in vegetation cover may cause a decrease in microbial community composition as well as have an effect on soil formation processes (Aiko et al., 2000; Nierop et al., 2001). Microbial activity may also be influenced by the decrease in vegetation cover due to the lost of vegetation contributing to continues addition of organic material to the soil.

3.3.1.2 Soil physical and chemical properties

A summary of the physical properties of platinum mine tailings are presented in Table 3.2. Platinum mine tailings were characterised by a high sand percentage and a lower clay content than that of the natural veldt. Higher percentage sand and silt may contribute to surface erosion by wind or water (Bradshaw, 1997) if not protected by a suitable vegetation cover. Sandy soils have large pores, but a low total porosity, which may lead to free drainage from the soil. Low clay content contributes to the loss of retention of essential nutrients and especially an absence of soil organic matter. The woodchip-vermicompost site (NR_N) however, contained a higher clay percentage than the rest of the sites, which may contribute to better retention of essential nutrients and may have contributed to higher occurrence of vegetation cover on this site (Table 3.1).

The Principal Components Analysis (PCA) ordination diagram in Figure 3.1 illustrates the chemical properties of the woodchip-vermicompost sites over the sampling periods. Eigenvalues for the first two canonical axes were 0.656 and 0.696 respectively. Sites located on the old tailings dams are indicated by NR_1 and NR_2, while NR_N represents the site located on the new tailings dam. Chemical properties for the respective sites are presented in Tables 3.3. to 3.7.

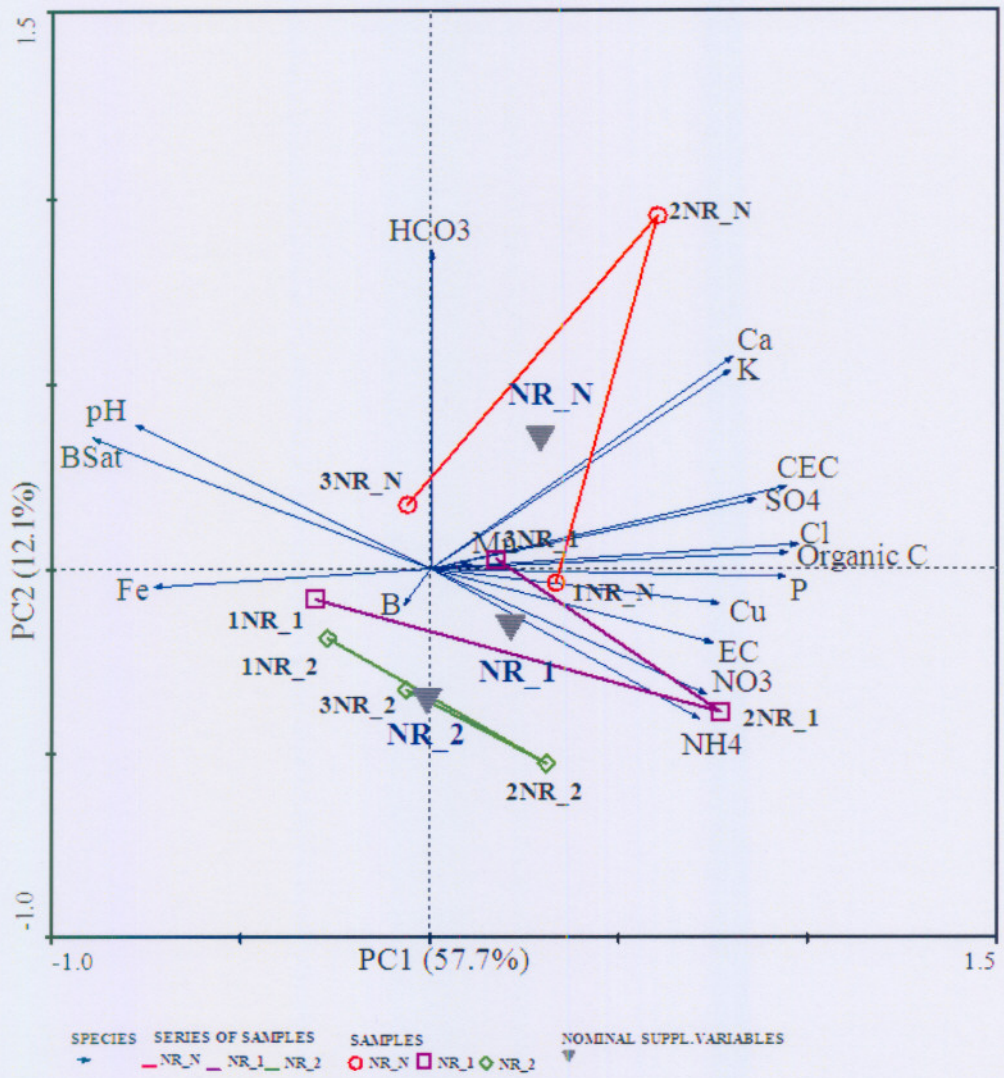


Figure 3.1. Principal Components Analysis ordination diagram of the chemical properties of the sites treated with woodchip-vermicompost. Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

The woodchip-vermicompost site NR_N was characterised by a higher cation exchange capacity (CEC) than the woodchip-vermicompost sites NR_1 and NR_2 and can be attributed to the higher clay content in this site (Table 3.2). The negative association between CEC and base saturation (BSats) illustrates that the addition of the woodchip-vermicompost contributed to a lower base saturation, which in turn contributes to an increase in CEC and water holding capacity. A decrease in CEC values from April 2004 to April 2005 was observed in NR_N, whereas NR_1 showed an increase in CEC value. The decreases in CEC values indicate a loss in soil organic

matter, which in turn might influence microbial activity within this site, causing a decrease in potential enzymatic activity and thus a decrease in cycling of essential nutrients. The increase in CEC value within the NR_1 may indicate the presence of additional organic matter. Organic material may be derived from the loss of vegetation cover as a decrease of 17% in vegetation cover was observed during the study period in this site.

Woodchip-vermicompost site NR_N was characterised by higher calcium and potassium content than Old_N and NR_2 (Table 3.6). According to results obtained by Clark et al. (1998), calcium and potassium have higher levels within soils treated with organic amendments than in soils treated with inorganic fertilisers. The increase in concentration of calcium can be associated with a decrease in boron concentration and vice versa, this was observed in both the woodchip-vermicompost and inorganic fertilised sites from April 2004 to April 2005 (Table 3.3 to 3.7). Increase in calcium was found to contribute to the decrease of boron uptake by plants (Sotiropoulos et al., 1999), which may lead to boron deficiency in plant, causing shoot leaves to become stunted (FSSA, 2003). The occurrence of higher Ca in the sites may be attributed to an increase in the leaching of Ca with annual rainfall as well as high content of Ca bearing minerals (Mengel & Kirby, 1987). Results obtained from a study conducted by Van Rensburg & Morgenthal (2004) indicated an increase in concentration of manganese, copper and iron in sites treated with woodchip-vermicompost. On the contrary, in this study a slight decrease in concentration of manganese and copper was observed in NR_N, while iron showed an increase during the last sampling period. The woodchip-vermicompost site NR_1 showed an increase in manganese and copper, with a decrease in iron levels, while NR_2 showed lower levels of all elements. The decrease in manganese and copper in NR_N may be attributed to an increase in pH, whereas NR_1 showed a decrease in pH which may have contributed to an increase in manganese and copper (Table 3.4).

The PCA diagram in Figure 3.2 illustrates the chemical properties of the site treated with inorganic fertilisers. Eigenvalues for the first two canonical axes were 0.656 and 0.696 respectively. The inorganic fertilised site located on the old tailings dam is

indicated as Old_O, whereas Old_N indicate the inorganic fertilised site located on the new tailings dam. Chemical properties of these sites are presented in Table 3.3 to 3.7.

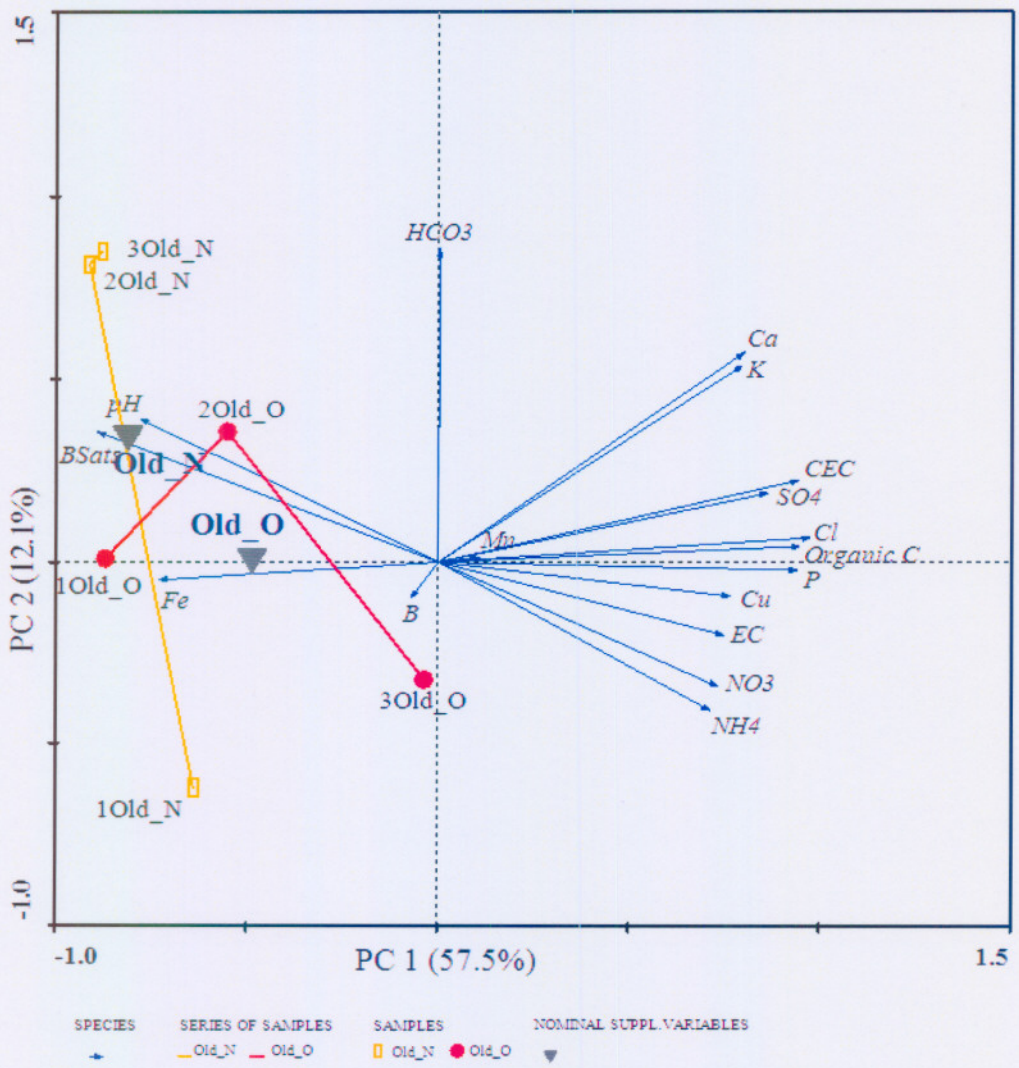


Figure 3.2. Principal Components Analysis ordination diagram of the chemical properties of the sites treated with inorganic fertilisers. Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

The low electrical conductivity (EC) values of Old_N and Old_O indicated that the salinity would not have an effect on the rehabilitation process. It also confirms the low nutrient status within these sites (Van Rensburg & Morgenthal, 2004). Inorganic fertilised sites were characterised by a higher pH, base saturation and iron levels.

From the PCA diagram (Figure 3.3) it can be concluded that the addition of woodchip-vermicompost contributes to the improvement of the chemical properties of the platinum mine tailings. By increasing the CEC and water holding capacity, the addition of the woodchip-vermicompost contributes to the availability of nutrients to plants.

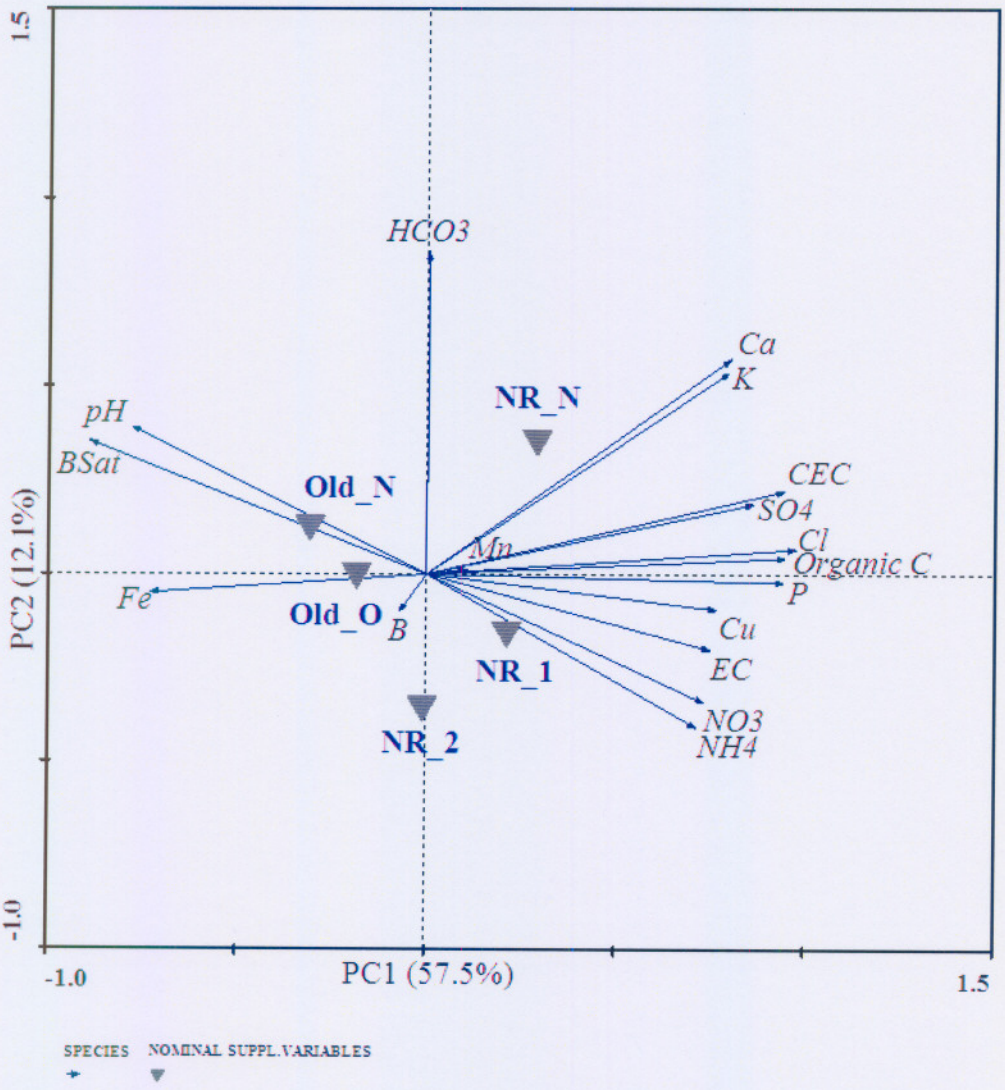


Figure 3.3. Principal Components Analysis ordination diagram indicating the chemical properties of the different treatments. NR_N represents the average of the three sampling periods in the woodchip-vermicompost site on the new tailings dam; NR_1 & NR_2 represent the average of the three sampling periods in the woodchip-vermicompost sites on the old tailings dam. Old_N and Old_O represent the average of the three sampling period in the inorganic fertilised sites on the new and old tailings dams, respectively.

3.3.1.3 Potential enzymatic activities

A summary of the potential enzymatic activities observed during the study period are presented in Table 3.8. A one-way ANOVA preformed on the enzymatic activities indicated an overall difference between the woodchip-vermicompost and inorganic fertilised sites with a probability level of 0.05 or less. A Redundancy Analysis (RDA) ordination diagram in Figure 3.4 illustrates the relationship between five different microbial enzymatic activities and the dominant independent soil chemical properties.

The eigenvalues of the first and second ordination axes were 0.435 and 0.132, respectively. The first two canonical axes contributed 56.7% to the observed variation in the data. Carbon (C) contributed to 74.3% (r^2 = -0.743) of the variance indicated by the first canonical axis while nitrate (NO_3) contributed to 31.8% (r^2 = 0.318) of the variance indicated by the second canonical axis. According to Monte Carlo Permutations test conducted with 499 permutations the first canonical axis was not statistically significant (p = 0.084). The overall effect of the chosen environmental variables on the microbial enzymatic activities were statistically significant (p = 0.026). Woodchip-vermicompost sites located on the old tailings dam are indicated as NR_1 and NR_2, while NR_N indicates the woodchip-vermicompost site located on the new tailings dam.

The RDA ordination diagram (Figure 3.4) indicated a seasonal fluctuation from the first sampling period in April 2004 to the third sampling period in April 2005. Soil potential enzymatic activity in NR_N and NR_1 increased from April 2004 to November 2004, whereafter the potential enzymatic activity decreased from November 2004 to April 2005. The woodchip-vermicompost site NR_2 showed a decrease in potential enzymatic activity from November 2004 to April 2005.

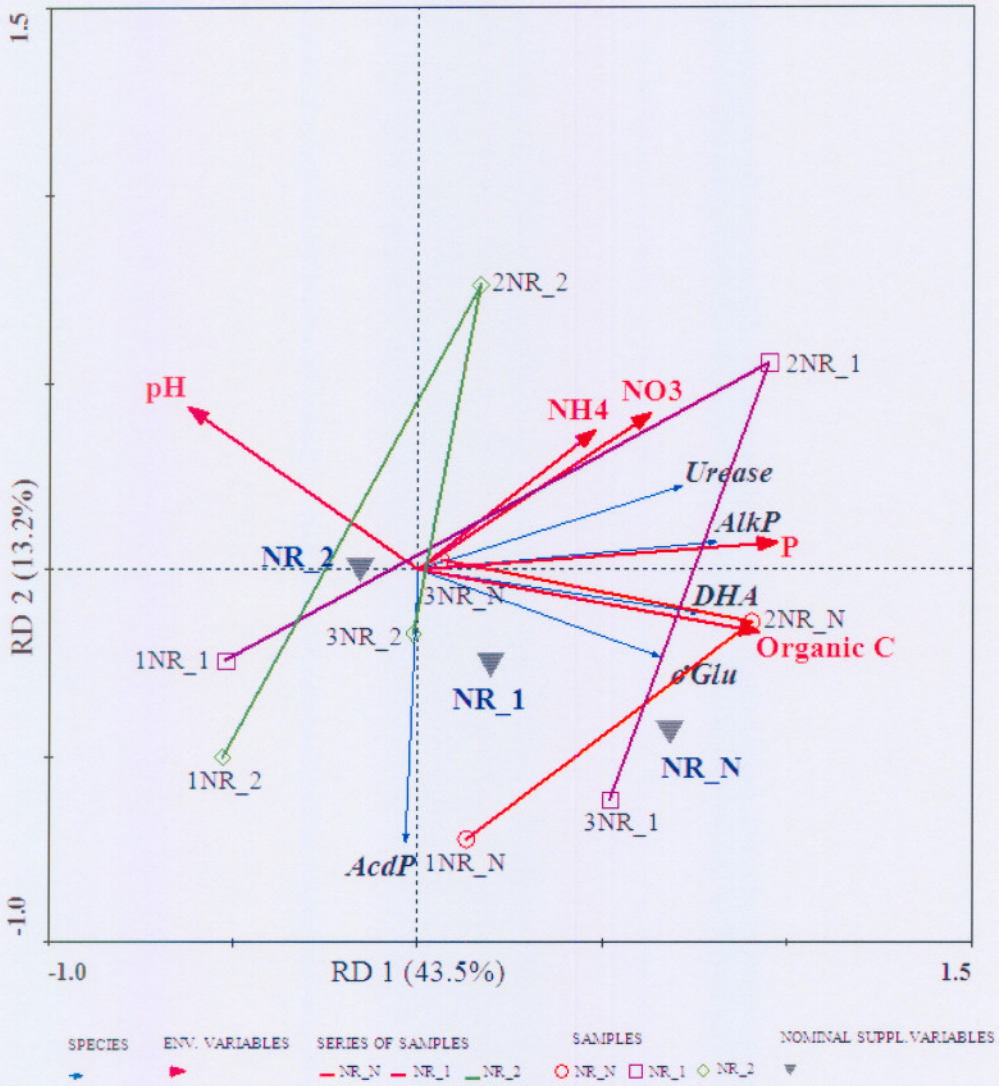


Figure 3.4. Redundancy Analysis ordination diagram illustrating the dominant independent environmental variables in relationship to potential enzymatic activities and their distribution in the woodchip-vermicompost sites. Potential enzymatic activities indicated on the ordination diagram are as follows: β-glucosidase (β-Glu), alkaline phosphatase activity (AlkP), acid phosphatase activity (AcdP), dehydrogenase activity (DHA) and urease. Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

Since dehydrogenase is mainly located in the plasma membrane of bacteria and mitochondrial membranes of fungi (Aon & Colaneri, 2001), it can be used as an indicator of overall microbial activity in environments such as semi-arid soils (Garcia et al., 1994). From the RDA ordination diagram (Figure 3.4), a correlation ($r = 0.37$, $p < 0.05$) between dehydrogenase activity and organic carbon can be observed. A correlation ($r = 0.87$, $p < 0.05$) was also observed between β-glucosidase activity and

organic carbon. Both the woodchip-vermicompost sites NR_N and NR_1 were characterised by higher β-glucosidase activity and dehydrogenase activity compared to NR_2.

Dehydrogenase activity within a soil ecosystem can be related to the organic carbon content (Bandick & Dick, 1999). The increase of dehydrogenase activity within the woodchip-vermicompost sites were consistent with the finding of Arancon et al. (2005) who reported an increase in dehydrogenase activity in sites treated with vermicompost compared to sites treated with inorganic fertilisers. The woodchip-vermicompost site NR_2 showed a slight decrease in dehydrogenase activity from April 2004 to November 2004, with a slight increase toward April 2005, but this activity was still lower than that of NR_N and NR_1 (Table 3.8). An increase in organic carbon (Table 3.7) was observed in NR_2 from April 2004 to November 2004, which may be attributed to the loss in vegetation cover (Table 3.1). The additional organic carbon was still not sufficient to contribute to higher dehydrogenase activity, similar to that found in NR_N and NR_1. The addition of the woodchip-vermicompost to NR_2, may therefore not have been sufficient to increase enzymatic activities, similar to that found in NR_N and NR_1. The woodchip-vermicompost site NR_2 may therefore be expected to have a lower nutrient cycling than NR_N and NR_1.

All woodchip-vermicompost sites were characterised by a lower β-glucosidase activity during April 2004. The β-glucosidase activity increased in the woodchip-vermicompost sites NR_N and NR_1 from April 2004 to November 2004, with NR_1 showing higher β-glucosidase activity in November 2004 compared to April 2004. Both these woodchip-vermicompost sites were characterised by higher organic carbon content (Table 3.7) than NR_2. Carbon content, which is an indicator of organic matter, contributes to the stabilisation and protection of extracellular enzymes such as β-glucosidase (Busto & Perez-Mateos, 2000). β-Glucosidase plays a central role in the cycling of soil organic carbon (Turner et al., 2002) and could therefore explain the correlation between β-glucosidase activity, dehydrogenase activity and carbon.

Phosphatase is an important enzyme in soil due to its role in hydrolyses of organically bound phosphorous to different forms of inorganic phosphorous which is assimilated by plants (Chen, 2003). A positive correlation ($r = 0.77$, $p < 0.05$) existed between alkaline phosphatase and phosphorous. The woodchip-vermicompost sites NR_N, NR_1 and, NR_2 were characterised by higher acid phosphatase activity during April 2004. In November 2004, alkaline phosphatase activity was higher in NR_N and NR_1 compared to NR_2. During the third sampling period in April 2005, the acid phosphatase activity was higher within both NR_1 and NR_2 than in NR_N, whereas alkaline phosphatase activity was higher in NR_N compared to both NR_1 and NR_2. Alkaline phosphatase activity is derived from bacterial and fungal communities whereas acid phosphatase activity is predominantly derived from plant roots (Criquet et al., 2004). The shift from acid phosphatase activity to alkaline phosphatase activity may therefore be attributed to the increase in microbial activity. It may therefore be concluded that phosphatase activity within NR_N, NR_1 and, NR_2 have been stimulated by the addition of organic matter (Pascual et al., 2002). Verchot & Borelli (2005) suggested that different phosphatase activities have specific pH preferences, with alkaline phosphatase activity being higher in alkaline soils and acid phosphatase activity being higher soils with a lower pH. From the RDA ordination diagrams (Figure 3.4), a negative correlation was observed between alkaline ($r = -0.62$, $p < 0.05$) and acid phosphatase ($r = -0.48$, $p < 0.05$) and pH. No significant association existed between pH and all enzymatic activities during this study.

Urease activity correlated with ammonium (NH_4) ($r = 0.46$, $p < 0.05$) and nitrate (NO_3) ($r = 0.70$, $p < 0.05$) according to Figure 3.4. This could be attributed to the involvement of urease in the hydrolysis of organic nitrogen components into ammonium and nitrate (Gil-Sortres et al., 2005). Jones (1982) suggested that nitrogen (N) is mineralised in the forms of ammonium (NH_4) or nitrate (NO_3) when the C/N ratio is smaller than 20:1, while immobilisation of nitrogen takes place in soils with a high C/N ratio (Bengtsson et al., 2003). It may therefore expected that the C/N ratio in the woodchip-vermicompost sites may have a ratio smaller than 20:1, leading to the mineralisation of nitrogen to ammonium or nitrate. The woodchip-vermicompost sites NR_N, NR_1 and NR_2 showed an increase in urease activity from April 2004 to

November 2004. In April 2005, NR_N was characterised by a higher urease activity than NR_1 and NR_2. Pascaul et al. (2002) found that urease activity increases over time in sites treated with composted organic material. This was explained by the proportion of immobilised enzymes, which were more stable in compost causing a slower release of this enzyme. Therefore, an increase in urease activity occured over time and might therefore explain the increase in urease activity in the woodchip-vermicompost sites.

The RDA ordination diagram in Figure 3.5 illustrates the potential enzymatic activity in relation with independent environmental variables as obtained in sites treated with inorganic fertilisers. The eigenvalues of the first and second ordination axes were 0.435 and 0.132, respectively. The first two canonical axes contributed 56.7% to the observed variation in the data. Carbon (C) contributed to 74.3% (r^2 = -0.743) of the variance indicated by the first canonical axis while nitrate (NO_3) contributed to 31.8% (r^2 = 0.318) of the variance indicated by the second canonical axis. According to Monte Carlo Permutations test conducted with 499 permutations the first canonical axis was not statistically significant (p = 0.084). The overall effect of the chosen environmental variables on the potential enzymatic activities were statistically significant (p = 0.026). Old_O and Old_N represent the inorganic fertilised sites on the old tailings dam and new tailings dam, respectively.

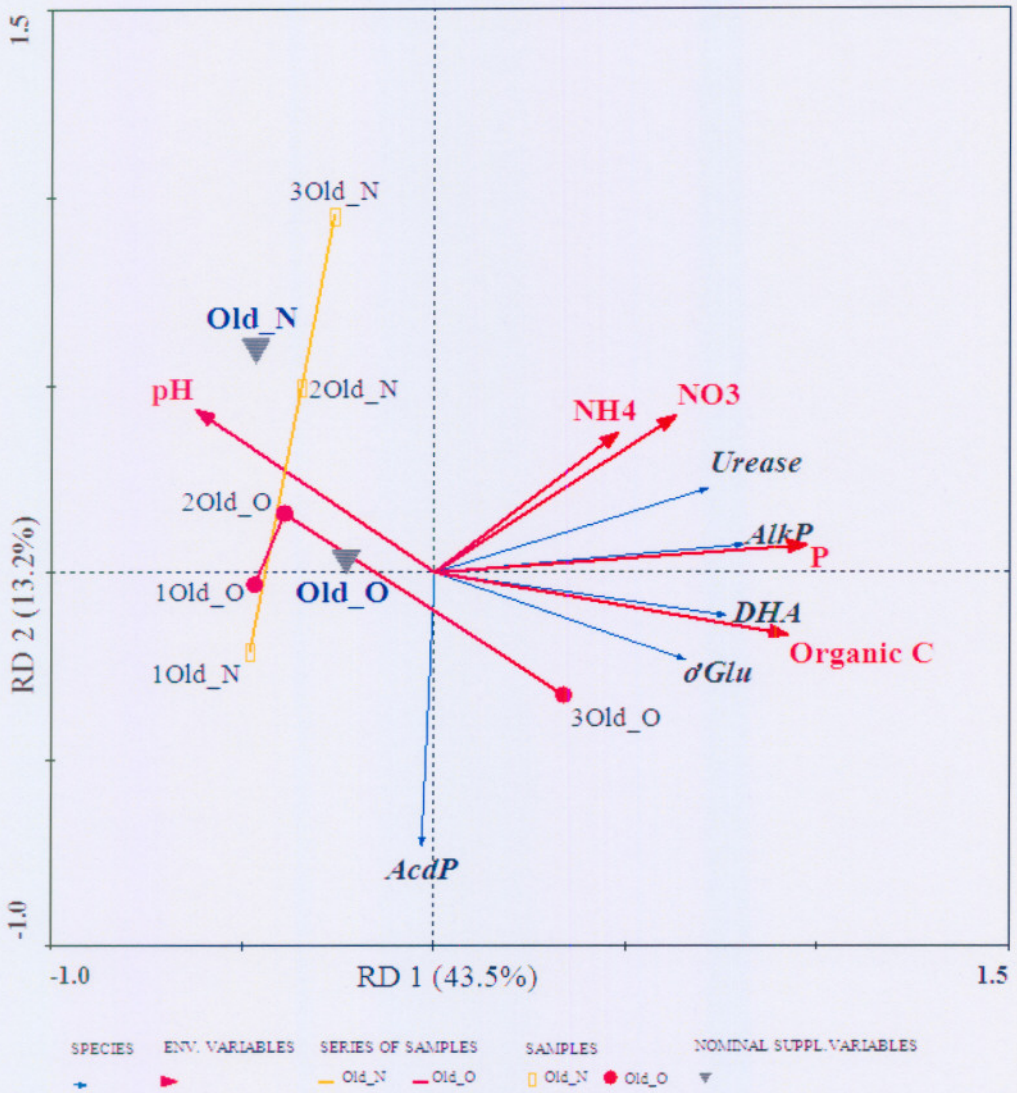


Figure 3.5. Redundancy Analysis ordination diagram illustrating the environmental variables in relationship to potential enzymatic activities and their distribution in the inorganic fertilised sites. Potential enzymatic activities indicated on the ordination diagram are as follows: β-glucosidase (β-Glu), alkaline phosphatase activity (AlkP), acid phosphatase activity (AcdP), dehydrogenase activity (DHA) and Urease. Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

Sites treated with inorganic fertilisers were characterised by lower potential enzymatic activity, than the woodchip-vermicompost sites. The inorganic fertilised sites contained lower organic carbon and thus lower organic colloid content. The decrease in organic colloids within these sites may lead to a decrease in stability of enzymes (Knight & Dick, 2004), resulting in a decrease in enzymatic activity. The lower dehydrogenase activity within the inorganic fertilised sites Old_N and Old_O may be due to progressive erosion because of low plant cover (Table 3.1) and lower organic

matter within these sites. The inorganic fertilised site Old_O did show an increase in β-glucosidase activity, indicating an increase in carbon cycling in this site. This increase may be due to the loss of extensive plant cover, which may have been the source of the organic matter, since a decrease of 13% in vegetation cover was observed during the study in this site (Table 3.1). Inorganic fertilised sites were also characterised by lower alkaline and acid phosphatase activity, indicating lower cycling of phosphorous in these sites.

The RDA ordination diagram in Figure 3.6 illustrates the potential enzymatic activities in relation to the independent environmental variables. From the RDA ordination diagram (Figure 3.6) it can be observed that sites to the right of the first ordination axis (NR_N and NR_1) had the highest potential enzymatic activity during the study, whereas sites located to the left had the lowest potential enzymatic activity.

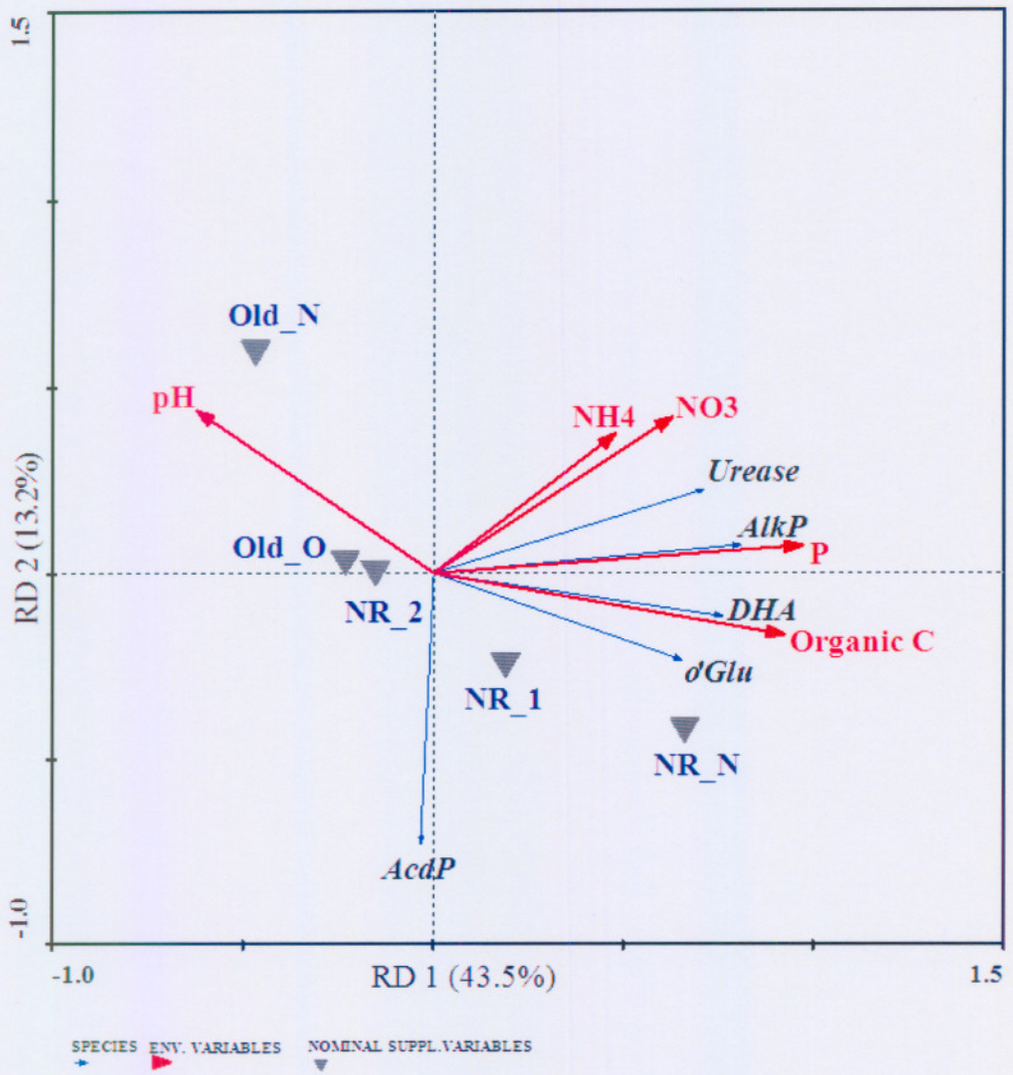


Figure 3.6. Redundancy Analysis ordination diagram illustrating the potential enzymatic activities in relation to the independent environmental variables. NR_N represents the average of the three sampling periods in woodchip-vermicompost site on the new tailings dam; NR_1 & NR_2 represent the average of the three sampling periods in the woodchip-vermicompost sites on the old tailings dam. Old_N and Old_O represent the average of the three sampling periods in the inorganic fertilised sites on the new and old tailings dams, respectively.

The higher potential enzymatic activity within the woodchip-vermicompost sites can be attributed to the addition of organic material (Ros et al., 2003), in this case representing woodchip-vermicompost. The lower potential enzymatic activity in Old_N and Old_O may be attributed to the low organic matter and low vegetation cover (Table 3.1).

Soil microbial activity contributes to the maintenance of soil quality by contributing to the biogeochemical cycling of essential nutrients, the soil formation process and regulating the decomposition of organic matter (Turco et al., 1994). The woodchip-vermicompost sites may therefore have a higher cycling of essential soil nutrient than the sites treated with the inorganic fertilisers. The higher potential enzymatic activity in the woodchip-vermicompost sites emphasises the importance of the use of composted organic matter during the rehabilitation process. The addition of woodchip-vermicompost could be a rapid approach in the remediation of mine tailings in order to establish a growth medium sufficient for the establishment of vegetation.

No significant differences have been observed between the enzymatic activities of the two tailings dams based on rehabilitation age. The woodchip-vermicompost site NR_2 could, however, be grouped with the inorganic fertilised sites based on lower potential enzymatic activity. The lower potential enzymatic activity in this site may be due to poor management practices during the implementation of the woodchip-vermicomposting at this site.

3.3.2 Reference area

3.3.2.1 Soil physical and chemical properties

The physical properties of the natural veldt are presented in Table 3.2. The natural veldt showed a high sand, silt and clay content. The high clay content contributes to the retention of essential nutrients with in these soils. A higher percentage of particles greater than 2 mm were expected within the natural veldt due to the occurrence of rock particles.

The PCA ordination diagram in Figure 3.7 illustrates the chemical properties distribution of the natural veldt, which was used as reference area during the study. Eigenvalues for the two principal component axes are 0.656 and 0.696, respectively. Table 3.3 to 3.7 summarises the chemical properties of the natural veldt.

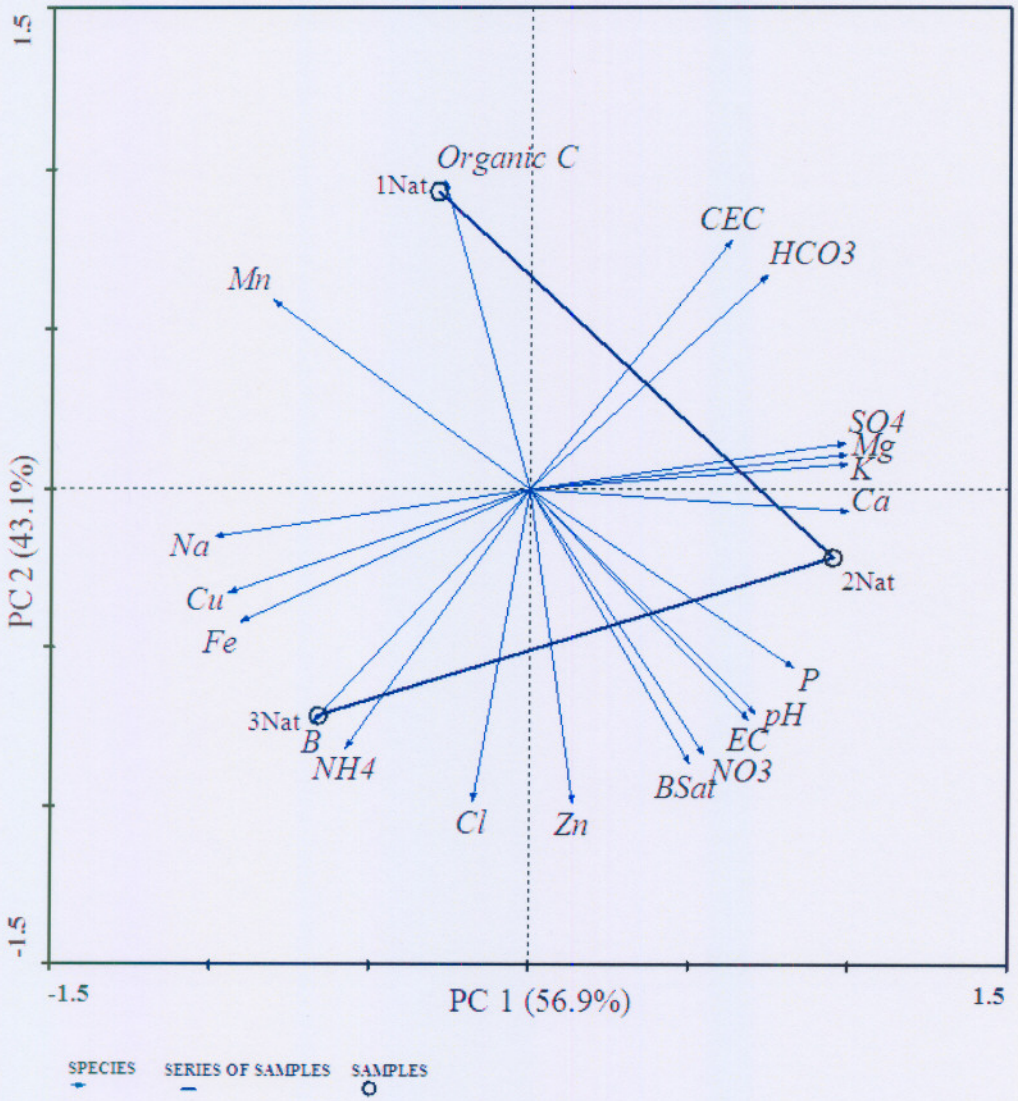


Figure 3.7. Principal Components Analysis ordination diagram of the chemical properties of the natural veldt used as reference site. Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

As expected, the PCA ordination diagram (Figure 3.7) showed a remarkable difference between the chemical properties of the platinum mine tailings (Figure 3.3) and that of the surrounding natural veldt. The overall chemical properties of the natural veldt, with emphasis on organic carbon, EC and CEC, has a tendency to be significantly higher than that found on the platinum mine tailings dams. Conversely, the natural veldt showed a lower pH and base saturation (Figure 3.7) compared to that of platinum mine tailings (Figure 3.3).

3.3.2.2 Potential enzymatic activities

The RDA ordination diagram presented in Figure 3.8 illustrates the relation between potential enzymatic activities and the dominant independent chemical properties of the natural veldt. A summary of the potential enzymatic activity of the natural veldt can be obtained from Tables 3.8. The eigenvalues for the first two axes were 0.911 and 0.090, respectively. Total observed variance of the first two canonical axes was 100%. Phosphorous (P) contributed to 99.8% (r^2 = -0.9985) of the variance indicated by the first canonical axis while ammonium (NH_4) contributed to 99.7% (r^2 = 0.9974) of the variance indicated by the second canonical axis. According to Monte Carlo Permutations test conducted with 499 permutations the first canonical axis was not statistically significant (p = 1.000). The overall effect of the chosen environmental variables on the microbial enzymatic activities was also not statistically significant.

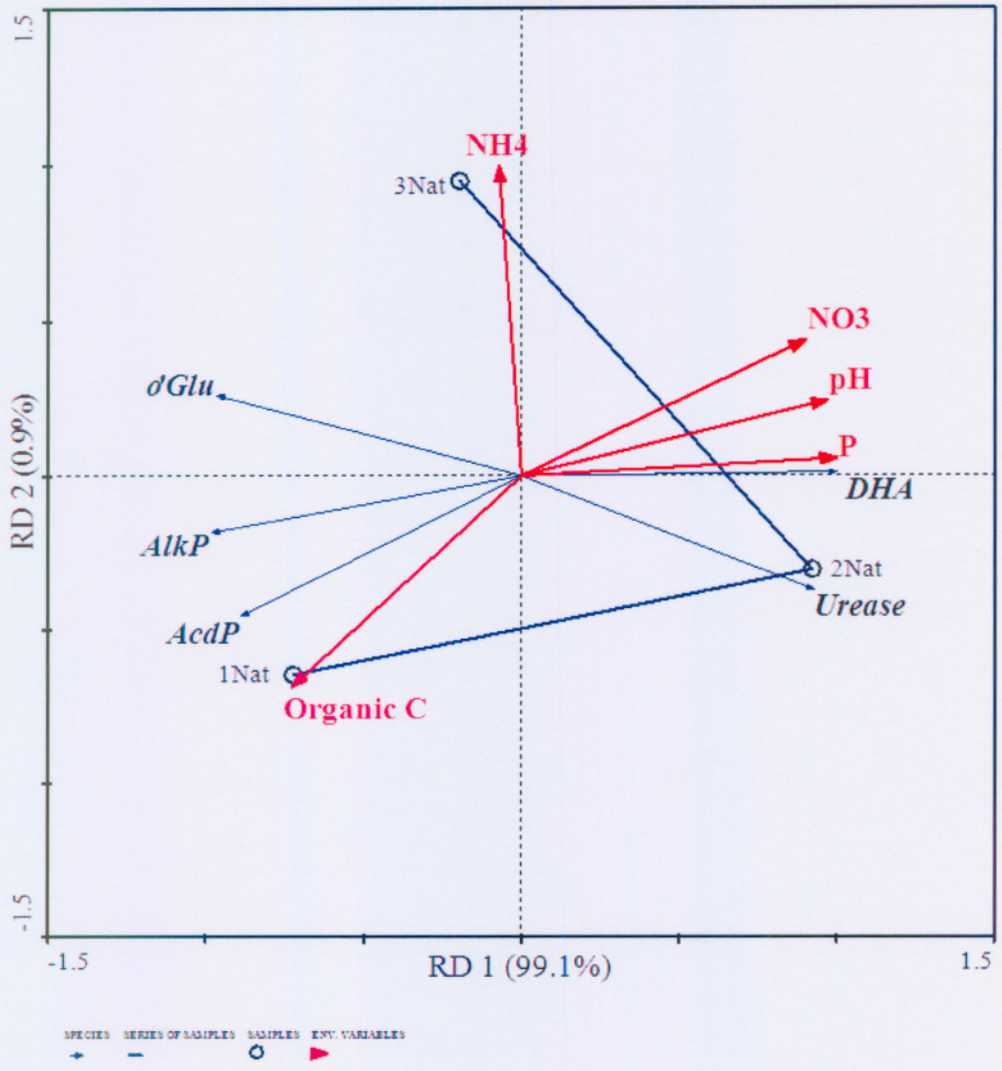


Figure 3.8. Redundancy Analysis ordination diagram illustrating the environmental variables in relation to the potential enzymatic activities and their distribution in the natural veldt. Potential enzymatic activities indicated on the ordination diagram are as follows: β-glucosidase (β-Glu), alkaline phosphatase activity (AlkP), acid phosphatase activity (AcdP), dehydrogenase activity (DHA) and Urease. Sampling periods at the different sites are indicated as follows: (1) April 2004, (2) November 2004 and (3) April 2005.

Dehydrogenase and urease activity showed an association with pH. A weak association existed between urease and ammonium (NH_4) however, urease could be associated with nitrate (NO_3). Both acid and alkaline phosphatase activities showed a weak association with phosphorous and pH. Seasonal fluctuation of potential enzymatic activities was observed in the natural veldt. The overall potential enzymatic activities observed were much higher in the natural veldt than in either the woodchip-vermicompost or inorganic fertilised sites.

3.4. Conclusion

The accumulation of organic matter (organic carbon) in mine tailings as well as the activation of this organic matter by microbial activity is an important component of the soil development process in post mining rehabilitation (Šantrůčková et al., 2004). According to the potential enzymatic activities, the addition of organic material, in this case woodchip-vermicompost, may be essential in the progress of the rehabilitation process. Sites treated with woodchip-vermicompost showed a higher overall potential enzymatic activity, whereas inorganic fertilisers showed a lower potential enzymatic activity. These findings can be supported by the results obtained by Atiyeh et al. (2002) who found an increase in microbial activity within sites treated with vermicompost. Enzymatic activity contributes to the biogeochemical transformation of essential elements such as phosphorous (P), nitrogen (N) and carbon (C), which in turn contribute to plant growth. The addition of the woodchip-vermicompost may be a more preferable rehabilitation process compared to the addition of inorganic fertilisers, due to its ability to improve the chemical properties of the substrate by increasing CEC (causing more cations to be absorbed). Thus, supplying metabolic energy for microorganisms, as well as contributing to the growth and development of plant and by stimulating enzymatic activity, which contribute to the biogeochemical cycling of essential nutrients (FSSA, 2003). Further studies need to be conducted over a longer time period in order to establish the sustainability of these organically enriched systems. Aftercare may be required for the woodchip-vermicompost sites by adding additional organic matter to these sites to ensure and maintain a sufficient nutrient status and organic carbon within these sites.

Table 3.1. Summary of vegetation crown cover of the different treatments.

	New Tailings Dam				Old Tailings Dam					
	NR_N		Old_N		NR_1		NR_2		Old_O	
	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05
Vegetation Cover (%)	64	64	34	26	72	55	66	53	38	23
Bare ground (%)	37	37	66	74	28	45	34	47	62	78

Table 3.2. Summary of the particle size distribution of platinum mine tailings.

Particle size distribution	> 2mm	Clay (%)	Silt (%)	Sand (%)
NR_N	3.5	5.2	14.1	80.7
NR_1	2.5	2.4	13.5	84.1
NR_2	0.7	2.3	4.8	92.9
Old_N	0.6	2.3	13.4	84.3
Old_O	0.5	2.2	12.4	85.4
Natural Veldt	12.4	6.6	23.4	70.0

Table 3.3. Summary of the chemical composition of platinum tailings: 1:2 (v/v) water extraction.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_05	Nov_05	Apr_05	Apr_04	Nov_04	Apr_05
1:2 Extraction						
Ca (mmol/l)	0.63 ± 0.13	1.14 ± 0.40	0.39 ± 0.17	0.33 ± 0.05	0.17 ± 0.07	0.11 ± 0.02
Mg (mmol/l)	0.46 ± 0.11	0.47 ± 0.10	0.19 ± 0.09	0.12 ± 0.03	0.02 ± 0.15	0.13 ± 0.03
K (mmol/l)	0.17 ± 0.04	0.38 ± 0.72	0.25 ● 0.06	0.17 ± 0.05	0.09 ± 0.02	0.10 ± 0.02
Na (mmol/l)	0.22 ± 0.04	0.38 ± 0.09	0.13 ± 0.06	0.05 ± 0.01	0.08 ± 0.01	0.03 ± 0.00
SO4 (mmol/l)	0.81 ± 0.31	1.10 ± 0.52	0.39 ± 0.24	0.42 ± 0.10	0.03 ± 0.002	0.06 ± 0.02
NO3 (mmol/l)	0.01 ± 0.00	0.49 ± 0.04	0.04 ± 0.02	0.08 ± 0.03	0.07 ± 0.02	0.02 ± 0.00
NH4 (mmol/l)	0.12 ± 0.04	0.08 ± 0.03	0.05 ± 0.00	0.05 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
Cl (mmol/l)	0.25 ± 0.04	0.34 ± 0.07	0.18 ± 0.04	0.06 ± 0.08	0.03 ± 0.01	0.09 ± 0.01
HCO3 (mmol/l)	0.40 ± 0.05	0.58 ± 0.13	0.33 ± 0.04	0.23 ± 0.01	0.47 ± 0.15	0.40 ± 0.13
	Old Tailings Dam					
	NR_1			NR_2		
Ca (mmol/l)	0.17 ± 0.07	1.29 ± 0.03	0.52 ± 0.22	0.19 ± 0.06	0.73 ± 0.22	0.27 ± 0.17
Mg (mmol/l)	0.09 ± 0.04	0.70 ± 0.11	0.44 ± 0.18	0.03 ± 0.02	0.40 ± 0.15	0.22 ± 0.09
K (mmol/l)	0.06 ± 0.01	1.12 ± 0.09	0.20 ± 0.08	0.11 ± 0.01	0.42 ± 0.04	0.12 ± 0.06
Na (mmol/l)	0.02 ± 0.01	0.21 ± 0.03	0.16 ± 0.09	0.02 ± 0.01	0.14 ± 0.06	0.12 ± 0.06
SO4 (mmol/l)	0.10 ± 0.05	0.94 ± 0.26	0.59 ± 0.39	0.09 ± 0.01	0.61 ± 0.24	0.15 ± 0.08
NO3 (mmol/l)	0.03 ± 0.01	2.16 ± 0.26	0.16 ± 0.04	0.04 ± 0.02	0.93 ± 0.16	0.42 ± 0.35
NH4 (mmol/l)	0.03 ± 0.01	0.23 ± 0.05	0.02 ± 0.01	0.04 ± 0.01	0.26 ± 0.05	0.02 ● 0.00
Cl (mmol/l)	0.05 ± 0.04	0.37 ± 0.02	0.16 ± 0.05	0.05 ± 0.01	0.26 ± 0.17	0.12 ± 0.03
HCO3 (mmol/l)	0.33 ± 0.13	0.23 ± 0.02	0.25 ± 0.06	0.35 ± 0.20	0.23 ± 0.02	0.12 ± 0.02
	Old Tailings Dam			Natural veldt		
	Old_O			Nat		
Ca (mmol/l)	0.23 ± 0.06	0.64 ± 0.48	0.21 ± 0.04	0.84 ± 0.17	1.23 ± 0.29	0.45 ± 0.18
Mg (mmol/l)	0.04 ± 0.02	0.21 ± 0.18	0.20 ± 0.02	0.48 ± 0.12	0. 40 ± 0.13	0.35 ± 0.25
K (mmol/l)	0.07 ± 0.01	0.24 ± 0.13	0.20 ± 0.03	0.85 ± 0.23	0.97 ± 0.21	1.34 ± 0.93
Na (mmol/l)	0.02 ± 0.00	0.06 ± 0.02	0.05 ± 0.02	0.02 ± 0.01	0.04 ± 0.03	0.01 ± 0.01
SO4 (mmol/l)	0.13 ± 0.02	0.65 ± 0.58	0.09 ± 0.02	0.35 ± 0.06	0.41 ± 0.13	0.23 ± 0.12
NO3 (mmol/l)	0.05 ± 0.01	0.33 ± 0.26	0.14 ± 0.07	2.18 ± 0.34	2.37 ± 0.68	1.75 ± 1.24
NH4 (mmol/l)	0.04 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.23 ± 0.14	0.04 ± 0.01	0.04 ± 0.01
Cl (mmol/l)	0.06 ± 0.01	0.11 ± 0.08	0.14 ± 0.02	0.17 ± 0.02	0.22 ± 0.08	0.31 ± 0.23
HCO3 (mmol/l)	0.35 ± 0.13	0.38 ± 0.12	0.22 ± 0.03	0.42 ± 0.26	0.33 ± 0.10	0.17 ± 0.03

Value indicated as mean ± SEM

Table 3.4. Summary of the micro-element properties of the platinum mine tailings

New Tailings Dam						
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Micro-elements						
Fe (mmol/l)	16.39 ± 2.97	8.66 ± 4.05	21.52 ± 6.64	30.8 ± 6.20	26.78 ± 2.16	20.16 ± 6.34
Mn (mmol/l)	2.56 ● 0.94	1.45 ± 0.49	1.98 ● 0.27	1.64 ± 0.29	1.43 ± 0.20	3.08 ± 1.67
Cu (mmol/l)	3.44 ± 0.96	2.54 ± 0.12	1.96 ± 0.25	0.64 ± 0.03	1.29 ± 0.51	1.65 ± 0.47
Zn (mmol/l)	1.23 ± 0.44	0.98 ± 0.47	0.49 ± 0.21	0.36 ± 0.06	0.43 ± 0.20	0.28 ± 0.23
B (mmol/l)	62.7 ± 0.21	4.16 ± 4.15	4.70 ± 4.69	0.46 ± 0.36	2.90 ± 1.45	3.01 ± 2.99
Old Tailings Dam						
	NR_1			NR_2		
Fe (mmol/l)	18.18 ± 5.75	11.38 ± 1.63	12.33 ± 3.03	31.92 ± 5.60	14.77 ± 2.32	11.16 ± 3.27
Mn (mmol/l)	1.07 ± 0.23	2.24 ± 0.12	2.09 ± 0.07	1.85 ± 0.22	2.53 ± 0.49	1.11 ± 0.33
Cu (mmol/l)	0.65 ± 0.16	2.50 ± 0.34	2.08 ± 0.26	1.35 ± 0.31	3.48 ± 0.16	1.77 ± 0.41
Zn (mmol/l)	0.26 ± 0.01	1.57 ± 0.92	0.92 ± 0.37	0.23 ± 0.07	0.55 ± 0.29	0.19 ± 0.11
B (mmol/l)	49.4 ± 0.94	0.01 ± 0.00	5.91 ± 0.89	48.3 ± 1.11	0.01 ± 0.00	6.48 ± 3.64
	Old Tailings Dam			**Natural veldt**		
	Old_O			Nat		
Fe (mmol/l)	24.91 ± 5.63	31.09 ± 16.26	15.52 ± 3.45	37.66 ± 13.97	17.61 ± 4.70	69.65 ± 27.52
Mn (mmol/l)	1.43 ± 0.22	3.54 ± 1.15	1.17 ± 0.08	6.53 ± 1.20	0.80 ± 0.32	4.24 ± 123
Cu (mmol/l)	0.85 ± 0.20	0.75 ± 0.36	2.41 ± 0.22	0.56 ± 0.07	0.02 ± 0.00	1.16 ± 0.16
Zn (mmol/l)	0.40 ± 0.07	0.09 ± 0.06	0.18 ± 0.03	0.32 ± 0.03	0.65 ± 0.19	0.72 ± 0.44
B (mmol/l)	0.10 ± 0.00	4.80 ± 2.59	1.67 ± 0.66	0.1 ± 0.00	0.01 ± 0.00	1.34 ± 1.33

Value indicated as mean ± SEM

Table 3.5. Summary of the chemical properties of platinum tailings: ammonium acetate extraction method.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Nutrients						
Ca (mg/kg)	1063.00 ± 81.61	1526.67 ± 317.92	804.83 ± 153.69	333.83 ± 12.70	562.33 ± 274.01	483.50 ± 127.40
Mg (mg/kg)	190.50 ± 9.37	226.33 ± 64.81	121.17 ± 34.92	45.83 ± 8.82	26.17 ± 2.62	56.50 ± 16.53
K (mg/kg)	65.17 ± 6.66	208.17 ± 83.51	86.83 ± 13.92	6.83 ± 0.17	45.33 ± 15.55	43.00 ± 25.08
Na (mg/kg)	60.67 ± 6.36	36.33 ± 3.32	45.50 ± 16.91	49.5 ± 1.04	37.33 ± 4.57	81.83 ± 12.89
P (mg/kg)	57.70 ± 13.28	144.68 ± 41.93	53.58 ± 21.15	7.10 ± 1.84	10.88 ± 1.56	10.73 ± 1.28
	Old Tailings Dam					
	NR_1			NR_2		
Ca (mg/kg)	333.52 ± 52.61	1189.50 ± 31.19	950.00 ± 416.97	290.33 ± 44.07	576.83 ± 140.10	579.83 ± 83.16
Mg (mg/kg)	34.83 ± 6.21	181.67 ± 10.76	161.33 ± 67.24	41.50 ± 6.45	71.50 ± 17.30	115.33 ± 23.29
K (mg/kg)	2.17 ± 0.33	230.67 ± 10.94	102.50 ± 63.00	4.83 ± 2.05	71.83 ± 18.50	39.83 ● 9.90
Na (mg/kg)	36.83 ± 3.53	52.67 ± 2.24	69.17 ± 32.71	46.00 ± 8.39	31.67 ± 0.60	96.83 ± 7.86
P (mg/kg)	3.25 ± 0.13	191.00 ± 23.68	95.75 ± 35.84	4.45 ± 0.70	95.10 ± 25.17	71.03 ± 25.22
	Old Tailings Dam			Natural Veldt		
	Old_O			Nat		
Ca (mg/kg)	304.00 ± 39.24	454.17 ± 135.44	680.17 ± 74.32	2282.8 ± 356.97	3292.67 ± 203.74	2741.67 ± 259.23
Mg (mg/kg)	42.00 ± 1.00	33.67 ± 13.31	104.67 ± 22.11	339.33 ± 71.95	433.50 ± 60.60	402.17 ± 84.80
K (mg/kg)	1.00 ± 0.29	37.67 ± 11.42	65.33 ± 16.18	423.00 ± 88.74	577.83 ± 39.83	1050.00 ± 529.64
Na (mg/kg)	47.67 ± 14.09	49.00 ± 5.30	99.17 ± 4.51	55.67 ± 6.43	39.83 ± 2.91	91.17 ± 2.49
P (mg/kg)	4.20 ± 0.40	8.35 ● 0.63	85.80 ± 13.19	64.70 ± 15.68	135.50 ± 14.78	90.85 ± 9.20

Value indicated as mean ± SEM

Table 3.6 Summary of the exchangeable cations found in the platinum mine tailings.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Exchangeable cations						
Ca (cmol(+)/kg)	5.30 ± 0.41	7.62 ± 1.59	4.02 ± 0.77	1.67 ± 0.06	2.81 ± 1.37	2.41 ± 0.64
Mg (cmol(+)/kg)	1.57 ± 0.08	1.86 ± 0.53	1.00 ± 0.29	0.38 ± 0.07	0.22 ± 0.02	0.47 ± 0.14
K (cmol(+)/kg)	0.17 ± 0.02	0.53 ± 0.21	0.22 ± 0.04	0.02 ± 0.00	0.12 ± 0.04	0.11 ± 0.06
Na (cmol(+)/kg)	0.26 ± 0.03	0.16 ± 0.01	0.20 ± 0.07	0.22 ± 0.00	0.16 ± 0.02	0.36 ± 0.06
	Old Tailings Dam					
	NR_1			NR_2		
Ca (cmol(+)/kg)	1.66 ± 0.26	5.94 ± 0.16	4.74 ± 2.08	1.45 ± 0.22	2.88 ± 0.70	2.89 ± 0.42
Mg (cmol(+)/kg)	0.29 ± 0.05	1.50 ± 0.09	1.33 ± 0.55	0.34 ± 0.05	0.60 ± 0.14	0.95 ± 0.19
K (cmol(+)/kg)	0.01 ± 0.00	0.59 ± 0.03	0.26 ± 0.16	0.01 ± 0.05	0.18 ± 0.05	0.10 ± 0.03
Na (cmol(+)/kg)	0.16 ± 0.02	0.23 ± 0.01	0.30 ± 0.14	0.2 ± 0.04	0.14 ± 0.00	0.42 ± 0.03
	Old Tailings Dam			Natural Veldt		
	Old_O			Nat		
Ca (cmol(+)/kg)	1.51 ± 0.19	2.27 ± 0.68	3.39 ± 0.37	1.78 ± 1.78	16.43 ± 1.01	13.68 ± 1.29
Mg (cmol(+)/kg)	0.35 ± 0.01	0.28 ± 0.11	0.86 ± 0.18	2.79 ± 0.59	3.57 ± 0.50	3.31 ± 0.70
K (cmol(+)/kg)	0.00 ± 0.00	0.10 ± 0.03	0.17 ± 0.04	1.09 ± 0.23	1.48 ± 0.29	2.69 ± 1.36
Na (cmol(+)/kg)	0.21 ± 0.06	0.21 ± 0.02	0.43 ± 0.02	0.24 ± 0.03	0.17 ± 0.01	0.40 ± 0.01

Value indicated as mean ± SEM

Table 3.7. Summary of important chemical properties of the platinum mine tailings.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Total C (%)	0.51 ± 0.04	1.72 ± 0.28	0.71 ± 0.25	0.09 ± 0.003	0.13 ± 0.06	0.12 ± 0.03
pH	6.51 ± 0.23	7.09 ± 0.22	6.89 ± 0.22	6.87 ± 0.12	7.30 ± 0.26	7.07 ± 0.17
EC (mS/cm)	0.27 ± 0.06	0.41 ± 0.10	0.16 ± 0.06	0.12 ± 0.02	0.06 ± 0.01	0.07 ± 0.01
pH(H2O)	6.51 ± 0.06	6.69 ± 0.19	7.10 ± 0.19	6.98 ± 0.09	7.53 ± 0.27	7.85 ± 0.03
pH(KCl)	5.97 ± 0.09	6.18 ± 0.25	6.43 ± 0.28	6.84 ± 0.06	7.01 ± 0.42	7.08 ± 0.05
CEC (cmol(+)/kg)	8.99 ± 0.39	12.10 ± 3.14	5.66 ± 1.40	2.34 ± 0.10	3.21 ± 1.27	3.10 ± 0.80
Base sat (%)	81.26 ± 4.03	85.46 ± 8.82	99.12 ± 6.45	97.47 ± 3.58	100.60 ± 5.02	107.82 ± 0.85
P (mmol/l)	0.13 ± 0.02	0.16 ± 0.06	0.10 ± 0.02	0.01 ± 0.00	0.01 ● 0.00	0.01 ± 0.01
	Old Tailings Dam					
	NR_1			NR_2		
Total C (%)	0.07 ± 0.01	2.19 ± 0.13	1.57 ± 0.68	0.12 ± 0.02	0.94 ± 0.53	0.36 ± 0.22
pH	6.89 ± 0.02	6.29 ± 0.01	6.18 ± 0.33	6.64 ± 0.45	6.45 ± 0.12	6.26 ± 0.12
EC (mS/cm)	0.07 ± 0.02	0.55 ± 0.03	0.23 ± 0.08	0.07 ± 0.02	0.31 ● 0.09	0.13 ± 0.06
pH(H2O)	7.04 ± 0.06	6.39 ± 0.03	6.78 ± 0.36	6.86 ± 0.12	6.62 ± 0.15	6.83 ± 0.14
pH(KCl)	6.92 ± 0.42	5.79 ± 0.06	6.19 ± 0.44	6.31 ± 0.48	5.72 ± 0.20	6.11 ± 0.13
CEC (cmol(+)/kg)	2.08 ± 0.16	11.98 ± 0.53	8.59 ± 3.39	2.21 ● 0.08	5.12 ± 1.64	4.99 ± 1.05
Base sat (%)	101.2 ± 2.60	69.08 ± 2.19	83.59 ± 14.89	90.46 ± 5.49	77.97 ± 6.97	90.27 ± 6.78
P (mmol/l)	0.01 ± 0.00	0.29 ± 0.05	0.18 ± 0.05	0.01 ± 0.00	0.15 ± 0.01	0.10 ± 0.02
	Old Tailings Dam			Natural Veldt		
	Old_O			Nat		
Total C (%)	0.08 ± 0.01	0.57 ± 0.26	6.28 ± 0.29	6.20 ± 0.38	6.88 ± 1.09	6.51 ± 1.38
pH	7.21 ± 0.19	7.16 ± 0.20	6.41 ± 0.21	6.37 ± 0.48	6.71 ± 0.22	5.82 ± 0.13
EC (mS/cm)	0.07 ± 0.01	0.21 ± 0.15	0.11 ± 0.02	0.38 ± 0.09	0.43 ± 0.10	0.31 ± 0.17
pH(H2O)	7.15 ± 0.05	7.33 ± 0.37	6.87 ± 0.17	5.93 ± 0.29	6.58 ± 0.20	6.27 ± 0.11
pH(KCl)	6.84 ± 0.08	6.97 ± 0.43	6.28 ± 0.29	5.05 ± 0.483	6.03 ± 0.21	5.21 ± 0.12
CEC (cmol(+)/kg)	1.98 ± 0.20	2.83 ± 0.68	5.42 ± 0.78	27.20 ± 1.02	26.64 ± 3.82	32.34 ± 5.11
Base sat (%)	104.3 ± 2.08	100.26 ± 2.50	91.06 ± 6.54	57.01 ± 8.89	83.22 ± 6.83	62.60 ± 6.19
P (mmol/l)	0.01 ± 0.00	0.00 ± 0.00	0.15 ± 0.01	0.12 ± 0.09	0.19 ± 0.03	0.15 ± 0.02

Value indicated as mean ± SEM

Table 3.8. Summary of potential enzymatic activity.

	New Tailing Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Dehydrogenase ($\mu g\ INFg^{-1}\ 2h^{-1}$)	122.29 ± 6.82^{a}	347.68 ± 119.12^{a}	191.77 ± 64.43^{a}	122.19 ± 14.87^{a}	79.87 ± 20.24^{a}	80.34 ± 17.86^{a}
β-Glucosidase ($mg\ PNPg^{-1}\ h^{-1}$)	126.75 ± 26.83^{a}	241.55 ± 58.20^{a}	182.93 ± 55.82^{a}	108.61 ± 15.22^{a}	35.24 ± 20.78^{a}	91.81 ± 55.62^{a}
Alkaline Phosphatase ($mg\ PNPg^{-1}\ h^{-1}$)	617.40 ± 49.33^{ab}	1220.53 ± 9.66^{c}	912.57 ± 178.62^{ab}	767.11 ± 86.87^{b}	15.68 ± 10.45^{d}	432.86 ± 126.26^{ab}
Acid Phosphatase ($mg\ PNPg^{-1}\ h^{-1}$)	1148.74 ± 105.36^{a}	837.19 ± 51.30^{ab}	724.86 ± 214.96^{a}	1402.66 ± 12.56^{a}	321.94 ± 163.83^{ab}	195.47 ± 20.44^{a}
Urease ($\mu gNH_4\text{-}Ng^{-1}\ 2h^{-1}$)	131.40 ± 12.72^{a}	1075.35 ± 262.56^{a}	83.20 ± 18.81^{a}	138.28 ± 11.22^{a}	174.47 ± 2.04^{a}	116.34 ± 27.03^{a}
	Old Tailings Dam					
	NR_1			NR_2		
Dehydrogenase ($\mu g\ INFg^{-1}\ 2h^{-1}$)	112.86 ± 7.03^{ab}	205.59 ± 59.45^{a}	122.33 ± 50.65^{a}	100.45 ± 5.07^{a}	78.01 ± 23.36^{a}	87.73 ± 11.11^{a}
β-Glucosidase ($mg\ PNPg^{-1}\ h^{-1}$)	82.34 ± 6.85^{a}	144.16 ± 13.95^{a}	84.79 ± 15.35^{a}	100.73 ± 10.84^{a}	63.07 ± 28.21^{a}	90.77 ± 11.31^{a}
Alkaline Phosphatase ($mg\ PNPg^{-1}\ h^{-1}$)	549.90 ± 66.29^{abc}	1238.39 ± 56.93^{c}	652.82 ± 215.26^{ab}	606.59 ± 82.57^{ab}	673.29 ± 161.75^{ab}	278.01 ± 33.69^{ab}
Acid Phosphatase ($mg\ PNPg^{-1}\ h^{-1}$)	1265.60 ± 52.65^{a}	852.10 ± 110.82^{a}	721.92 ± 230.35^{ab}	1347.44 ± 40.90^{a}	273.17 ± 190.04^{ab}	516.13 ± 19.24^{ab}
Urease ($\mu gNH_4\text{-}Ng^{-1}\ 2h^{-1}$)	141.40 ± 16.46^{a}	755.20 ± 79.17^{a}	66.17 ± 12.35^{a}	141.60 ± 16.83^{a}	361.06 ± 115.09^{a}	64.71 ± 0.72^{a}
	Old Tailings Dam			Natural veldt		
	Old_O			Nat		
Dehydrogenase ($\mu g\ INFg^{-1}\ 2h^{-1}$)	63.73 ± 21.80^{bc}	94.49 ± 53.35^{a}	153.52 ± 20.69^{a}	32.63 ± 5.93^{c}	1229.58 ± 54.46^{b}	526.82 ± 22.58^{b}
β-Glucosidase ($mg\ PNPg^{-1}\ h^{-1}$)	87.30 ± 19.42^{a}	86.03 ± 47.94^{a}	173.07 ± 48.35^{a}	73.08 ± 11.01^{a}	923.58 ± 117.44^{b}	1176.50 ± 92.82^{b}
Alkaline Phosphatase ($mg\ PNPg^{-1}\ h^{-1}$)	387.68 ± 123.18^{ac}	224.13 ± 97.03^{ad}	621.87 ± 5.33^{ab}	195.38 ± 35.19^{c}	572.32 ± 55.26^{ab}	1103.57 ± 223.79^{c}
Acid Phosphatase ($mg\ PNPg^{-1}\ h^{-1}$)	794.77 ± 312.11^{a}	438.45 ± 117.22^{ab}	975.22 ± 72.62^{b}	309.42 ± 57.24^{b}	235.31 ± 115.23^{b}	504.91 ± 145.74^{ab}
Urease ($\mu gNH_4\text{-}Ng^{-1}\ 2h^{-1}$)	87.27 ± 32.09^{a}	151.60 ± 18.45^{a}	127.20 ± 26.28^{a}	84.31 ± 4.72^{a}	2343.81 ± 243.29^{b}	83.97 ± 2.93^{a}

Value indicated as mean ± SEM, Sites per sample period with the same superscript indicate no significant difference between the sites.

PNP – *p*-nitrophenyl phosphate

INF – Iodonitrotetrazolium chloride-formazan

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CHAPTER 4

MICROBIAL COMMUNITY STRUCTURE AS INDICATOR OF ECOSYSTEM DEVELOPMENT DURING PLATINUM MINE TAILINGS REHABILITATION

ABSTRACT

Due to the increase in the demand for platinum, the production of mine wastes (tailings) has increased. Platinum mine tailings may contribute to environmental pollution if not properly rehabilitated. The establishment of vegetation on platinum mine tailings contribute to the reduction of possible environmental pollution. Microbial community structure plays an important role in an ecosystem due to its involvement in soil formation processes and biogeochemical cycling of essential nutrients. Phospholipid fatty acids provide a unique 'fingerprint' of the viable microbial community within an ecosystem and were use to determine microbial community structure. Multivariate statistical analyses were used to evaluate the effectiveness of the addition of woodchip-vermicompost and inorganic fertilisers during the rehabilitation of platinum mine tailings. The addition of woodchip-vermicompost contributed to the establishment of microbial communities within nearly biologically sterile platinum mine tailings. Both the addition of woodchip-vermicompost and inorganic fertilisers illustrated that bacteria may have contributed largely to the decomposition of organic material within these sites. The woodchip-vermicompost sites showed a higher estimated viable biomass than the sites treated with inorganic fertilisers.

Microbial community structure, Phospholipid fatty acids, Platinum mine tailings, Woodchip-vermicompost

4.1 Introduction

Platinum mines produce a large amount of waste material in the form of tailings, which are generated during the processing of excavated materials to obtain the desired product (Ledin & Pedersen, 1996). Tailings material consists of a small particle size, which is liable to wind and water erosion and could contribute to dust pollution and the siltation of rivers (Wong, 2003). The law requires developers to include the cost of ecological rehabilitation within their operation cost and compile an Environmental Management Plan (EMP) (Milton, 2001). The rehabilitation of mine tailings is made difficult by the physical and chemical properties of these tailings. Platinum mine tailings are an essentially biologically sterile medium, which contains a high concentration of non-essential elements that may be phytotoxic and which may leach in to surrounding water bodies (Van Rensburg & Morgenthal, 2004).

Vegetation cover contributes to the above-ground improvement of platinum mine tailings by reducing soil surface erosion as well as contributing to the accumulation of small particles (Tordoff et al., 2000). It facilitates below-ground improvement through root growth, which contributes to the accumulation of organic matter and microbial activity that prevent soil compaction by lowering the bulk density (Bradshaw, 1997). The establishment of vegetation may be hindered by a lack of organic matter, insufficient nutrients and virtually no microbial activity within platinum mine tailings. The success of establishing vegetation on platinum mine tailings, therefore, depends on the accumulation of organic matter and the regeneration of microbial diversity (DeGrood et al., 2005).

During underground blasting a large amount of woodchip debris, originating from wooden buttresses, are excavated with waste rock and separated from rock material during the screening process (Van Rensburg & Morgenthal, 2004). These woodchips can be mixed with other industrial wastes such as sewage sludge, whereafter it can be decomposed to form woodchip-vermicompost, which may serve as an organic amendment to the tailings. The addition of this woodchip-vermicompost as organic amendment may contribute to the stimulation of microbial activity (Zeller et al., 2001). Microbial community structure may serve as an indicator of the rehabilitation

process since it is responsive to changes in the soil ecosystem (Turco et al., 1994; Kandeler et al., 1999).

"The large variety of phospholipid fatty acids (PLFAs) present within organisms may provide a unique 'fingerprint' of the viable microbial community within a given soil, at a given time" (Lundquist et al., 1999). Therefore, soil microbial communities can be characterised by the variation in fatty acid components within the different microbial taxons (Ibekwe & Kennedy, 1998). For example, polyenoic fatty acids are characteristic of eukaryotes, while Gram-positive bacteria contain cyclopropane fatty acids and Gram-negative bacteria contains monoenic fatty acids (Ponder & Tadros, 2002). The composition of phospholipids, which are a major component in membranes of living cells (Böhme et al., 2005), is an important criteria to evaluate the physiological condition of microbial communities (Zelles, 1999). The total PLFAs may give an indication of the size of the viable biomass within the tailings (DeGrood et al., 2005), which is an important factor since it is the main soil component through which all organic matter must pass. Phospholipid fatty acid ratios may be used to evaluate stress conditions, which may occur within the microbial community (Van Bruggen & Semenov, 2000). Thus, by studying the PLFAs an indication of the recovery of microbial diversity, biomass and complete microbial community, which is essential for ecosystem health, within the platinum mine tailings can be monitored.

The aim of this study was to evaluate the effectiveness of woodchip-vermicompost as alternative amendments over the use of inorganic fertilisers, in order to establish a self-sustaining vegetation cover on platinum mine tailings dams in a semi-arid region of the North-West province of South Africa. Rehabilitation success was monitored as changes within microbial community structure using PLFAs.

4.2 Materials and Methods

4.2.1 Experimental design

Experimental sites were situated at Impala Platinum Mine, Rustenburg, South Africa and consisted of five sites divided between two tailings dams. Three sites measuring

30m x 10m in size were located on an old tailings dam, which has been dormant production since 1976. Two of the three sites were treated with woodchip-vermicompost and one site was treated with inorganic fertilisers. One woodchip-vermicompost treated site measuring 30m x 30m and one inorganic fertilised site of the same size was located on the new tailings dam, which was still in production. Fertilisation rate for optimum growth was previously determined in a study conducted by Van Rensburg & Morgenthal (2004). The aspect of all experimental sites was in a northerly direction. Woodchip-vermicompost of 50 tons per hectare was manually applied to the sites and worked in to a depth of 30 cm. Woodchips were obtained from mine blasting and separated from excavated rock material during the screening phase (Van Rensburg & Morgenthal, 2004), mixed with sewage and water and decomposed using *Eisenia fetida*. Sites were sown with a mixture of 12 different grass species. A natural veldt in the vicinity of the mine was selected as a reference area, since no suitable rehabilitated mine tailings dam, which could serve a as reference could be found. Sites were sampled during April 2004, November 2004 and April 2005, during which period the sites did not receive any irrigation. Average rainfall for the experimental site during the study period was

474. 4 mm.

4.2.2 Soil sampling and vegetation cover

A total of three samples per site (n = 18) were taken for the five sites on the two tailings dams and one reference site. The top organic matter layer was removed from the soil surface before soil samples were taken. Samples were obtained from the first 10 to 15 cm of the soil surface and placed in plastic bags whereafter they were frozen on site. Samples were homogenised and a sub-samples were taken from the original soil sample for PLFA analysis and stored at -80°C. Vegetation cover was determined as the percentage vegetation cover to bare ground. Twenty $1m^2$ quadrants were placed at random locations within the various sites. The mean representative value was obtained to serve as vegetation cover estimated for the specific site (Kent & Coker, 1997).

4.2.3 Analyses

4.2.3.1 Soil physical and chemical analyses

Physical and chemical analyses were performed on the soil samples of the various sites as described in Van Rensburg & Morgenthal, (2004).

4.2.3.2 Phospholipid fatty acid analyses

Phospholipid fatty acids were extracted according to the method of Bligh & Dyer (1959) as modified by White et al. (1979). Before PLFA extraction, soil samples were lyophilised and plant roots and stones removed (Peacock et al., 2001). Lipids were extracted from lyophilised soil using a single-phase chloroform-methanol-buffer system (White et al., 1979). Total lipid extract was fractionated by re-dissolving the dried extract in chloroform and loaded on silicic acid packed columns, which were pre-eluted with acetone and chloroform. The extract was fractionated into neutral lipids, glycolipids and polar lipids (Peacock et al., 2001). The polar lipid fractions were treated with methanolic KOH. The fatty acids methyl esters (FAMES) were obtained from the sample by adding hexane, glacial acetic acid (1N) and nano-pure water. Fatty acids methyl esters (FAMES) were analysed using capillary gas chromatography with flame ionization detection on a Hewlett-Packard 5890 series II chromatograph. Peaks were identified by comparing with acid methyl ester (C:19) internal standard (N-5377, Sigma Chemical Inc.). Fatty acid nomenclature used was as described in Bååth & Anderson (2003) and Ibekwe & Kennedy (1998).

4.2.4 Statistical analyses

PLFA data was analysed using STATISTICA 7 (StatSoft, Inc ©). A one-way ANOVA was preformed on all data sets. A three-tiered statistical approach was used where hypothesis testing was first made using analysis of variance (ANOVA) followed by the application of multivariate analyses such as Principal Components Analysis (PCA) using CANOCO (Canoco for Windows, Version 4.5, GLW-CPRO ©). A Redundancy Analysis (RDA) ordination diagram was constructed to illustrate the association between the most related independent environmental data and the phospholipid fatty acid group-data. Environmental data used were selected through

the forward selection procedure provided in CANOCO, to obtain the most significant soil chemical properties (Claassens et al., 2005).

Fungal-to-bacterial ratio was calculated using the following fatty acids: 18,2ω6,9c for fungi; and i15:0, a15:0, 15:0, i16:0, 16:1v5c, i17:0, a17:0, cy17:0, 17:0, 18:1v7c, cy19, characteristic of bacteria (Klamer & Bååth., 1998). Cyclopropyl fatty acid-to-*cis*-monoenoic fatty acid (cy19:18:1ω7c, cy17:16:1ω7c) ratio was used to determine physiological stress (Macdonald et al., 2005), Saturated-to-unsaturated ratio and *trans-to-cis* ratio were determined to assist with the interpretation of the occurrence of possible nutrient stress.

4.3 Results and Discussion

4.3.1 Experimental sites

4.3.1.1 Vegetation cover

Percentage vegetation cover to bare ground results obtained during the study are summarised in Table 4.1. The perennial species *Cenchrus ciliaris* (L.) var. *ciliaris*, *Chloris gayana* Kunth, *Melinis repens* (Wild) Zizka and *Heteropogon contortus* (L.) Beauvand the stoloniferous species *Cynodon dactylon* (L.) Pers., *Digitaria eriantha* Steud., which was all part of the sown grass seed mixture, was most likely to have contributed to the high percentage of vegetation on the woodchip-vermicompost sites. A vegetation cover to bare ground ratio of 64%:37% was observed on NR_N, while NR_1 and NR_2 showed a vegetation cover to bare ground ratio of 72%:28% and 66%:34%, respectively. The inorganic fertilised sites showed the lowest vegetation cover to bare ground ratio with Old_N showing a ratio of 34%:66% and Old_O showed a ratio 38%:62%. This is consistent with the findings of De Mora et al. (2005), which found an increase in vegetation cover in sites treated with organic amendments. The vegetation cover on NR_1 and NR_2 showed a decrease in vegetation cover of 17% and 13% respectively, at the end of the study period, while the vegetation cover on NR_N did not show any change in vegetation cover. A decrease in vegetation cover was also observed on Old_O and Old_N, which showed a decrease of 13% and 8% in vegetation cover. The decrease in vegetation cover on both the inorganic fertilised sites and woodchip-vermicompost sites may be attributed

to a decrease in rainfall. The rainfall decreased from 719.1 mm rain received during the first growth season from November 2003 to February 2004 to 413.5 mm rain received during the second growth season November 2004 to February 2005.

4.3.1.2 Soil physical and chemical properties

The physical properties of the tailings are presented in Table 4.2. Both the woodchip-vermicompost and inorganic fertilised sites showed a high sand percentage and lower clay, which contributes to the easy erosion of this material. The woodchip-vermicompost site NR_N showed higher clay percentage than the other sites indicating a higher retention of essential nutrients in this site.

The PCA ordination diagram in Figure 4.1 illustrates the chemical properties distribution in sites treated with woodchip-vermicompost. Eigenvalues for the first two canonical axes were 0.656 and 0.696 respectively. Sites treated with woodchip-vermicompost on the old tailings dam are indicated as NR_1 and NR_2, while NR_N represents the woodchip-vermicompost site located on the new tailings dam. A summary of the results obtained from the chemical analyses is presented in Tables 4.3 to 4.7.

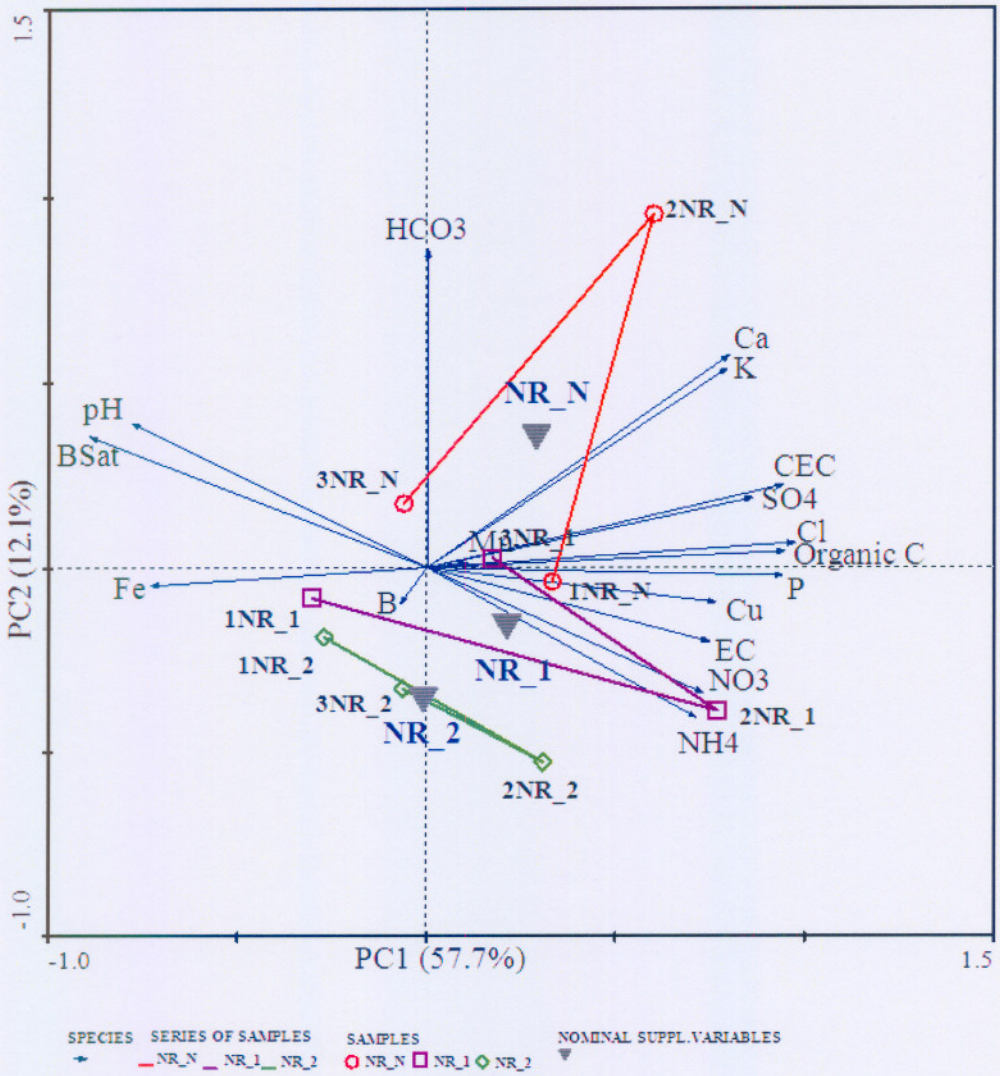


Figure 4.1 Principal Components Analysis ordination diagram of the chemical properties within the sites treated with woodchip-vermicompost. Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

The woodchip-vermicompost sites showed a higher CEC value than sites treated with inorganic fertiliser, with NR_N being characterised by a higher CEC value than NR_1 and NR_2. The higher CEC value of NR_N can be attributed to the higher clay content of this site. A decrease in CEC value was observed in NR_N, while an increase was observed in NR_1 from April 2004 to April 2005. The woodchip-vermicompost site NR_N was characterised by a higher calcium and potassium content, which may be attributed to the addition of the woodchip-vermicompost. Manganese and copper concentrations decreased, while iron increased in April 2005

in NR_N. An increase in manganese and copper was observed in NR_1, with a decrease in iron for the period April 2004 to November 2004. This was in contradiction what was observed by Van Rensburg & and Morgenthal (2004), which reported an increase in concentrations of manganese, copper and iron with the addition of woodchip-vermicompost. The fluctuation in the concentrations of these elements may be attributed to the fluctuation in pH within these sites (Table 4.4).

The PCA ordination diagram (Figure 4.2) illustrates the chemical property distribution in sites treated with inorganic fertilisers. Eigenvalues for the first two axes were 0.656 and 0.696, respectively. The site treated with inorganic fertilisers located on the new tailings dam are indicated as Old_N, while the site located on the old tailings dam are indicated as Old_O.

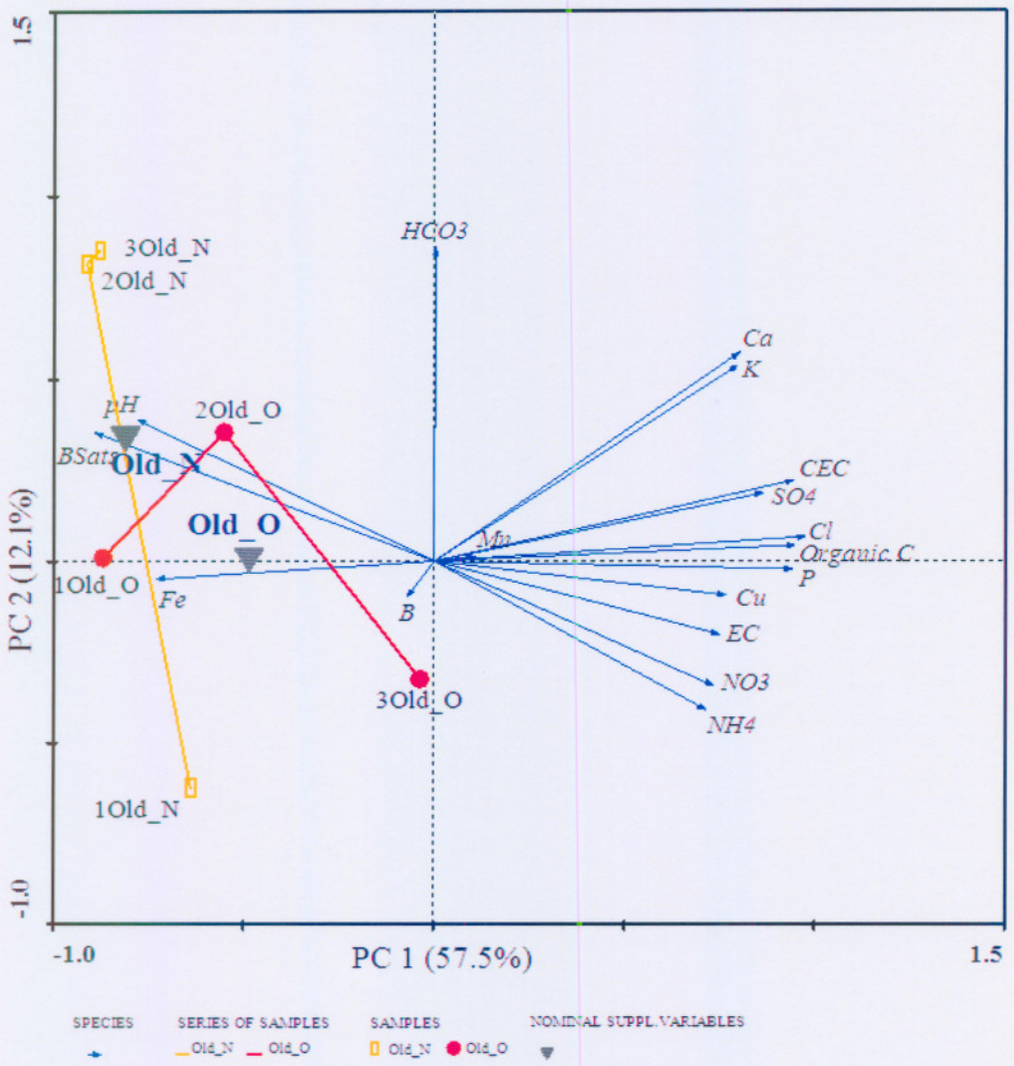


Figure 4.2 Principal Components Analysis ordination diagram of the chemical properties of the sites treated with inorganic fertilisers. Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

The inorganic fertilised sites were characterised by a higher pH and base saturation (BSats) and lower EC values. The lower EC values indicate that salinity will have no effect on the rehabilitation process, as well as confirms the low nutrient status of these sites (Van Rensburg & Morgenthal, 2004).

Figure 4.3 illustrates the PCA ordination diagram of the distribution of the chemical properties among the different treatments. Eigenvalues of the first two ordination axis were 0.656 and 0.696, respectively. The woodchip-vermicompost sites are indicated

as NR_N, NR_1 and NR_2, whereas Old_N and Old_O represent the inorganic fertilised sites.

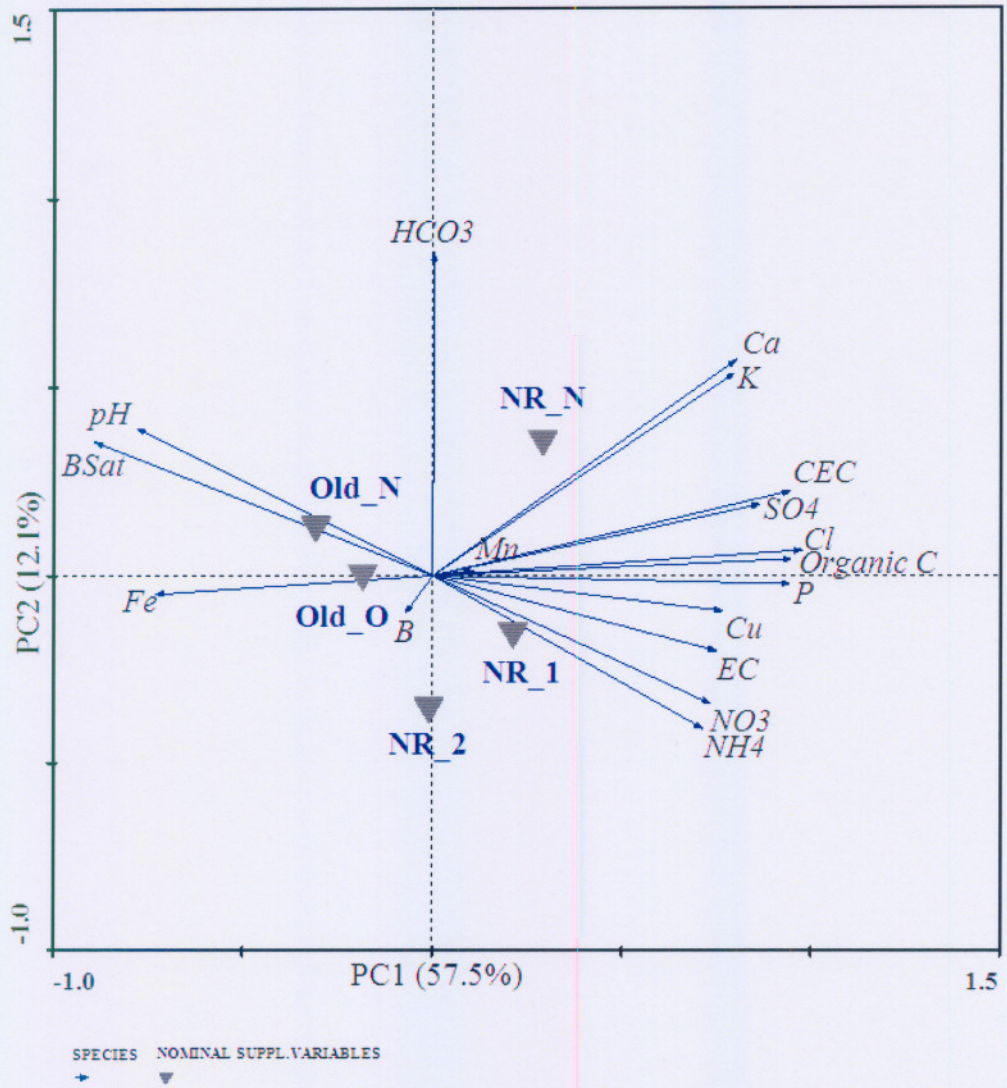


Figure 4.3. Principal Components ordination diagram indicating the chemical properties of the different treatments. NR_N represents the average of the three sampling periods in the woodchip-vermicompost site on the new tailings dam; NR_1 & NR_2 represent the average of the three sampling periods in the woodchip-vermicompost sites on the old tailings dam. Old_N and Old_O represent the average of the three sampling period in the inorganic fertilised sites on the new and old tailings dams, respectively.

The PCA ordination diagrams (Figure 4.3) indicated that sites treated with woodchip-vermicompost differ in chemical properties from sites treated with inorganic fertilisers. The variation in the chemical properties between the different treatments,

therefore, could have an effect on the microbial community structure in these sites as well as the self-sustainability of the vegetation cover.

4.3.1.3 Microbial community structure

Phospholipid fatty acids can be classified into two main groups namely, the first being the *saturated fatty acids* which includes, (1) normal saturated fatty acids (Nsats) with biomarkers: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; (2) mid-chain branched saturated fatty acids (MBSats) with biomarkers: 10Me16:0, 12Me16:0 10Me17:0, 10Me18:0, 12Me18:0; (3) terminal branched saturated fatty acids (TBSats) with biomarkers: i15:0, a15:0, i16:0, i17:0, a17:0. The second group, the *unsaturated fatty acids* include: (1) monounsaturated fatty acids (Monos) with biomarkers: 16:1ω7c, 16:1ω7t, 18:1ω9c, 18:1ω7c, 18:1ω7t, 18:1, 20:1ω9c and the (2) poly-unsaturated fatty acids (Polys) with biomarker: 18:2ω6,9c (Böhme et al., 2005). The average mole percentage (mol %) fraction of phospholipid fatty acids within each site is presented in Figures 4.3 to 4.5 and Tables 4.8.1 to 4.8.3.

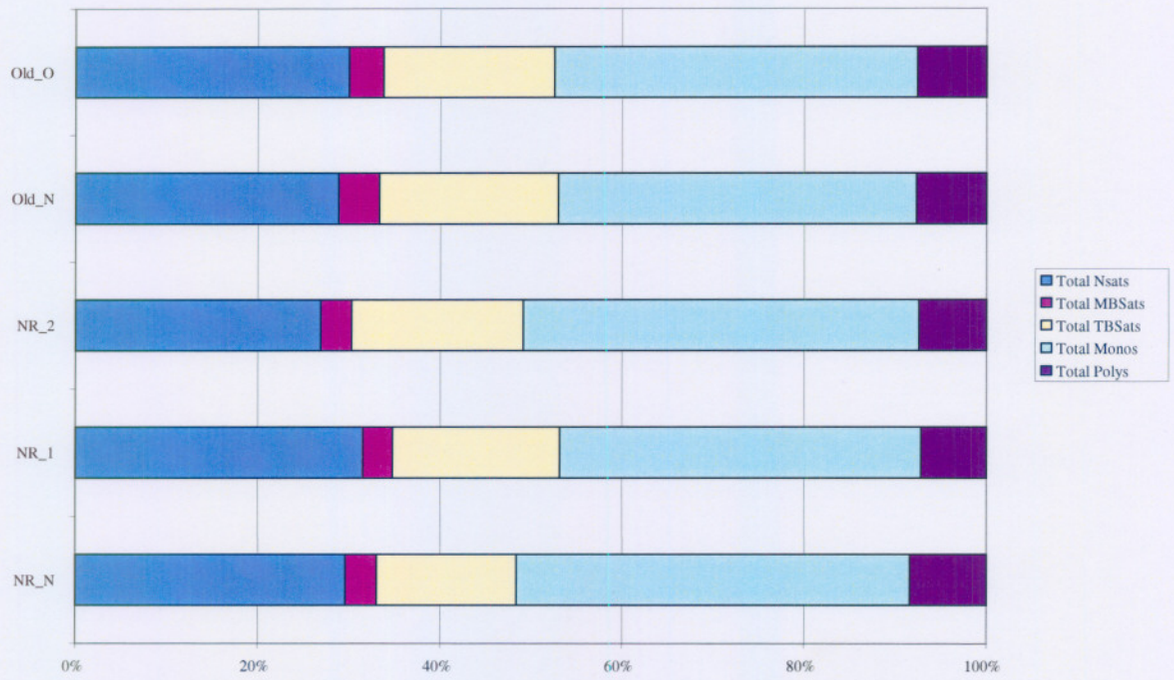


Figure 4.3 Microbial community structure based on the average mole percentage fraction of the major phospholipid fatty acid groups as found in the different treatments during April 2004.

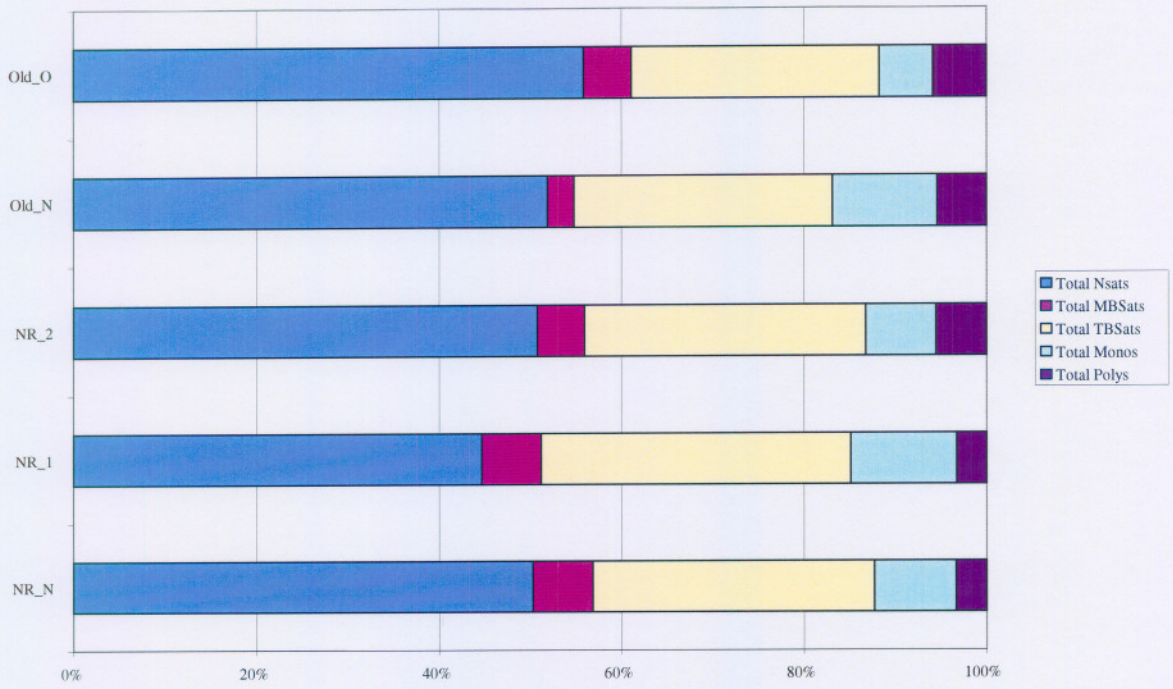


Figure 4.4 Microbial community structure based on the average mole percentage fraction of the major phospholipid fatty acid groups as found in the different treatments during November 2004.

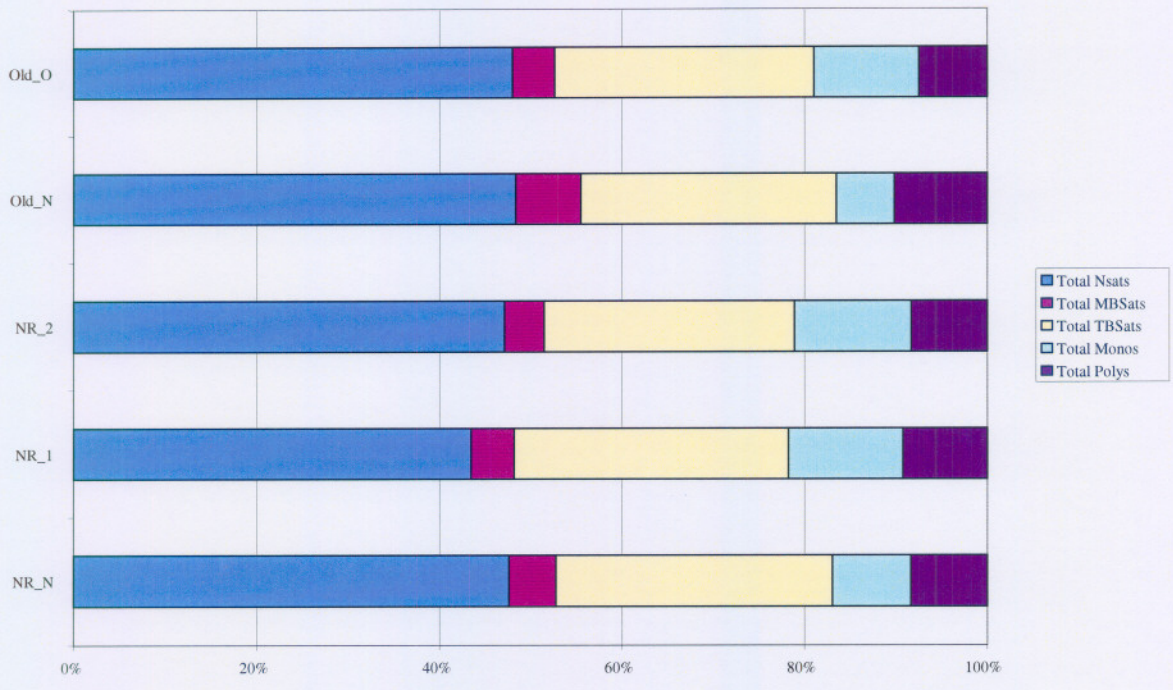


Figure 4.5 Microbial community structure based on the average mole percentage fraction of the major phospholipid fatty acid groups as found in the different treatments during April 2005.

An increase in mole % normal saturated fatty acids was observed from April 2004 to November 2004 in all sites, whereafter it decreased (Figures 4.3 to 4.5). Normal saturated fatty acids are a general biomarker, which occurs in both prokaryotic and eukaryotic organisms (Ponder & Tadros, 2002). Sites treated with inorganic fertilisers, Old_N and Old_O showed higher mole % normal saturated fatty acids during November 2004 than sites treated with woodchip-vermicompost. No statistically significant difference in normal saturated fatty acids was observed at the end of the study between the two treatments (Figures 4.5; Tables 4.8.1 to 4.8.3).

Mid-branched saturated fatty acids (MBSats) are indicative of Actinomycetes (Bassio et al., 1998). All sites except Old_O showed an increase in mole % mid-branched saturated fatty acids from April 2004 to November 2004 (Tables 4.8.1 to 4.8.3). The woodchip-vermicompost sites NR_N and NR_1 showed higher mole % mid-branched saturated fatty acids in November 2004. The inorganic fertilised site Old_N showed an increase in mole % mid-branched saturated fatty acids from November 2004 to April 2005. The increase in the occurrence of Actinomycetes has been found to relate to an increase in soil pH after the addition of lime (Frostegård et al., 1993; Hackl et al., 2005). An increase in pH was observed within the different sites, therefore, the increase in Actinomycetes might be attributed to the increase in pH. The increase in mole % mid-branched saturated fatty acids may further be attributed to the addition of woodchip-vermicompost, since Bassio et al. (1998) found that 10Me18:0 increased after manuring of soils. An increase in organic carbon was observed in all sites during April 2004 to November 2004. The increase in Actinomycetes, which occurred in Old_N during April 2005, may be attributed to the loss of vegetation cover (Table 4.1), which may have contributed to organic material in this site. Additionally the increase in pH (Table 4.7) might have further attributed to the increase in Actinomycetes from November 2004 to April 2005.

Higher mole % monounsaturated fatty acids (Monos), which is indicative of Gram-negative bacteria (Böhme et al., 2005) was present in all sites during April 2004 (Figure 4.3), whereafter mole % monounsaturated fatty acids decrease during April 2004 to November 2004. From November 2004 to April 2005, an increase in mole %

monounsaturated fatty acids was observed in NR_1, NR_2 and Old_O, whereas NR_N and Old_N showed a further decrease. Peacock et al. (2001) found that monounsaturated fatty acids increase with an increase in available carbon sources. He also suggested that monounsaturated fatty acids decrease in soils subjected to disturbance. The increase in mole % monounsaturated fatty acids in NR_1, NR_2 and Old_O may be attributed to burning of vegetation cover, which may have contributed to an increase in organic carbon (Table 4.7). Boerner et al. (2005) found an increase in organic carbon in sites after burning. A decrease in organic carbon was observed from November 2004 to April 2005 in NR_N and Old_N (Table 4.7), which may have contributed to the decrease in mole percentage monounsaturated fatty acids.

Terminal branched saturated fatty acids (TBSats) which are indicative of Gram-positive bacteria (Zelles, 1999), increased from April 2004 to November 2004 (Figures 4.3 & 4.4). During April 2005, a decrease in mole % terminal branched saturated fatty acids was observed in NR_1 and NR_2 (Figure 4.5). Marschner et al. (2003) and Bassio et al. (1998) found an increase in Gram-positive bacterial PLFAs in sites treated with sewage sludge amendment compared to sites treated with mineral fertilisers. Bardgett et al. (1999) and Fierer et al. (2003) however found that Gram-positive bacteria were more abundant in unfertilised grasslands and in sites with lower carbon contents and reduced carbon quality. The decrease in mole % terminal branched saturated fatty acids in NR_1 and NR_2 from November 2004 to April 2005 may be associated with the decrease in organic carbon observed during this period (Table 4.7). The decrease in mole % terminal branched saturated fatty acids may have been due to the decrease in organic carbon. The woodchip-vermicompost site NR_N and the inorganic fertilised site Old_N showed an increase in organic carbon but only showed a modest change in mole % terminal branched fatty acids, indicating that the availability of the organic carbon may have played a role.

Polyunsaturated fatty acids (Polys) indicative of fungi, decreased in all sites from April 2004 to November 2004 (Tables 4.3 & 4.4), whereafter mole % polyunsaturated fatty acids increased from November 2004 to April 2005 (Tables 4.4 & 4.5). The inorganic fertilised site Old_N and the woodchip-vermicompost site NR_1 showed

higher mole % polyunsaturated fatty acids during April 2005 (Figure 4.5). Fierer et al. (2003) found a higher occurrence of fungi at the soil surface with high carbon input. An increase in organic carbon was observed in all sites from April 2004 to November 2004, however, a decrease in mole % polyunsaturated fatty acids occurred. Fungi have been found to decrease with an increase in pH (Bååth & Anderson, 2003). The increase in pH could have contributed to the decrease in mole % polyunsaturated fatty acids.

The estimated viable biomass as determined for each site can be obtained from Table 4.8.1 to 4.8.3. Estimated viable biomass was expressed as pmol per gram dry weight (pmol/g dry weight) and presented in Figure 4.6. The woodchip-vermicompost sites NR_N, NR_1 and, NR_2 showed a higher initial estimated viable biomass than Old_N and Old_O during April 2004. Estimated viable biomass was the highest in NR_1 and NR_2 during this period. This would have been expected since the addition of organic material typically correlates with an increase in microbial biomass (Lundquist et al., 1999). The estimated viable biomass in NR_N, NR_1 and NR_2 decreased during November 2004, while the estimated viable biomass in Old_N and Old_O increased. The inorganic fertilised site Old_N showed a higher estimated viable biomass during November 2004. The estimated viable biomass decreased in Old_N and NR_1 from November 2004 to April 2005. The woodchip-vermicompost sites NR_N and NR_2 and the inorganic fertilised site Old_O showed an increase in estimated viable biomass from November 2004 to April 2005. Bardgett et al. (1999) suggested that the change in microbial biomass correlates with changes in microbial community structure, since the amount of phospholipids per unit biomass differ between the different organisms (Olsson, 1999). The change in estimated viable biomass observed may therefore be attributed to the change in microbial community structure. Monounsaturated fatty acids may have contributed to a large proportion of the estimated viable biomass, while terminal branched- and mid-branched saturated fatty acids only contributes to a small proportion, leading to the observed decrease in estimated viable biomass with a decrease in monounsaturated fatty acids.

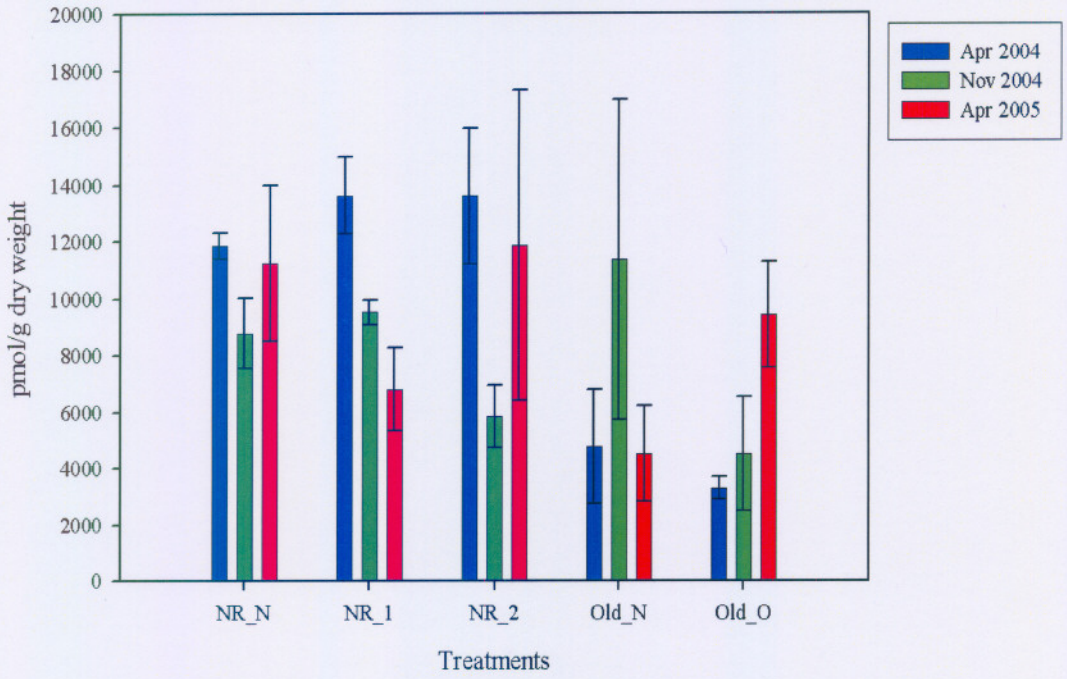


Figure 4.6 The estimated viable biomass expressed in pmol/g dry weight present in the different sites during the study period from April 2004 to April 2005.

Bailey et al. (2002) suggested that a fungal-to-bacterial ratio of 1 indicates that fungi and bacteria contribute equally to the microbial activity within soils. In a natural ecosystem the fungal community dominates and therefore the fungal-to-bacterial ratio illustrate values greater than 1. Values smaller than 1 indicate that bacterial activity dominates within the ecosystem and is indicative of an improved ecosystem (Bardgett & McAlister, 1999). The fungal-to-bacterial ratios observed during the study in the different sites are presented in Tables 4.8.1 to 4.8.3. All sites showed a fungal-to-bacterial ratio lower than 1, indicating that the bacteria contributed the most to the overall microbial activity within these sites. Results obtained were similar to those of Bardgett et al. (1999) who found the fungal-to-bacterial ratio to be lowest in managed systems. Leckie et al. (2004) suggested that forests soils containing higher fungal PLFAs than bacterial PLFAs was indicative that the soils have lower fertility, nitrogen mineralisation rates and nitrogen availability. The addition of the both the inorganic fertilisers and woodchip-vermicompost may therefore be said to have contributed to soil fertility, nitrogen mineralisation and validity of nitrogen. Frostegård & Bååth (1996) found that the contribution of fungi and bacteria activity to

soil processes differ amongst different soil types with ratios of 0.03 to 0.13 found in grasslands and garden soils. All sites showed a fungal-to-bacterial ratio within these observed ratios and might therefore indicate that fungi and bacteria contribute to the soil processes in the same ratios observed within other grasslands. Fluctuations observed between the different sampling periods vary within this range. Bååth & Anderson (2003) also found that pH might have an effect on the fungal-to-bacterial ratio, indicating that the ratio decreased with an increase in pH. All sites showed an increase in pH, it could therefore be said that pH may be one of the factors contributing to the lower fungal-to-bacterial ratio observed. No statistically significant difference in fungal-to-bacterial ratio was found between the woodchip-vermicompost and the inorganic fertilised sites.

The ratio of cyclopropyl fatty acids to *cis*-monoenoic fatty acids (cy19:0/,18:1ω7c, cy17:0/16:1ω7c) precursors can be used to determine whether the microbial community within an ecosystem experiences physiological stress (Leckie, 2005). A high cyclopropyl fatty acids (cy19:0, cy17:0) to *cis*-monoenoic fatty acids precursors ratio (>0.1) may be indicative of a decrease in microbial growth rates of some bacteria as well as insufficient carbon resources (Fierer et al., 2003). All treatments indicated a cyclopropyl fatty acids to *cis*-monoenoic fatty acids ratio greater than 0.1 during the first sampling period in April 2004 (Table 4.8.1 to 4.8.3), indicating that the microbial community have already entered a period of stationary growth (Jackson et al., 2003). The cy19:0/18:1ω7c ratios decreased in all treatments from April 2004 to November 2004 and April 2005 illustrating values lower than 0.1. Smith et al. (2000) found that cyclopropyl fatty acid to monoenoic fatty acid ratios less than 0.05 indicates exponential growth. The decrease in cy19:0/18:1ω7c ratio may indicate that the proportion of the microbial community containing the cy19:0 fatty acids were undergoing exponential growth due to the increase in organic carbon availability during this period (Table 4.7). Bååth & Anderson (2003) found that cy19:0 decreased with an increase in pH. As mentioned an increase in pH was observed in all sites during November 2004 except in Old_O and NR_2 and may therefore have contributed to the decrease in cy19:0/18:1ω7c ratios. Bassio et al. (1998) also found that cy19:0 and its precursor decreased in organic systems, which further decreased or

showed an increase over time. Organic material in the inorganic fertilised sites may be attributed to the loss of vegetation cover, whereas the addition of the woodchip-vermicompost in the woodchip-vermicompost sites, might have contributed to the decrease in cy19:0.

An increase in cy17:0/16:1ω7c was observed from April 2004 to November 2004 (Tables 4.8.1 to 4.8.3) in all sites indicating that the proportion of the microbial community containing cy17:0 fatty acids once again enters stationary growth phase. A study conducted by Frostegård et al. (1993) showed that the fatty acid cy17:0 increased in mole percentage with an increase in pH as found in forest soils treated with lime. The increase in cy17:0 might therefore further be attributed to the increase in pH that occurred within the sites (Table 4.7), causing the occurrence of higher cy17:0/16:1ω7c ratio.

Another indicator of stress is the *trans/cis* ratio of 16:1ω7 and 18:1ω7 (Heipieper et al., 1996) and may be used to indicate nutrient status of a microbial community (Frostergård et al., 1993). The woodchip-vermicompost sites NR_N, NR_1 and NR_2 showed an increase in the *trans/cis* (18:1ω7t/18:1ω7c) ratio, the increase was observed from April 2004 to November 2004 and April 2005. Both the inorganic fertilised sites illustrated a *trans/cis* ratio of lower than 0.1. White et al. (1996) reported that a *trans/cis* ratio of less than 0.5 might be indicative of microbial community under stress. The decrease in estimated viable biomass within the woodchip-vermicompost sites during November 2004 to April 2005 may be attributed the increase in stress within these sites due to management practices. The inorganic fertilised sites showed relatively lower levels of stress than that of the woodchip-vermicompost and might therefore explain the increase in estimated viable biomass during November 2004 and April 2005. It may therefore indicate that the conditions in inorganic fertilised sites may have been more favourable for the growth microorganisms, during this period. The saturated-to-unsaturated fatty acid ratio showed an increase during November 2004 to April 2005 (Tables 4.8.1 to 4.8.3), indicating an increase in terminal branched fatty acids and a decrease in monounsaturated fatty acids within both the inorganic fertilised sites and woodchip-

vermicompost sites. The increase of saturated fatty acids over monounsaturated fatty acids is subjected to starvation conditions (Bassio et al., 1998), which might be due to a decrease in availability of nutrients form the woodchip-vermicompost for microbial use. Lundquist et al. (1999) found that soil moisture plays an important role in the availability of nutrients for microbial use. In the study conducted by him a decrease in soil moisture was observed to contribute to a decrease in dissolved organic carbons as well as a decrease in diffusion of other nutrients to microorganisms, contributing to starvation and an increase in nutrient stress. November being mid-summer and platinum mine tailings containing a high percentage of sand and silt which contribute to less retention of soil moisture, may therefore have contributed to the reduced availability of nutrients to microorganisms.

The RDA ordination diagram (Figure 4.7) illustrates the distribution of PLFA biomarkers in the woodchip-vermicompost sites and their relation with the dominant independent environmental factors organic carbon (C), nitrate (NO_3), ammonium (NH_4), phosphorous (P) and pH (Figure 4.7). The eigenvalues of the first and second ordination axes were 0.487 and 0.068, respectively. The first two canonical axes contributed 55.5% to the total observed variance. Phosphorous (P) contributed (r^2 = -0.578) to the variance indicated by the first canonical axis while pH contributed (r^2 = -0.570) to the variance indicated by the second canonical axis. According to Monte Carlo Permutations test conducted with 499 permutations the first canonical axis was statistically significant (p = 0.002). The overall effect of the chosen environmental variables on the microbial community structure was statistically significant (p = 0.004). Woodchip-vermicompost sites located on the old tailings dam are indicated as NR_1 and NR_2, while NR_N indicate the woodchip-vermicompost site located on the new tailings dam.

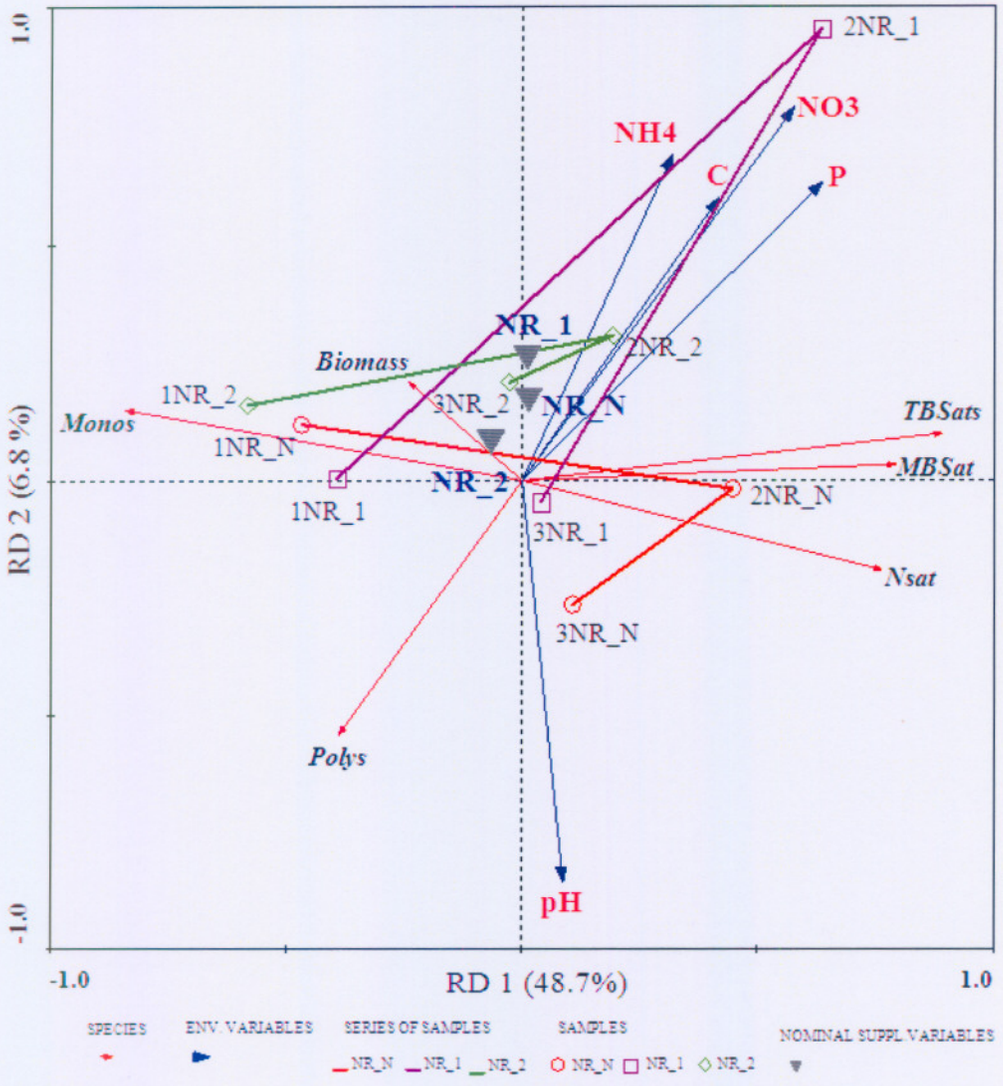


Figure 4.7. Redundancy Analysis ordination diagram illustrating the environmental variables in relationship with the PLFAs and their distribution among the woodchip-vermicompost sites. Phospholipid fatty acid biomarkers are indicated as Monounsaturated (Monos), Polyunsaturated (Polys), Normal saturated (Nsat), Terminal branched Saturated (TBSats) and Mid-branched Saturated (MBSats). Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

During April 2004, the woodchip-vermicompost sites NR_N, NR_1 and, NR_2 were characterised by higher estimated viable biomass and monounsaturated fatty acids (Monos) fatty acids. Estimated viable biomass showed a positive correlation with organic carbon (C) ($r = 0.66$, $p < 0.05$). The higher estimated viable biomass in the woodchip-vermicompost sites may be attributed to the recent addition of woodchip-vermicompost. The addition of a suitable nutrient supply, like available carbon, leads to the increase in r-strategists, typically, Gram-negative bacteria indicated by

monounsaturated fatty acids (Peacock et al., 2001). From April 2004 to November 2004, a decrease in estimated viable biomass and mole % monounsaturated fatty acids (Tables 4.8.1 & 4.8.2) was observed in NR_N and NR_1, which may indicate a decrease in organic carbon. The woodchip-vermicompost site (NR_2) was characterised by a higher estimated viable biomass and mole % monounsaturated fatty acids during April 2005 (Table 4.8.2).

Terminal branched saturated fatty acids (TBsats) increase in soils with mature vegetation and decrease in soils with low vegetation (Waldrop et al., 2000). A positive correlation existed between terminal branched saturated fatty acids and nitrate ($r = 0.49$, $p < 0.05$) and phosphorous ($r = 0.66$, $p < 0.05$) (Figure 4.7). The woodchip-vermicompost site NR_N were characterised by higher mole % terminal branched fatty acids and mid-branched saturated fatty acids in November 2004 and April 2005 (Table 4.8.1). The higher mole % terminally branched saturated fatty acids in this site may be associated with the high vegetation cover (Table 4.1). Polyunsaturated fatty acids showed a positive correlation with pH ($r = 0.64$, $p < 0.05$). The woodchip-vermicompost sites NR_N and NR_1 were characterised by higher mole % polyunsaturated fatty acids during April 2005 than NR_2 (Tables 4.8.1 & 4.8.2).

As mentioned earlier, terminal branched saturated fatty acids and mid-branched saturated fatty acids may serve as indicators of environments with reduced quality and availability of carbon. An increase in organic carbon was observed from April 2004 to November 2004. The change in community structure that occurs during November 2004 may indicate that the availability of organic carbon may have decreased.

The RDA ordination diagram presented in Figure 4.8 illustrates the PLFAs distribution in sites treated with inorganic fertilisers and their relation with the dominant independent environmental factors, carbon (C), nitrate (NO_3), ammonium (NH_4) and pH. The eigenvalues of the first and second ordination axes were 0.487 and 0.068, respectively. The first two canonical axes contributed 55.5% to the total observed variance. Phosphorous (P) contributed ($r^2 = -0.578$) to the variance indicated by the first canonical axis while pH contributed ($r^2 = -0.570$) to the variance indicated

by the second canonical axis. According to Monte Carlo Permutations test conducted with 499 permutations the first canonical axis was statistically significant (p = 0.002). The overall effect of the chosen environmental variables on the microbial community structure was statistically significant (p = 0.004). The inorganic fertilised site on the old tailings dam is indicated by Old_O, while Old_N indicates the inorganic fertilised site on the new tailings dam.

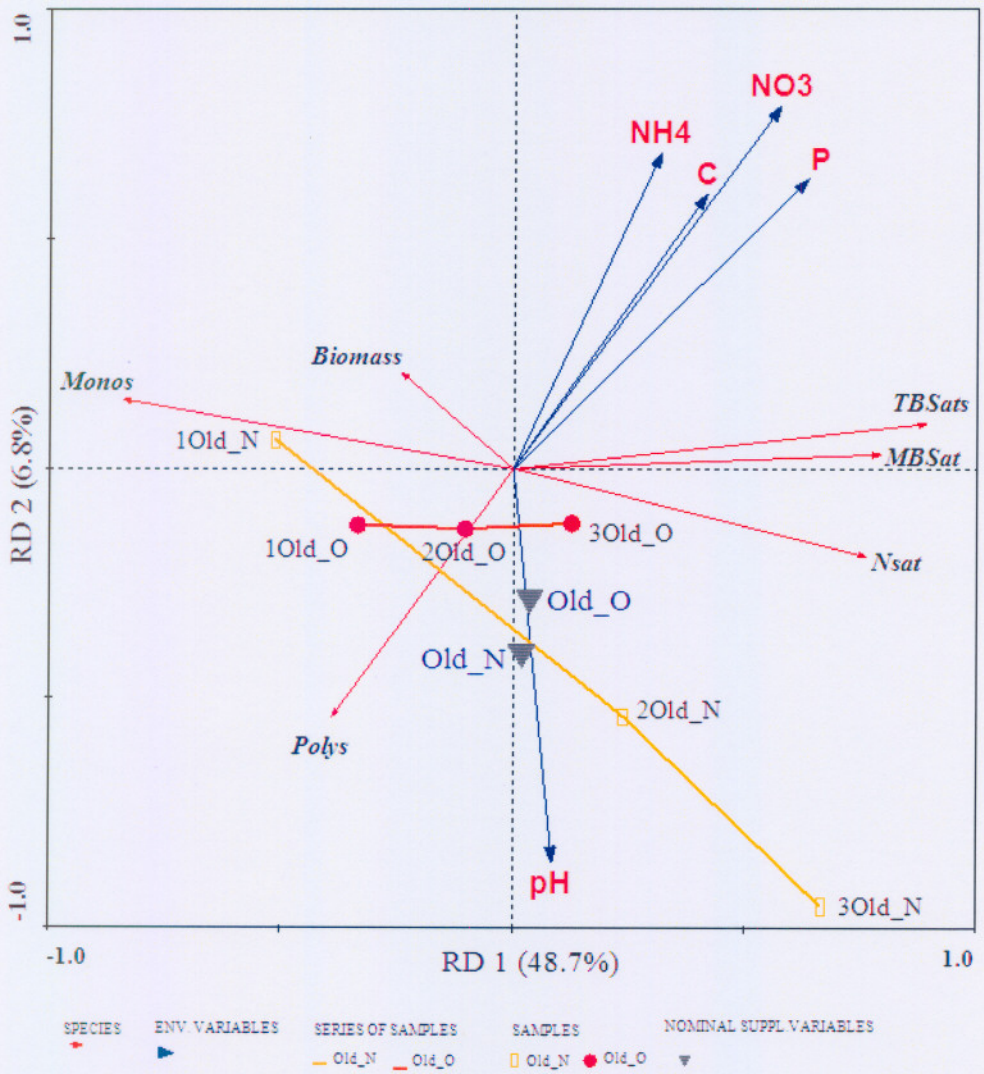


Figure 4.8 Redundancy Analysis ordination diagram illustrates the environmental variables in relationship with the PLFAs and their distribution among the inorganic fertilised sites. Phospholipid fatty acid biomarkers are indicated as Monounsaturated (Monos), Polyunsaturated (Polys), Normal saturated (Nsat), Terminal branched Saturated (TBSats) and Mid-branched Saturated (MBSats). Sampling periods at the different sites are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

The inorganic fertilised site Old_N was characterised by a higher mole % monounsaturated fatty acids during April 2004 (Table 4.8.1). During November 2004, the Old_O was characterised by higher mole % polyunsaturated fatty acids, which showed an association with pH. The inorganic fertilised site Old_O showed to be characterised by higher mole % normal saturated fatty acids during April 2005 (Table 4.8.3).

The RDA ordination diagram presented in (Figure 4.9) illustrates the comparison between the woodchip-vermicompost sites and inorganic fertilised sites in relation to the distribution of phospholipid fatty acids. The eigenvalues of the first and second ordination axes were 0.487 and 0.068, respectively. The first two canonical axes contributed 55.5% to the total observed variance. Phosphorous (P) contributed (r^2 = -0.578) to the variance indicated by the first canonical axis while pH contributed (r^2 = -0.570) to the variance indicated by the second canonical axis. According to Monte Carlo Permutations test conducted with 499 permutations the first canonical axis was statistically significant (p = 0.002). The overall effect of the chosen environmental variables on the microbial community structure was statistically significant (p = 0.004). Sites treated with the woodchip-vermicompost are indicated as NR_1, NR_2, sites on the old tailings dam and NR_N site on the new tailings dam. Old_N represents the inorganic treated site on the new tailings dam. Old_O represents the inorganic fertilised site on the old tailings dam.

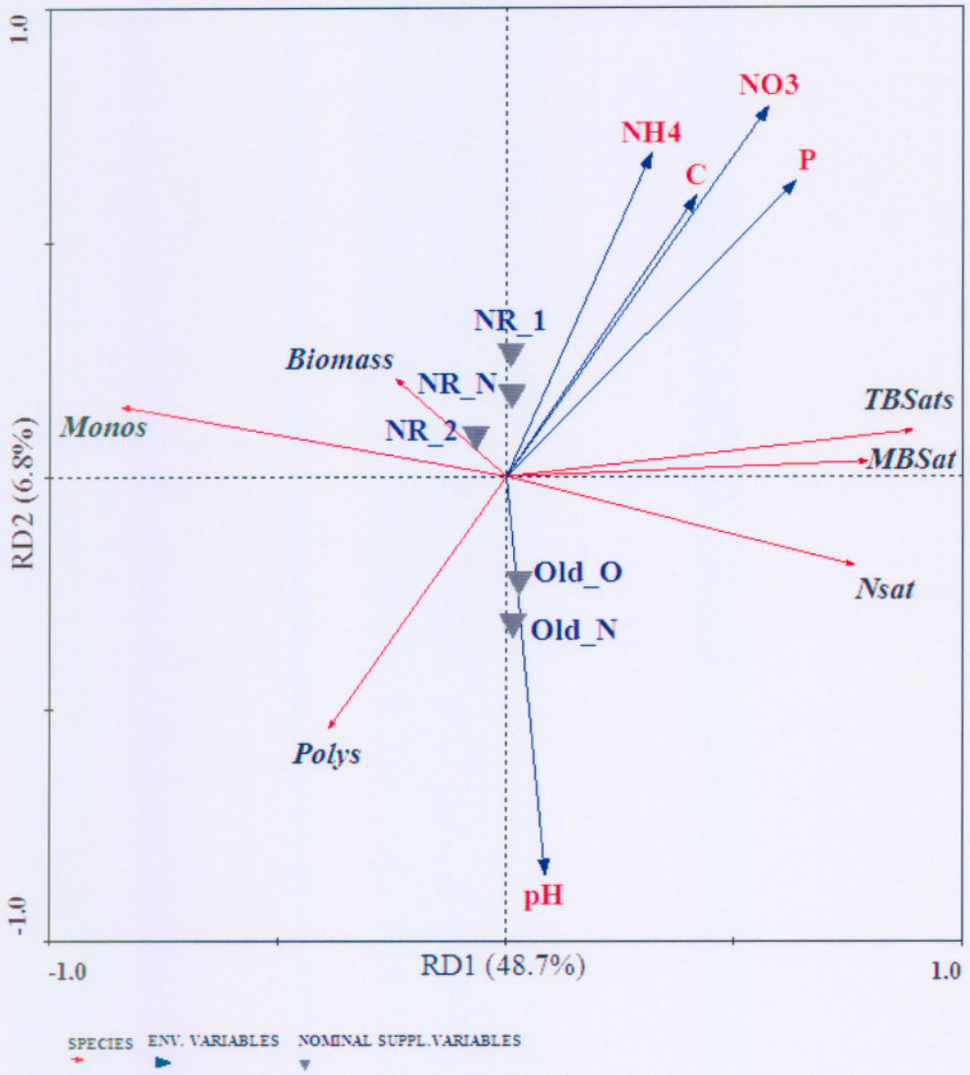


Figure 4.9. Redundancy Analysis ordination diagram illustrates the comparison in phospholipid fatty acids distribution among the woodchip-vermicompost sites and inorganic fertilised sites and their correlation with the dominant independent environmental variables. Phospholipid fatty acid biomarkers are indicated as Monounsaturated (Monos), Polyunsaturated (Polys), Normal saturated (Nsat), Terminal branched Saturated (TBSats) and Mid-branched Saturated (MBSats).

From the RDA ordination diagram (Figure 4.9) a difference in the microbial community structure can be observed between the woodchip-vermicompost and inorganic fertilised sites. The woodchip-vermicompost sites were characterised by a higher occurrence of the essential nutrients. However, no statistically significant ($p < 0.05$) difference in microbial community was observed between the two treatments.

4.3.2. Reference area

4.3.2.1 Soil physical and chemical properties

A summary of the physical properties of the natural veldt can be obtained from Table 4.2. As expected, the natural veldt differ in physical properties from that of the platinum mine tailings.

A summary of the chemical properties of the natural veldt are presented in Table 4.3 to 4.7. A PCA ordination diagram presented by Figure 4.10 illustrates the chemical properties obtained from the soil chemical analyses of the natural veldt. Eigenvalues for the first two canonical axes are 0.569 and 0.431 respectively. The chemical properties of the natural veldt, as expected, showed a statistically significant ($p < 0.05$) difference from the chemical properties of the platinum mine tailings.

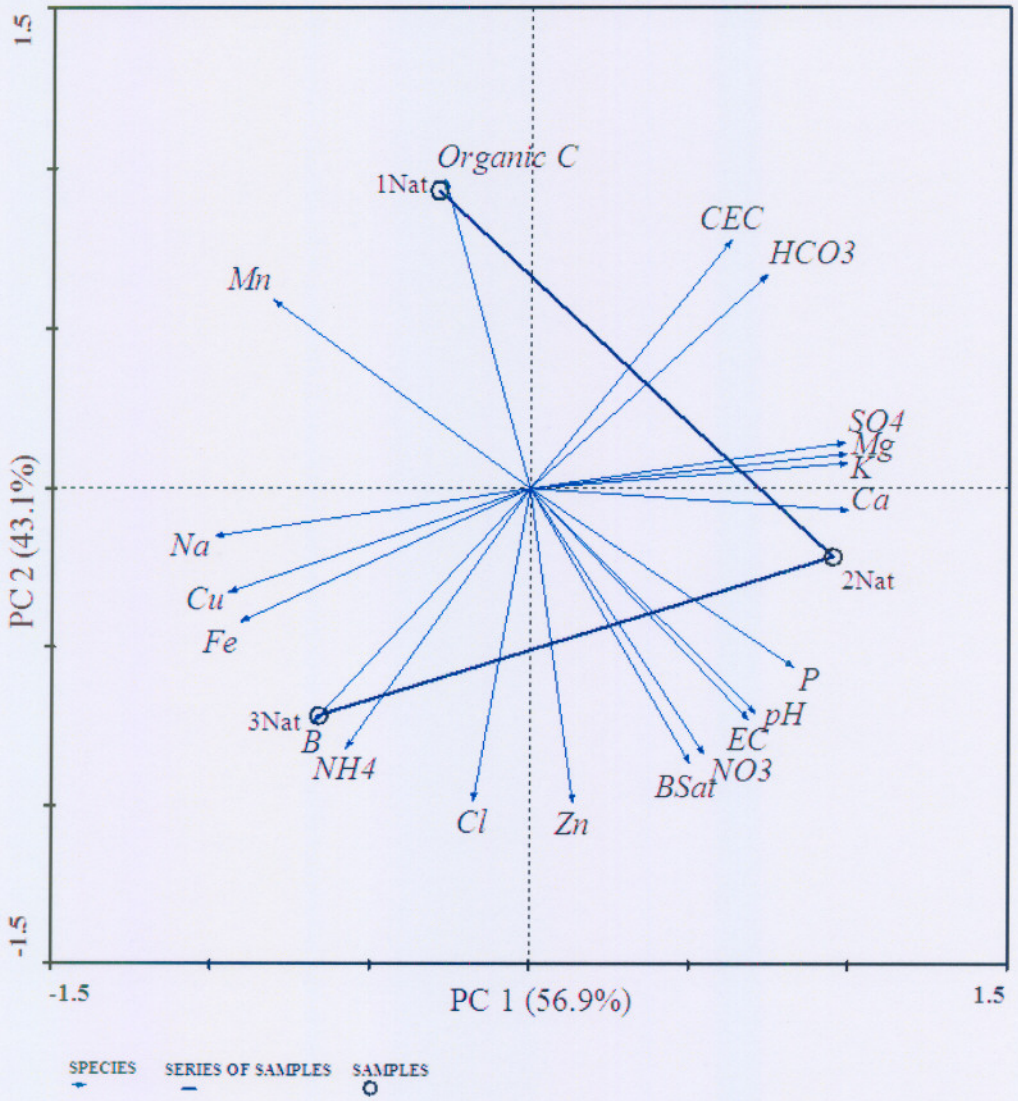


Figure 4.10. Principal Components Analysis ordination diagram of the chemical properties of the natural veldt used as reference site. Sampling periods at the site are indicated as follows: (1) April 2004; (2) November 2004 and (3) April 2005.

4.3.2.2 Microbial community structure

Figure 4.11 illustrates the average mole percentage fraction of PLFAs in the natural veldt as obtained during the study. Mole percentage fractions are presented in Table 4.8.3.

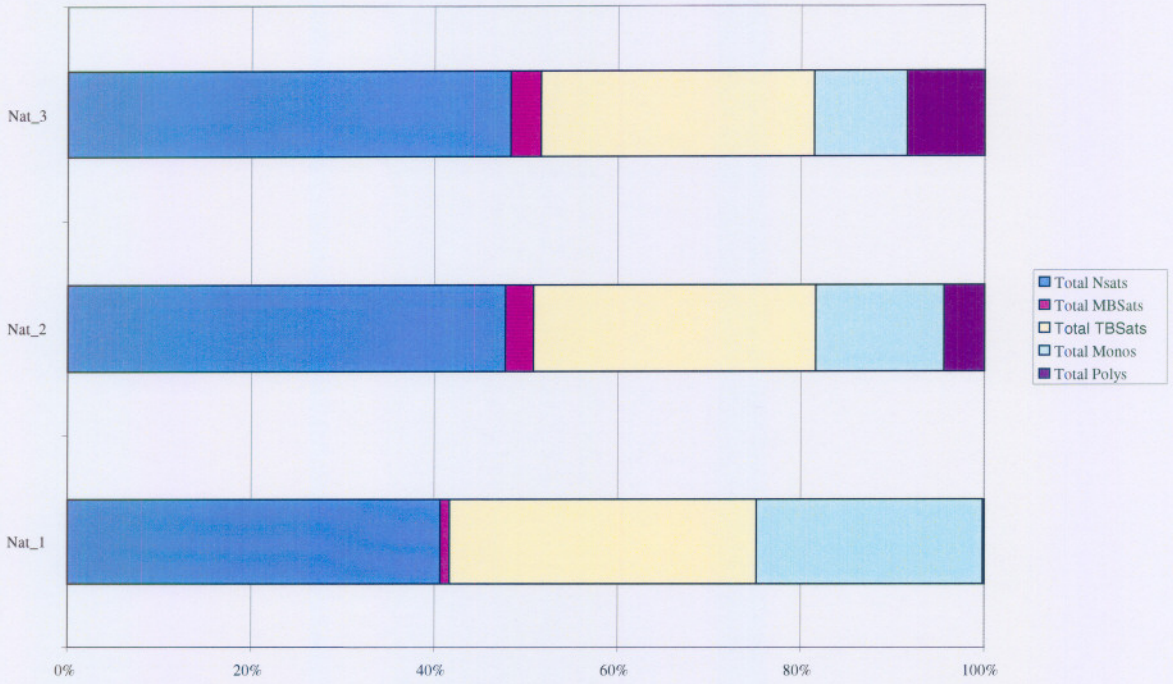


Figure 4.11. Microbial community structure based on the average mole percentage fraction of the major phospholipid fatty acid groups as found in the natural veldt. Nat_1, represents the microbial community structure during April 2004, Nat_2, represents microbial community structure during November 2004 and Nat_3 represents microbial community structure during April 2005.

No statistically significant ($p < 0.05$) difference was observed between the microbial community structure of the natural veldt and that of either the inorganic or woodchip-vermicompost treated sites. The natural veldt showed a decrease in mole % monounsaturated fatty acids and terminal branched saturated fatty acids from April 2004 to April 2005 (Table 4.8.3). The mid-branched fatty acids and poly-unsaturated fatty acids increased from November 2004 to April 2005.

Figure 4.12 illustrates the estimated viable biomass as obtained within the natural veldt during the study.

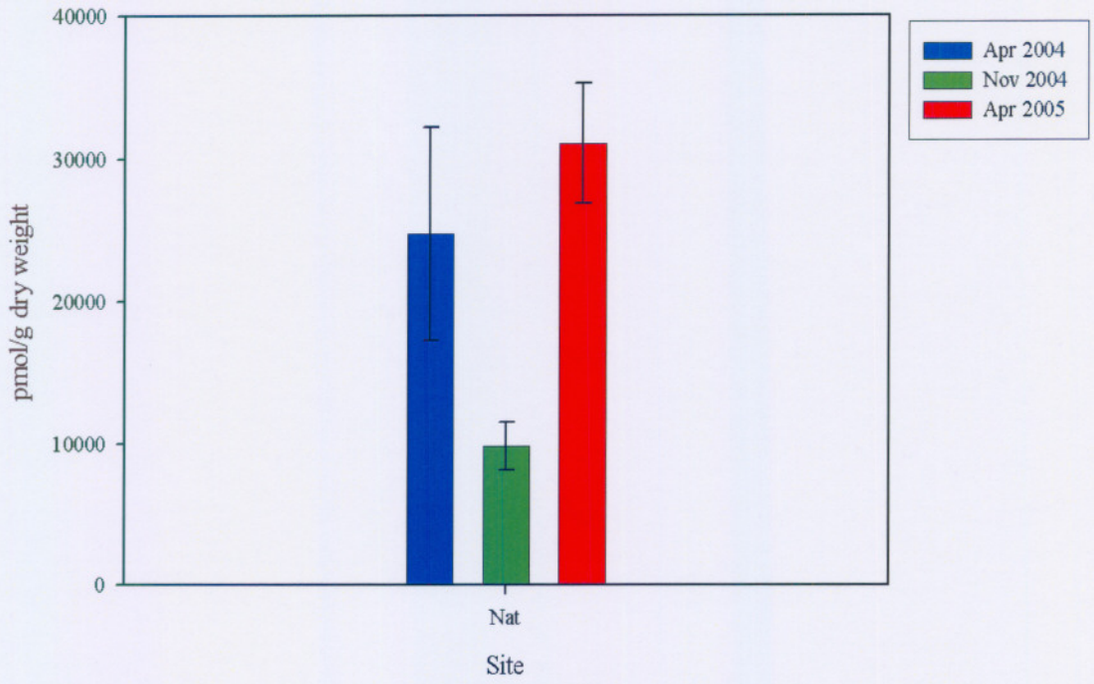


Figure 4.12. The estimated viable biomass expressed in pmol/g dry weight as occurring in the natural veldt during the present study.

The estimated viable biomass within the natural veldt (Figure 4.12) was higher than that observed in both the woodchip-vermicompost and inorganic fertilised sites during April 2004 (Figure 4.6). The natural veldt showed a decrease in estimated viable biomass during November 2004, whereafter the estimated viable biomass increased in April 2005. A statistically significant ($p < 0.05$) difference was observed between the estimated viable biomass within the natural veldt and both the inorganic fertilised and woodchip-vermicompost sites on the platinum tailings dams.

The RDA ordination diagram presented in Figure 4.13 illustrates the relation between the different phospholipid fatty acid biomarker groups and the dominant independent environmental factors organic carbon (C), nitrate (NO_3), ammonium (NH_4), phosphorous (P) and pH (Figure 4.13). The eigenvalues of the first two ordination axes was 0.810 and 0.190, respectively. The first two canonical axes contributed 100% to the total observed variance. Carbon (C) contributed to ($r^2 = 0.975$) the variance indicated by the first canonical axis while phosphorous contributed to ($r^2 = 0.795$) the variance indicated by the second canonical axis. According to Monte Carlo

Permutations test conducted with 499 permutations the first canonical axis was not statistically significant (p = 1.000). The overall effect of the chosen environmental variables on the microbial community structure was not statistically significant (p = 1.000).

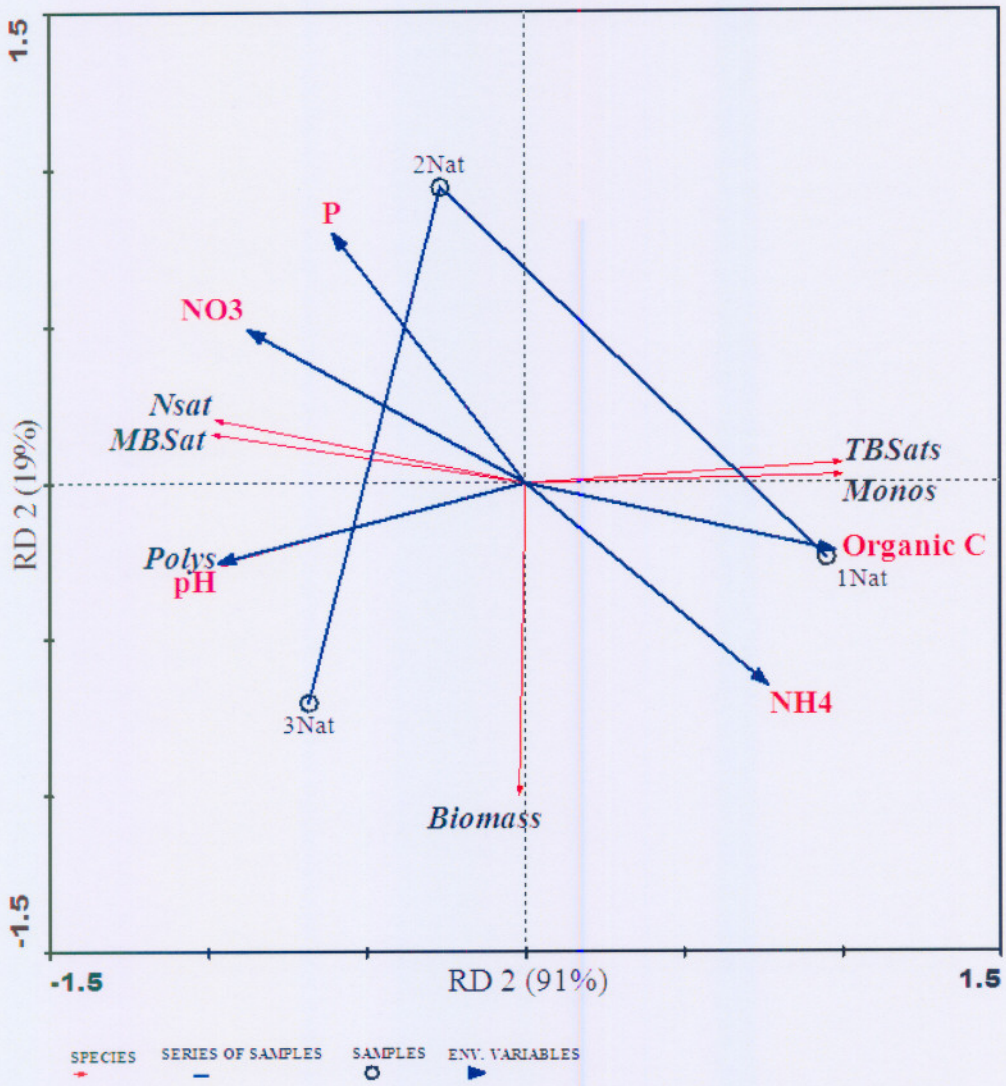


Figure 4.13. Redundancy Analysis ordination diagram illustrates the environmental variables in relationship to PLFA and their distribution in the natural veldt. Phospholipid fatty acid biomarkers are indicated as Monounsaturated (Monos), Polyunsaturated (Polys), Normal saturated (Nsat), Terminal branched Saturated (TBSats) and Mid-branched Saturated (MBSats). Sampling periods at the different sites are indicated as follows, (1) April 2004; (2) November 2004 and (3) April 2005.

The natural veldt showed a change in microbial community structure from a higher mole % monounsaturated- and terminally branched saturated fatty acid in April 2004, to a community containing higher mole % mid-branched saturated fatty acids in November 2004. The terminally branched saturated fatty acids and monounsaturated fatty acids showed an association with soil organic carbon (C). A negative association existed between organic carbon and mid-branched fatty acid. Polyunsaturated fatty acids showed an association with pH. An increase in mole % polyunsaturated fatty acids was observed in during April 2005.

4.4 Conclusion

No statistically significant ($p < 0.05$) difference was observed between the inorganic fertilised sites and the woodchip-vermicompost sites in terms of microbial community structure. The increase in mole % terminal branched saturated fatty acids and decrease in mole % monounsaturated fatty acids in both the woodchip-vermicompost and inorganic fertilised sites indicated that although an increase in organic carbon took place, the availability of the carbon to the microorganisms might have decreased. The decrease in availability of organic carbon, as well as other nutrients may have been due to a decrease in soil moisture. It can therefore be concluded that although the addition of the woodchip-vermicompost may have contributed to the enrichment of microorganisms in a previously biological sterile medium, similar to that found in the inorganic sites. Many factors, other than the addition of the woodchip-vermicompost may, have played a role in the composition of the microbial community structure, such as soil moisture and soil pH and vegetation cover. Further studies need to be conducted in order to determine the effect of soil moisture within the platinum mine tailings on the microbial community structure. Studies on the long-term stability to the woodchip-vermicompost in providing organic material should also be conducted.

Table 4.1. Summary of vegetation crown cover of the different treatments.

	New Tailings Dam				Old Tailings Dam					
	NR_N		Old_N		NR_1		NR_2		Old_O	
	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05
Vegetation Cover (%)	64	64	34	26	72	55	66	53	38	23
Bare ground (%)	37	37	66	74	28	45	34	47	62	78

Table 4.2. Summary of the particle size distribution of platinum mine tailings.

Particle size distribution	> 2mm	Clay (%)	Silt (%)	Sand (%)
NR_N	3.5	5.2	14.1	80.7
NR_1	2.5	2.4	13.5	84.1
NR_2	0.7	2.3	4.8	92.9
Old_N	0.6	2.3	13.4	84.3
Old_O	0.5	2.2	12.4	85.4
Natural Veldt	12.4	6.6	23.4	70.0

Table 4.3. Summary of the chemical composition of platinum tailings: 1:2 (v/v) water extraction.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_05	Nov_05	Apr_05	Apr_04	Nov_04	Apr_05
1:2 Extraction						
Ca (mmol/l)	0.63 ± 0.13	1.14 ± 0.40	0.39 ± 0.17	0.33 ± 0.05	0.17 ± 0.07	0.11 ± 0.02
Mg (mmol/l)	0.46 ± 0.11	0.47 ± 0.10	0.19 ± 0.09	0.12 ± 0.03	0.02 ± 0.15	0.13 ± 0.03
K (mmol/l)	0.17 ± 0.04	0.38 ± 0.72	0.25 ± 0.06	0.17 ± 0.05	0.09 ± 0.02	0.10 ± 0.02
Na (mmol/l)	0.22 ± 0.04	0.38 ± 0.09	0.13 ± 0.06	0.05 ± 0.01	0.08 ± 0.01	0.03 ± 0.00
SO4 (mmol/l)	0.81 ± 0.31	1.10 ± 0.52	0.39 ± 0.24	0.42 ± 0.10	0.03 ● 0.002	0.06 ± 0.02
NO3 (mmol/l)	0.01 ± 0.00	0.49 ± 0.04	0.04 ± 0.02	0.08 ● 0.03	0.07 ± 0.02	0.02 ± 0.00
NH4 (mmol/l)	0.12 ± 0.04	0.08 ± 0.03	0.05 ± 0.00	0.05 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
Cl (mmol/l)	0.25 ± 0.04	0.34 ± 0.07	0.18 ± 0.04	0.06 ± 0.08	0.03 ± 0.01	0.09 ± 0.01
HCO3 (mmol/l)	0.40 ± 0.05	0.58 ± 0.13	0.33 ± 0.04	0.23 ± 0.01	0.47 ± 0.15	0.40 ± 0.13
	Old Tailings Dam					
	NR_1			NR_2		
Ca (mmol/l)	0.17 ± 0.07	1.29 ± 0.03	0.52 ± 0.22	0.19 ± 0.06	0.73 ± 0.22	0.27 ● 0.17
Mg (mmol/l)	0.09 ± 0.04	0.70 ± 0.11	0.44 ± 0.18	0.03 ± 0.02	0.40 ± 0.15	0.22 ± 0.09
K (mmol/l)	0.06 ± 0.01	1.12 ± 0.09	0.20 ± 0.08	0.11 ± 0.01	0.42 ± 0.04	0.12 ± 0.06
Na (mmol/l)	0.02 ± 0.01	0.21 ± 0.03	0.16 ± 0.09	0.02 ± 0.01	0.14 ± 0.06	0.12 ± 0.06
SO4 (mmol/l)	0.10 ± 0.05	0.94 ± 0.26	0.59 ± 0.39	0.09 ± 0.01	0.61 ± 0.24	0.15 ± 0.08
NO3 (mmol/l)	0.03 ± 0.01	2.16 ± 0.26	0.16 ± 0.04	0.04 ± 0.02	0.93 ± 0.16	0.42 ± 0.35
NH4 (mmol/l)	0.03 ± 0.01	0.23 ± 0.05	0.02 ± 0.01	0.04 ± 0.01	0.26 ± 0.05	0.02 ± 0.00
Cl (mmol/l)	0.05 ± 0.04	0.37 ± 0.02	0.16 ± 0.05	0.05 ± 0.01	0.26 ± 0.17	0.12 ± 0.03
HCO3 (mmol/l)	0.33 ± 0.13	0.23 ± 0.02	0.25 ± 0.06	0.35 ± 0.20	0.23 ± 0.02	0.12 ± 0.02
	Old Tailings Dam			Natural veldt		
	Old_O			Nat		
Ca (mmol/l)	0.23 ± 0.06	0.64 ± 0.48	0.21 ± 0.04	0.84 ± 0.17	1.23 ± 0.29	0.45 ± 0.18
Mg (mmol/l)	0.04 ± 0.02	0.21 ± 0.18	0.20 ± 0.02	0.48 ± 0.12	0. 40 ± 0.13	0.35 ± 0.25
K (mmol/l)	0.07 ± 0.01	0.24 ± 0.13	0.20 ± 0.03	0.85 ± 0.23	0.97 ± 0.21	1.34 ± 0.93
Na (mmol/l)	0.02 ± 0.00	0.06 ± 0.02	0.05 ± 0.02	0.02 ± 0.01	0.04 ± 0.03	0.01 ± 0.01
SO4 (mmol/l)	0.13 ± 0.02	0.65 ± 0.58	0.09 ± 0.02	0.35 ± 0.06	0.41 ± 0.13	0.23 ± 0.12
NO3 (mmol/l)	0.05 ± 0.01	0.33 ± 0.26	0.14 ± 0.07	2.18 ± 0.34	2.37 ± 0.68	1.75 ± 1.24
NH4 (mmol/l)	0.04 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.23 ± 0.14	0.04 ± 0.01	0.04 ± 0.01
Cl (mmol/l)	0.06 ± 0.01	0.11 ± 0.08	0.14 ± 0.02	0.17 ± 0.02	0.22 ± 0.08	0.31 ± 0.23
HCO3 (mmol/l)	0.35 ± 0.13	0.38 ± 0.12	0.22 ± 0.03	0.42 ± 0.26	0.33 ± 0.10	0.17 ± 0.03

Value indicated as mean ± SEM

Table 4.4 Summary of micro-element properties in the platinum mine tailings

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Micro-elements						
Fe (mmol/l)	16.39 ± 2.97	8.66 ± 4.05	21.52 ± 6.64	30.8 ± 6.20	26.78 ± 2.16	20.16 ± 6.34
Mn (mmol/l)	2.56 ± 0.94	1.45 ± 0.49	1.98 ± 0.27	1.64 ± 0.29	1.43 ± 0.20	3.08 ± 1.67
Cu (mmol/l)	3.44 ± 0.96	2.54 ± 0.12	1.96 ± 0.25	0.64 ± 0.03	1.29 ± 0.51	1.65 ± 0.47
Zn (mmol/l)	1.23 ± 0.44	0.98 ± 0.47	0.49 ± 0.21	0.36 ± 0.06	0.43 ± 0.20	0.28 ± 0.23
B (mmol/l)	62.7 ± 0.21	4.16 ± 4.15	4.70 ± 4.69	0.46 ± 0.36	2.90 ± 1.45	3.01 ± 2.99
	Old Tailings Dam					
	NR_1			NR_2		
Fe (mmol/l)	18.18 ± 5.75	11.38 ± 1.63	12.33 ± 3.03	31.92 ± 5.60	14.77 ± 2.32	11.16 ± 3.27
Mn (mmol/l)	1.07 ± 0.23	2.24 ± 0.12	2.09 ± 0.07	1.85 ± 0.22	2.53 ± 0.49	1.11 ± 0.33
Cu (mmol/l)	0.65 ± 0.16	2.50 ± 0.34	2.08 ± 0.26	1.35 ± 0.31	3.48 ± 0.16	1.77 ± 0.41
Zn (mmol/l)	0.26 ± 0.01	1.57 ± 0.92	0.92 ± 0.37	0.23 ± 0.07	0.55 ± 0.29	0.19 ± 0.11
B (mmol/l)	49.4 ± 0.94	0.01 ± 0.00	5.91 ± 0.89	48.3 ± 1.11	0.01 ± 0.00	6.48 ± 3.64
	Old Tailings Dam			Natural veldt		
	Old_O			Nat		
Fe (mmol/l)	24.91 ± 5.63	31.09 ± 16.26	15.52 ± 3.45	37.66 ± 13.97	17.61 ± 4.70	69.65 ± 27.52
Mn (mmol/l)	1.43 ± 0.22	3.54 ± 1.15	1.17 ± 0.08	6.53 ± 1.20	0.80 ± 0.32	4.24 ± 123
Cu (mmol/l)	0.85 ± 0.20	0.75 ± 0.36	2.41 ± 0.22	0.56 ± 0.07	0.02 ± 0.00	1.16 ± 0.16
Zn (mmol/l)	0.40 ± 0.07	0.09 ● 0.06	0.18 ± 0.03	0.32 ± 0.03	0.65 ± 0.19	0.72 ± 0.44
B (mmol/l)	0.10 ± 0.00	4.80 ± 2.59	1.67 ± 0.66	0.1 ± 0.00	0.01 ± 0.00	1.34 ± 1.33

Value indicated as mean + SEM

Table 4.5 Summary of the chemical properties of platinum tailings: ammonium acetate extraction method.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Nutrients						
Ca (mg/kg)	1063.00 ± 81.61	1526.67 ± 317.92	804.83 ± 153.69	333.83 ± 12.70	562.33 ± 274.01	483.50 ± 127.40
Mg (mg/kg)	190.50 ± 9.37	226.33 ± 64.81	121.17 ± 34.92	45.83 ± 8.82	26.17 ± 2.62	56.50 ± 16.53
K (mg/kg)	65.17 ± 6.66	208.17 ± 83.51	86.83 ± 13.92	6.83 ± 0.17	45.33 ± 15.55	43.00 ± 25.08
Na (mg/kg)	60.67 ± 6.36	36.33 ± 3.32	45.50 ± 16.91	49.5 ± 1.04	37.33 + 4.57	81.83 ± 12.89
P (mg/kg)	57.70 ± 13.28	144.68 ± 41.93	53.58 ± 21.15	7.10 ± 1.84	10.88 ± 1.56	10.73 ± 1.28
	Old Tailings Dam					
	NR_1			NR_2		
Ca (mg/kg)	333.52 ± 52.61	1189.50 ± 31.19	950.00 ± 416.97	290.33 ± 44.07	576.83 + 140.10	579.83 ± 83.16
Mg (mg/kg)	34.83 ± 6.21	181.67 ± 10.76	161.33 ± 67.24	41.50 ± 6.45	71.50 ± 17.30	115.33 ± 23.29
K (mg/kg)	2.17 ± 0.33	230.67 ± 10.94	102.50 ± 63.00	4.83 ± 2.05	71.83 ± 18.50	39.83 ± 9.90
Na (mg/kg)	36.83 ± 3.53	52.67 ± 2.24	69.17 ± 32.71	46.00 ± 8.39	31.67 ± 0.60	96.83 ± 7.86
P (mg/kg)	3.25 ± 0.13	191.00 ± 23.68	95.75 ± 35.84	4.45 ± 0.70	95.10 ± 25.17	71.03 ± 25.22
	Old Tailings Dam			Natural Veldt		
	Old_O			Nat		
Ca (mg/kg)	304.00 ± 39.24	454.17 ± 135.44	680.17 ± 74.32	2282.8 ± 356.97	3292.67 ± 203.74	2741.67 ± 259.23
Mg (mg/kg)	42.00 ± 1.00	33.67 ± 13.31	104.67 ± 22.11	339.33 ± 71.95	433.50 ± 60.60	402.17 ± 84.80
K (mg/kg)	1.00 ± 0.29	37.67 ± 11.42	65.33 ± 16.18	423.00 ● 88.74	577.83 ± 39.83	1050.00 ± 529.64
Na (mg/kg)	47.67 ± 14.09	49.00 ± 5.30	99.17 ± 4.51	55.67 ± 6.43	39.83 ± 2.91	91.17 ± 2.49
P (mg/kg)	4.20 ± 0.40	8.35 ± 0.63	85.80 ± 13.19	64.70 ± 15.68	135.50 ± 14.78	90.85 ± 9.20

Value indicated as mean ● SEM

Table 4.6 Summary of the exchangeable cations found in the platinum mine tailings.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Exchangeable cations						
Ca (cmol(+)/kg)	5.30 ± 0.41	7.62 ± 1.59	4.02 ± 0.77	1.67 ± 0.06	2.81 ± 1.37	2.41 ± 0.64
Mg (cmol(+)/kg)	1.57 ± 0.08	1.86 ± 0.53	1.00 ± 0.29	0.38 ± 0.07	0.22 ± 0.02	0.47 ± 0.14
K (cmol(+)/kg)	0.17 ± 0.02	0.53 ± 0.21	0.22 ± 0.04	0.02 ± 0.00	0.12 ± 0.04	0.11 ± 0.06
Na (cmol(+)/kg)	0.26 ± 0.03	0.16 ± 0.01	0.20 ± 0.07	0.22 ± 0.00	0.16 ± 0.02	0.36 ± 0.06
	Old Tailings Dam					
	NR_1			NR_2		
Ca (cmol(+)/kg)	1.66 ± 0.26	5.94 ± 0.16	4.74 ± 2.08	1.45 ± 0.22	2.88 ± 0.70	2.89 ± 0.42
Mg (cmol(+)/kg)	0.29 ± 0.05	1.50 ± 0.09	1.33 ± 0.55	0.34 ± 0.05	0.60 ± 0.14	0.95 ± 0.19
K (cmol(+)/kg)	0.01 ± 0.00	0.59 ± 0.03	0.26 ± 0.16	0.01 ± 0.05	0.18 ± 0.05	0.10 ± 0.03
Na (cmol(+)/kg)	0.16 ± 0.02	0.23 ± 0.01	0.30 ± 0.14	0.2 ± 0.04	0.14 ± 0.00	0.42 ± 0.03
	Old Tailings Dam			Natural Veldt		
	Old_O			Nat		
Ca (cmol(+)/kg)	1.51 ± 0.19	2.27 ± 0.68	3.39 ± 0.37	1.78 ± 1.78	16.43 ± 1.01	13.68 ± 1.29
Mg (cmol(+)/kg)	0.35 ± 0.01	0.28 ± 0.11	0.86 ± 0.18	2.79 ± 0.59	3.57 ± 0.50	3.31 ± 0.70
K (cmol(+)/kg)	0.00 ± 0.00	0.10 ± 0.03	0.17 ± 0.04	1.09 ± 0.23	1.48 ± 0.29	2.69 ± 1.36
Na (cmol(+)/kg)	0.21 ± 0.06	0.21 ± 0.02	0.43 ± 0.02	0.24 ± 0.03	0.17 ± 0.01	0.40 ± 0.01

Value indicated as mean ± SEM

Table 4.7 Summary of important chemical properties of the platinum mine tailings.

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Total C (%)	0.51 ± 0.04	1.72 ± 0.28	0.71 ± 0.25	0.09 ± 0.003	0.13 ± 0.06	0.12 ± 0.03
pH	6.51 ± 0.23	7.09 ± 0.22	6.89 ± 0.22	6.87 ± 0.12	7.30 ± 0.26	7.07 ± 0.17
EC (mS/cm)	0.27 ± 0.06	0.41 ± 0.10	0.16 ± 0.06	0.12 ± 0.02	0.06 ± 0.01	0.07 ± 0.01
pH(H2O)	6.51 ± 0.06	6.69 ± 0.19	7.10 ± 0.19	6.98 ± 0.09	7.53 ± 0.27	7.85 ± 0.03
pH(KCl)	5.97 ± 0.09	6.18 ± 0.25	6.43 ± 0.28	6.84 ± 0.06	7.01 ± 0.42	7.08 ± 0.05
CEC (cmol(+)/kg)	8.99 ± 0.39	12.10 ± 3.14	5.66 ± 1.40	2.34 ± 0.10	3.21 ± 1.27	3.10 ± 0.80
Base sat (%)	81.26 ± 4.03	85.46 ± 8.82	99.12 ± 6.45	97.47 ± 3.58	100.60 ± 5.02	107.82 ± 0.85
P (mmol/l)	0.13 ± 0.02	0.16 ± 0.06	0.10 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01
	Old Tailings Dam					
	NR_1			NR_2		
Total C (%)	0.07 ± 0.01	2.19 ± 0.13	1.57 ± 0.68	0.12 ± 0.02	0.94 ± 0.53	0.36 ± 0.22
pH	6.89 ± 0.02	6.29 ● 0.01	6.18 ± 0.33	6.64 ± 0.45	6.45 ± 0.12	6.26 ± 0.12
EC (mS/cm)	0.07 ± 0.02	0.55 ± 0.03	0.23 ± 0.08	0.07 ± 0.02	0.31 ± 0.09	0.13 ± 0.06
pH(H2O)	7.04 ± 0.06	6.39 ± 0.03	6.78 ± 0.36	6.86 ± 0.12	6.62 ± 0.15	6.83 ± 0.14
pH(KCl)	6.92 ± 0.42	5.79 ± 0.06	6.19 ± 0.44	6.31 ± 0.48	5.72 ± 0.20	6.11 ± 0.13
CEC (cmol(+)/kg)	2.08 ± 0.16	11.98 ± 0.53	8.59 ± 3.39	2.21 ● 0.08	5.12 ± 1.64	4.99 ± 1.05
Base sat (%)	101.2 ± 2.60	69.08 ± 2.19	83.59 ± 14.89	90.46 ± 5.49	77.97 ± 6.97	90.27 ± 6.78
P (mmol/l)	0.01 ± 0.00	0.29 ± 0.05	0.18 ± 0.05	0.01 ± 0.00	0.15 ± 0.01	0.10 ± 0.02
	Old Tailings Dam			Natural Veldt		
	Old_O			Nat		
Total C (%)	0.08 ± 0.01	0.57 ± 0.26	6.28 ± 0.29	6.20 ± 0.38	6.88 ± 1.09	6.51 ± 1.38
pH	7.21 ± 0.19	7.16 ± 0.20	6.41 ± 0.21	6.37 ± 0.48	6.71 ± 0.22	5.82 ± 0.13
EC (mS/cm)	0.07 ± 0.01	0.21 ± 0.15	0.11 ± 0.02	0.38 ± 0.09	0.43 ± 0.10	0.31 ± 0.17
pH(H2O)	7.15 ± 0.05	7.33 ± 0.37	6.87 ± 0.17	5.93 ± 0.29	6.58 ± 0.20	6.27 ± 0.11
pH(KCl)	6.84 ± 0.08	6.97 ± 0.43	6.28 ± 0.29	5.05 ± 0.483	6.03 ± 0.21	5.21 ± 0.12
CEC (cmol(+)/kg)	1.98 ± 0.20	2.83 ± 0.68	5.42 ± 0.78	27.20 ± 1.02	26.64 ± 3.82	32.34 ± 5.11
Base sat (%)	104.3 ± 2.08	100.26 ± 2.50	91.06 ± 6.54	57.01 ± 8.89	83.22 ± 6.83	62.60 ± 6.19
P (mmol/l)	0.01 ± 0.00	0.00 ± 0.00	0.15 ± 0.01	0.12 ± 0.09	0.19 ± 0.03	0.15 ± 0.02

Value indicated as mean + SEM

Table 4.8.1 Summary of phospholipid fatty acid biomarkers (PLFAs).

	New Tailing Dam					
	NR_N			Old_N		
	04-Apr	04-Nov	05-Apr	04-Apr	04-Nov	05-Apr
Viable Biomass	11849.21 ± 468.30^{a}	8765.75 ± 1245.56^{a}	11241.13 ± 2746.58^{a}	4754.44 ± 2027.64^{a}	11349.63 ± 5620.89^{a}	4506.15 ± 1688.93^{a}
Neutral saturated	29.49 ± 0.48^{ab}	50.12± 1.51^{a}	47.08 ± 4.05^{a}	28.58 ± 0.91^{a}	51.59 ± 2.79^{c}	48.18 ± 5.34^{a}
Mid Branched saturated	3.42 ± 0.25ab	6.58 ± 0.44^{a}	5.08 ± 0.50^{ab}	4.48 ± 1.55^{c}	2.92 ± 1.52^{a}	7.10 ± 0.71^{c}
***Terminal* Branched saturated**	15.26 ± 0.19^{a}	30.80 ± 2.02^{a}	30.01 ± 2.17^{a}	19.49 ± 2.78^{ab}	28.23 ± 4.89^{a}	27.91 ± 0.53^{a}
Monounsaturated	43.04 ± 0.49^{a}	8.87 ± 0.68^{a}	8.51 ± 1.71^{a}	38.97 ± 2.60^{a}	11.39 ± 4.35^{a}	6.35 ± 3.20^{a}
Polyunsaturated	8.40 ± 0.77^{a}	3.38 ± 1.17^{a}	8.27 ± 2.49^{a}	7.67 ± 1.13^{a}	5.40 ± 3.05^{a}	10.12 ± 2.19^{a}
Ratios						
F:B ratio	0.23 ± 0.04^{a}	0.03 ± 0.01^{a}	0.16 ± 0.10^{a}	0.22 ± 0.03^{a}	0.13 ± 0.11^{a}	0.18 ± 0.14^{a}
cy19:0/18-1w7c	0.58 ± 0.02^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00	0.43 ± 0.09^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
cy17:0/16:1w7c	0.64 ± 0.02^{a}	2.73 ± 0.30^{a}	2.15 ± 0.92^{a}	0.27 ± 0.03^{a}	3.74 ± 2.09^{a}	7.73 ± 7.24^{a}
Sat-Unsaturated	1.12 ± 0.03^{a}	10.07 ± 1.11^{a}	10.67 ± 2.58^{a}	1.37 ± 0.14^{a}	10.17 ± 4.21^{a}	22.44 ± 10.13^{a}
18:1w7t/18:1w7c	0.00 ± 0.00^{a}	0.90 ± 0.01^{a}	0.98 ± 0.64^{a}	0.00 ± 0.00^{a}	0.27 ± 0.21^{a}	0.63 ± 0.63^{a}
16:1w7/:16:1w7c	0.03 ± 0.01^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values given as mean ± SEM

Sites per sample period with the same superscript indicate no significant difference between the sites.

Table 4.8.2 Summary of phospholipid fatty acid biomarkers (PLFAs).

	Old Tailings Dam					
	NR_1			NR_2		
	04-Apr	04-Nov	05-Apr	04-Apr	04-Nov	05-Apr
Viable Biomass	13617.03 ± 1370.54[a]	9504.92 ± 444.21[a]	6786.04 ± 1459.93[a]	13584.09 ± 2382.66[a]	5841.30 ± 1100.23[a]	11853.33 ± 5445.86[a]
Neutral saturated	31.33 ± 0.04[ab]	44.35 ± 4.23[a]	43.18 ± 2.46[a]	26.66 ± 4.62[a]	50.64 ± 0.70[a]	46.66 ± 2.95[a]
Mid Branched saturated	3.26 ± 0.20[ab]	6.47 ± 0.33[a]	4.65 ± 0.29[ab]	3.33 ± 0.37[a]	5.13 ± 0.36[a]	4.30 ± 0.33[ab]
Terminal Branched saturated	18.22 ± 1.02[a]	33.78 ± 2.22[a]	29.92 ± 1.58[a]	18.73 ± 2.91[ab]	30.77 ± 2.72[a]	27.22 ± 1.45[a]
Monounsaturated	39.48 ± 1.07[a]	11.53 ± 2.02[a]	12.45 ± 0.81[a]	43.10 ± 1.82[a]	7.69 ± 1.23[a]	12.71 ± 2.05[a]
Polyunsaturated	7.12 ± 0.16[a]	3.24 ± 0.34[a]	9.17 ± 1.43[a]	7.36 ± 0.87[a]	5.53 ± 2.84[a]	8.23 ± 0.23[a]
Ratios						
F:B ratio	0.19 ± 0.01[a]	0.03 ± 0.00[a]	0.26 ± 0.06[a]	0.18 ± 0.02[a]	0.12 ± 0.10[a]	0.24 ± 0.02[a]
cy19:0/18-1w7c	1.02 ± 0.10[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	1.12 ± 0.11[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
cy17:0/16:1w7c	0.61 ± 0.03[a]	2.34 ± 0.40[a]	0.82 ± 0.11[a]	0.60 ± 0.03[a]	4.28 ± 0.93[a]	1.01 ± 0.80[a]
Sat-Unsaturated	1.34 ± 0.06[a]	7.80 ± 1.32[a]	6.31 ± 0.48[a]	1.14 ± 0.11[a]	12.11 ± 2.71[a]	6.63 ± 1.46[a]
18:1w7t/18:1w7c	0.07 ± 0.00[a]	0.54 ± 0.19[a]	0.68 ± 0.30[a]	0.04 ± 0.02[a]	0.42 ± 0.40[a]	0.83 ± 0.40[a]
16:1w7t/16:1w7c	0.06 ± 0.01[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.06 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Values given as mean ± SEM
Sites per sample period with the same superscript indicate no significant difference between the sites.

Table 4.8.3 Summary of phospholipid fatty acid biomarkers (PLFAs).

	Old Tailings Dam			Natural veldt		
	Old_O			Nat		
	04-Apr	04-Nov	05-Apr	04-Apr	04-Nov	05-Apr
Viable Biomass	3284.92 ± 389.91[a]	4488.75 ± 2009.68[a]	9391.96 ± 1865.96[a]	24717.92 ± 7476.36[a]	9870.16 ± 1682.44[a]	31053.33 ± 4224.29[c]
Neutral saturated	29.85 ± 1.07[ab]	55.81 ± 5.23[a]	47.73 ± 1.79[a]	39.87 ± 3.70[c]	47.54 ± 2.84[a]	47.93 ± 7.71[a]
Mid Branched saturated	3.81 ± 0.04[ab]	5.25 ± 1.98[a]	4.60 ● 0.26[ab]	0.98 ± 0.17[a]	3.08 ± 0.46[a]	3.31 ± 0.63[a]
Terminal Branched saturated	18.67 ± 0.69[a]	27.20 ± 3.97[a]	28.25 ● 0.33[a]	32.85 ± 5.97[c]	30.62 ± 2.38[a]	29.61 ± 5.13[a]
Monounsaturated	39.76 ± 1.75[a]	5.89 ± 0.99[a]	11.45 ± 1.69[a]	24.18 ± 3.08[b]	13.96 ± 0.52[a]	10.05 ± 1.67[a]
Polyunsaturated	7.54 ± 0.33[a]	5.86 ± 3.41[a]	7.44 ± 0.49[a]	0.19 ± 0.05[b]	4.40 ± 0.44[a]	8.39 ± 0.41[ab]
Ratios						
F:B ratio	0.22 ± 0.02[a]	0.01 ± 0.01[a]	0.19 ± 0.02[a]	0.00 ± 0.00[b]	0.11 ± 0.01[a]	0.25 ± 0.02[a]
cy19:0/18-1w7c	0.37 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
cy17:0/16:1w7c	0.44 ± 0.03[a]	1.43 ± 1.43[a]	0.94 ± 0.09[a]	6.87 ± 0.86[b]	1.01 ± 0.02[a]	0.60 ± 0.07[a]
Sat-Unsaturated	1.32 ± 0.10[a]	15.74 ± 2.21[a]	7.36 ± 1.11[a]	3.18 ± 0.53[b]	3.85 ± 0.28[a]	8.70 ± 1.97[a]
18:1w7t/18:1w7c	0.00 ± 0.00[a]	0.42 ± 0.42[a]	0.34 ± 0.27[a]	0.13 ± 0.07[a]	0.94 ± 0.10[a]	0.05 ± 0.01[a]
16:1w7t/16:1w7c	0.00 ± 0.00[a]	0.00 ± 0.00	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[c]

Values given as mean ± SEM
Sites per sample period with the same superscript indicate no significant difference between the sites.

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CHAPTER 5

NEMATODES AS INDICATOR OF ECOSYSTEM DEVELOPMENT DURING PLATINUM MINE TAILINGS REHABILITATION

ABSTRACT

The success of two different methods of rehabilitation, which includes the use of woodchip-vermicompost and inorganic fertilisers, was evaluated using nematodes as indicators of ecosystem recovery. Nematodes respond rapidly to environmental disturbance by shifting toward a more opportunistic (r-strategist), fast-growing nematode community. The Maturity Index (MI), Plant-parasitic Index (PPI) and PPI/MI ratio were used to evaluate ecosystem recovery within platinum mine tailings. The addition of fertilisers (both woodchip-vermicompost and inorganic fertilisers) caused enrichment of the ecosystems, as indicated by a decrease in the MI values. A decrease in the MI value was consistent with an increase in the PPI. The woodchip-vermicompost site (NR_N) showed the highest initial number of nematodes, but showed a decrease from November 2004 to April 2005. The inorganic fertilised site (Old_O) had the highest number of nematodes at the end of the study period in April 2005. Bacterivorous nematodes, particularly the *Rhabditidae* (an enrichment opportunist) dominated the feeding functional groups. Plant-parasitic nematodes, which contributed to 34% of the feeding functional group, dominated in the woodchip-vermicompost sites during April 2004, with inorganic fertilised sites showing the highest number of plant-parasitic nematodes during April 2005. Results obtained were not significantly ($p < 0.05$) different between the different treatments and further studies as well as alternative measurements may be considered.

Maturity Index, Nematodes, Plant-parasitic Index, Rehabilitation, Woodchip-vermicompost

5.1 Introduction

Nematodes are widespread throughout terrestrial ecosystems (Háněl, 2002), contributing to important soil processes such as decomposition and nutrient cycling (Bulluck et al., 2002). They provide a holistic measurement of soil conditions due to their activity throughout the year (García-Álvarez et al., 2004). The structural composition of nematode communities is sensitive to disturbances caused to the soil ecosystem (Berkelmans et al., 2003). Nematode communities respond to disturbance by shifting towards a more opportunistic, fast growing population of bacterial feeding nematodes and slowly returning to a more diverse community including slow growing bacterial and fungal feeders and ultimately predators and omnivores (Fu et al., 2000). Due to the shift in community composition that occurs in nematodes after disturbance and the ability to identify specific taxa or functional groups, nematodes offer a great opportunity to be used as indicators in ecosystem recovery or degradation (Yeates & Bongers, 1999). Bongers (1990) proposed the use of a Maturity index (MI) as a tool to monitor colonisation and succession in ecosystem recovery. The Maturity Index is the weighted mean of five coloniser-persister (cp) values, which is based on the potential intrinsic rate of natural increase of nematodes as derived from their life-history traits and is ascribed to define the nematode taxa (Bongers et al., 1997). Plant-parasitic nematodes are excluded from the Maturity Index and calculated in a separate Plant-parasitic Index (PPI). The separation of non-parasitic nematodes in the Maturity Index from plant-parasitic nematodes in the Plant-parasitic index was due to the observations by Bongers et al. (1995) that under certain conditions the Plant-parasitic Index reacts oppositely to that of the Maturity Index.

Mining activities may be destructive to the ecosystem due to the removal of vegetation and soils in order to obtain the desired materials (Bradshaw, 1997). Mine waste in the form of tailings may be especially hazardous to both humans and the environment. Tailings may contain heavy metals and fine particle sized materials, which may cause heavy metal pollution due to leaching into nearby watercourses and dust pollution because of wind erosion (Tordoff et al., 2000).

Revegetation of these mine spoils contributes to aesthetic value by reducing erosion, stabilising the spoils and minimising the potential health impact they may have on human beings and the environment (Wong, 2003). Platinum mine tailings are a biologically sterile medium, which contain a large amount of heavy metals. The saline characteristics of the tailings hinder the establishment of vegetation (Walmsley, 1987). The rehabilitation process needs to be monitored in order to ensure that the rehabilitated area is restored to a stable, self-sustaining ecosystem similar to that of the surrounding area (Bell, 2001). The aim of this study was to evaluate the two different rehabilitation methods, including woodchip-vermicompost and inorganic fertilisers, on the trophic structure of nematode communities during the rehabilitation of platinum mine tailings. The possible use of nematodes as indicators during ecosystem development under these two management practices will also be evaluated, in order to promote the establishment of a self-sustaining vegetation cover the platinum mine tailings dam.

5.2 Materials and Methods

5.2.1 Experimental design

The experimental site was situated at Impala Platinum mine, Rustenburg, South Africa. Five sites were divided between an old tailings dam, which has been dormant since 1976 and a new tailings dam currently under production. Fertilisation rates for optimum growth were previously determined during a study conducted by Van Rensburg & Morgenthal (2004). Three sites of 30m x 10m were located on the old tailings dam of which two were treated with woodchip-vermicompost and one treated with inorganic fertiliser. Two sites (30m x 30m) were located on the new tailings dam, of which one was treated with the woodchip-vermicompost and one with inorganic fertilisers. The aspect of all experimental sites was in a northerly direction. Woodchips were obtained from mine blasting, separated from excavated rock during screening, mixed with sewage and water (Van Rensburg & Morgenthal, 2004) and decomposed using the earthworm species *Eisenia fetida*. Woodchip-vermicompost of 50 tons per hectare was manually mixed into the tailings to a depth of 30 cm. Sites were then sown with a premix of 12 different grass species. A natural veldt in the vicinity of the experimental sites were randomly selected as reference area as no

suitable rehabilitated platinum mine tailings site could be obtained. Sites were surveyed in April 2004, November 2004 and April 2005, during which it did not receive any irrigation. The average rainfall received at the sites during this period was 474.4 mm.

5.2.2 Soil sampling

Before soil was sampled, the organic matter was removed from the soil surface. Soil samples were then obtained from the first 10 – 15 cm of the soil surface, with each sample consisting of three core samples. Three samples were taken for each sample site (n = 18). Soil samples were handled carefully to avoid unnecessary pressure that might lead to crushing of soil organisms (Koehler, 1997). Samples were kept at room temperature (25°C) and extracted within days of sampling.

5.2.3 Vegetation cover

Vegetation cover for each site was determined by placing a $1m^2$ quadrant at random locations on each site. The vegetation cover was then estimated as the ratio of vegetation cover to bare soil within each quadrate. A total of twenty quadrants ($1m^2$) where placed on each site and the mean representative value of all quadrants surveyed served as vegetation cover estimation for the whole site (Kent & Coker, 1997).

5.2.4 Analyses

5.2.4.1 Nematode extraction

Nematodes found in the soil sample not only reflect the current population of nematodes in the soil at the time of sampling, but may also be affected by the sampling and extraction procedure (Yeates & Bongers, 1999). Nematodes were generally extracted according to the method (based on specific gravity) described by Jenkins (1964) with certain adaptations made for an increased efficacy rate. From each soil sample 200 cm^3 soil was added to 400 ml tap water until a volume of 600 ml was obtained. A sub sample was subsequently stirred thoroughly and poured through a kitchen sieve nested on a 5 L beaker to eliminate course debris and stones. Subsequently the 5 L soil, nematode and water mixture were allowed to settle for 30

seconds. The supernatant was decanted through a 750 µm-aperture sieve nested on a 45 µm-aperture sieve (McSorley & Frederick, 1991). The residue on the 25 µm-aperture sieve was collected and the process was repeated once more. The soil remaining on the 25 µm-aperture sieve was finally transferred into a 50 ml centrifuge tube and centrifuged for 5 minutes at 1750 rpm. The supernatant of the centrifuged sample was decanted and the pellet resuspended in a sucrose solution (624g/L H_2O) and centrifuged again (Jenkins, 1964). The supernatant containing the nematodes was poured onto a 25 µm-aperture sieve and thoroughly rinsed with tap water.

Nematodes were transferred into a sample flask (± 10 ml) for identification to genus level and counting under a dissection microscope. Dormant nematodes were not recorded due to the difficulties of identification. Coloniser-persister values were assigned to all nematode taxa after Bongers (1990). Nematodes were further assigned to different feeding functional groups based on morphological structures.

5.2.5 Statistical analyses

The Maturity Index was calculated as the weighted mean of the cp values of non parasitic free-living nematodes as described by Bongers (1990):

$$MI = \sum_{i=1} v(i) \times f(i)$$

where $v(i)$ is the cp value of the i'th taxon in the sample and $f(i)$ is the frequency of the i'th taxon. For plant-parasitic nematodes, the Plant-parasitic Index was calculated as:

$$PPI = \sum_{i=1} v(i) \times f(i)$$

where $v(i)$ is the cp value for plant-parasitic nematodes of the i'th taxon and $f(i)$ is the frequency of the plant-parasitic nematodes of the i'th taxon. The Modified Maturity Index was calculated as the combined cp values of both free-living and plant-parasitic nematodes:

$$MMI = \sum_{i=1} v(i) \times f(i)$$

The ratio of the Plant-parasitic Index to the Maturity Index was calculated for each sample. The representative percentage of each feeding functional group was determined for the experimental period. Statistical analyses were performed on the data using STATISTICA 7 (StatSoft, Inc ©). Data were tested for normality using the Shapiro-Wilk's test. One-way breakdown ANOVA as well as Tukey's honest significant difference (HSD) tests were performed.

5.3 Results and Discussion

5.3.1 Vegetation cover

The summary of the results obtained from the vegetation survey is presented in Table 5.4. Vegetation cover to bare ground of the woodchip-vermicompost sites was as follows: NR_N showed a ratio of 64%:37%, whereas NR_1 and NR_2 showed a ratio of 72%:28% and 66%:34%, respectively. The inorganic fertilised sites showed a vegetation cover to bare ground ratio of 34%:66% for Old_N and 38%:62% for Old_O. The woodchip-vermicompost sites NR_N, NR_1 and NR_2 therefore illustrated a higher overall vegetation cover than sites treated with inorganic fertilisers Old_N and Old_O.

A decrease in vegetation cover of 17% and 13% was observed in NR_1 and NR_2, whereas Old_N and Old_O showed a decrease of 8% and 13%, at the end of the study period. The woodchip-vermicompost site NR_N showed no difference in vegetation cover at the end of the study period. The perennial species *Cenchrus ciliaris* (L.) var. *ciliaris* and *Gaynday*, *Chloris gayana* Kunth, *Melinis repens* (Wild) Zizka and *Heteropogon contortus* (L.) Beauv. and the stoloniferous species *Cynodon dactylon* (L.) Pers., *Digitaria eriantha* Steud. of the sown seed mixture may have contributed to higher vegetation cover at the woodchip-vermicompost sites. Rainfall received decreased from 719.1 mm during the first growth season (November 2003 to February 2004) to 413.5 mm rain received during the second growth season of November 2004 to February 2005, which may have contributed to the decrease in vegetation cover.

5.3.2 Nematode community structure

The total number of nematodes present at the woodchip-vermicompost sites and inorganic fertilised sites are illustrated in Figure 5.1.

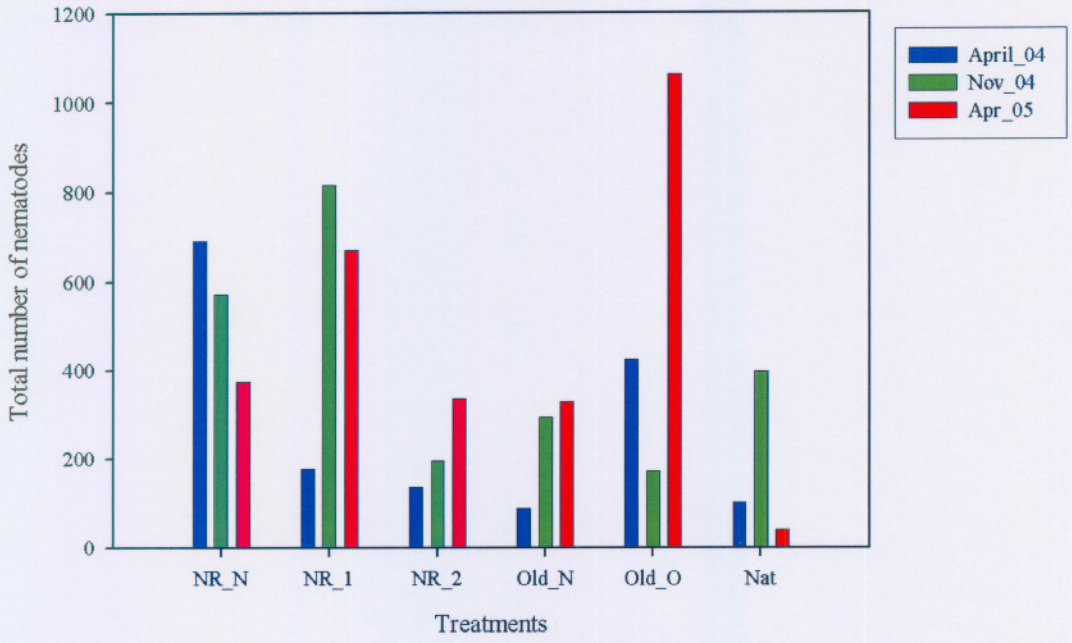


Figure 5.1 The total number of nematodes present in the different sites during the study period.

A total of 17 nematode genera were identified in the soil samples in the study period from April 2004 to April 2005. The woodchip-vermicompost site (NR_N) had the highest total number of individual nematodes during April 2004. All sites except NR_N and Old_O showed an increase in the number of individual nematodes. The inorganic fertilised site (Old_O) had the highest number of individual nematodes at the end of the study period in April 2005. The natural veldt showed an increase in nematode numbers from April 2004 to November 2004 with a decrease in number during April 2005. The lowest number of nematodes was present in the natural veldt during April 2005.

A summary of the nematode trophic groups obtained during the study are given in Tables 5.1 to 5.3. The total number of bacterivorous nematodes present at the different sites are illustrated in Figure 5.2.

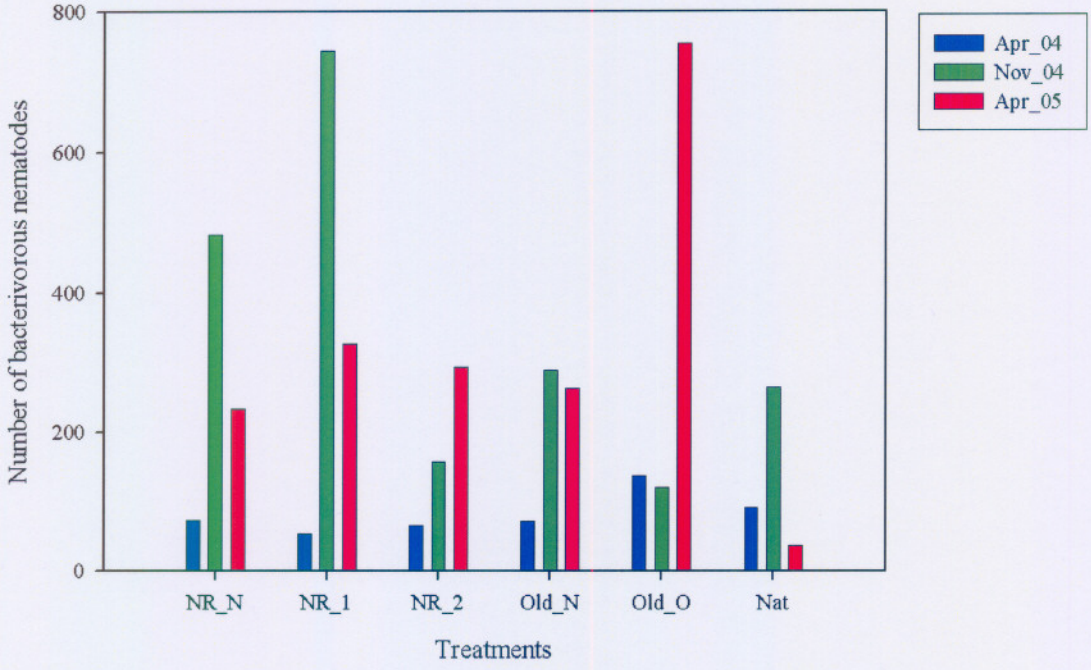


Figure 5.2 The total number of bacterivorous nematodes present at the sites during the study period.

Bacterivorous nematodes dominated and contributed to 65% of the community composition. Bacterivorous nematodes dominated in both the woodchip-vermicompost sites and inorganic fertilised sites. *Rhabditidae* was the dominated bacterivorous nematodes present. These nematodes are typical enrichment opportunistic nematodes with a short generation time and are classified as r-strategists. Enrichment opportunists are transported by insects and will only establish in areas with sufficient nutrient supply (Bongers & Bonger, 1998). *Rhabditidae* increased in numbers from the first sampling period in April 2004 to the second sample period in November 2004, whereafter their numbers decreased during April 2005 in NR_N, NR_1 and Old_N. The woodchip-vermicompost site NR_2 and inorganic fertilised site Old_O showed an increase in *Rhabditidae* numbers during April 2005. *Plectus* species (cp 2) was dominant in Old_N and Old_O during the first sampling period in April 2004. Bacterivorous nematodes showed an increase in numbers during November 2004, with *Rhabditidae* being the dominating species. The number of bacterivorous species shows a steep decrease in numbers during April 2005.

Fungivorous nematodes showed a slight increase during the second sampling period in November 2004, but numbers were still negligible (Table 5.1 – 5.3). *Tylenchus* species (cp 2) was only present in Old_N and NR_2 during the first sampling period in April 2004, whereafter its numbers decreased. During November 2004, an increase in numbers of *Tylenchus* species was observed in NR_N. *Paraphelenchus* species (cp 3) was present in NR_1 and NR_2 but were absent from the NR_N. No fungivorous nematodes were observed at these sites during April 2005. This is contrary to what would have been expected, since Sohlenius & Boström (1984) found an increase in cp 2 fungivorous nematodes and a decrease in cp 1 bacterivorous nematodes in organic matter, which was initially colonised by both cp 1 and cp 2 bacterivorous nematodes. Fungivorous nematodes were absent from Old_O throughout the study period. It can be concluded that the majority of decomposition at the woodchip-vermicompost sites and inorganic fertilised sites occurs mainly through bacterial decomposition, as indicated by the high abundance of bacterivorous nematodes. Bacterivorous nematodes contribute to the acceleration of the decomposition process and mineralisation and to the release of nutrients for plant growth (Neher, 2001).

Figure 5.3 illustrates the total number of plant-parasitic nematodes at the different sites as obtained during the present study.

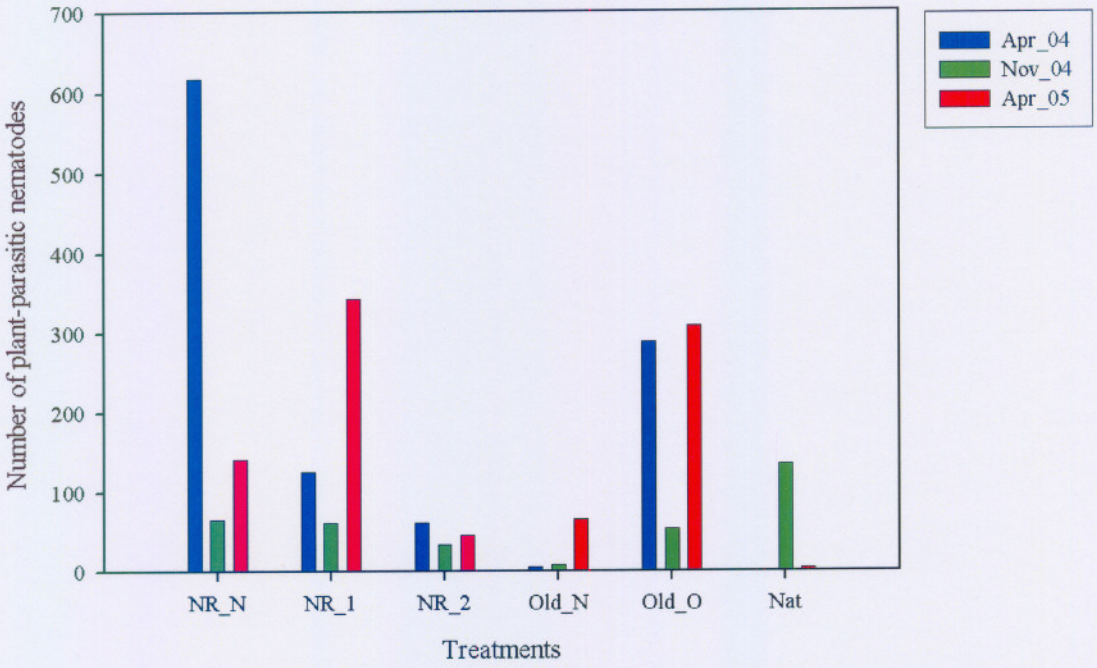


Figure 5.3. The total number of plant-parasitic nematodes at the sites during the study period.

Plant-parasitic nematodes contributed to 34% of the total nematode community. The majority of plant-parasitic nematodes present belong to both r- and K-strategists (cp 2 – 4). Háněl (2002) suggests that the presence of both r- and K-strategists may indicate that the persisters (K-strategists) act as colonisers under specific environmental conditions such as a high pH, high abundance of microorganisms' present and high diversity of herbaceous plant cover. Bongers (1990) suggested that persisters among the plant-parasitic nematodes occur under stressed conditions and that the increased in their numbers correlates to a gradual increase in primary production. All sites except Old_N showed a decrease in number of individual plant-parasitic nematodes during November 2004 (Figure 5.3). The number of individual plant-parasitic nematodes decreased in numbers in NR_2 during April 2005. An increase in plant-parasitic nematodes was observed in Old_N and Old_O as well as NR_N and NR_1 from November 2004 to April 2005. The total numbers of plant-parasitic nematodes in NR_N during April 2005 was lower than the initial number of plant-parasitic nematodes in April 2004 (Figure 5.3). The decrease in the plant-parasitic nematodes may be due to intraspecific competition between the different nematode species

owing to occupation of space, physical change of host plant or environmental factors, leading to natural selection of the most adaptable species (Brinkman et al., 2004).

Helicotylenchus species (cp 3) was present in the woodchip-vermicompost sites NR_N, NR_1 and, NR_2 as well as in the inorganic fertilised sites Old_N and Old_O (Table 5.1 -5.3). Villenave et al. (2001) found an increase in the abundance of *Helicotylenchus* species during the first year of cultivation of agricultural land after a long period of fallow. The woodchip-vermicompost sites were cultivated six months prior to the start of the experimental study, which could explain the occurrence of *Helicotylenchus* species at the woodchip-vermicompost sites. A decrease in *Paratylenchus* species was observed in NR_N and NR_1. The inorganic fertilised sites Old_N and Old_O contained none or little *Paratylenchus* species. The inorganic fertilised sites Old_N and Old_O contained *Tylenchorhynchus* species (cp 2), which showed an increase during November 2004 and April 2005 at these sites (Table 5.1 – 5.3). The absence of *Paratylenchus* species and the presence of *Tylenchorhynchus* species at the inorganic fertilised sites may indicate conditions that favour *Tylenchorhynchus* species over *Paratylenchus* species. Ectoparasites (*Tylenchorhynchus* species) are regulated by plant nutrient content, whereas well-nourished plants are more resistant to migratory endoparasites (*Paratylenchus* species). Vestergård (2004) found that ectoparasites (*Tylenchorhynchus* species) are stimulated by the addition of a balanced inorganic (NPK) fertiliser and endoparasites (*Paratylenchus* species) are inhibited by high and balanced fertilisation. This might explain the presence of *Tylenchorhychus* species in the inorganic fertilised sites. Plant-parasitic nematode populations were relatively low in April 2004 in the natural veldt, with numbers increasing during November 2004. *Hoplolaimidae* species dominated the plant-parasitic nematodes during November 2004. The plant-parasitic nematodes showed a decrease in numbers during April 2005 in the natural veldt.

Both the ecological and biological characteristics of individual nematode species in a particular community are considered in the Maturity Index and can therefore be seen as a semi-quantitative measurement (Poranzinska et al., 1999). The Maturity Index (MI) can be used to differentiate between different tillage regimes whereas the Plant-

parasitic index (PPI) can be used to indicate ecosystem fertility (Bongers & Ferris, 1999). The Plant-parasitic Index to Maturity Index ratio (PPI/MI) is a valuable measurement for assessing the state of an agroecosystem (Bongers et al., 1997). Frequency of disturbance is inversely correlated to Maturity Index value (Freckman & Ettema, 1993). The MI values were relatively low for all treatments during the study with values lower than two. According to Bongers & Ferris (1999), MI values lower than two are indicative of a nutrient enriched disturbed system. The low MI values in NR_N, NR_1 and NR_2 can be attributed to the addition of the woodchip-vermicompost. The enrichment of soil by adding organic material leads to an increase in microbial activity, which in turn causes an increase in the number of enrichment opportunists (cp 1). An increase in enrichment opportunists causes a decrease in the MI value. As the MI decreases, the PPI and the nutrient status of the ecosystem tends to increase (Bongers et al., 1997). A decrease in the MI values therefore, illustrates that both the woodchip-vermicompost sites and inorganic fertilised sites may not yet have reached stability, since Porazinska et al. (1999) suggested that a higher MI value illustrates a more stable and mature ecosystem.

Enrichment increases the carrying capacity of plants for nematodes, which leads to an increase in the PPI (Bonger & Ferris, 1999). The inorganic fertilised sites Old_N and Old_O showed a higher initial PPI value than the NR_N, NR_1 and, NR_2. The PPI values at the woodchip-vermicompost sites NR_N and NR_2 showed an increase in PPI values (Table 5.4). The PPI values are positively correlated to frequency of fertility (Freckman & Ettema, 1993). The inorganic fertilised sites may have had more available nutrients during April 2004 than the woodchip-vermicompost sites and may be attributed to the slow release of nutrients from organic fertilisers. The availability of nutrients may therefore explain the higher initial PPI value in the inorganic fertilised sites. The decrease in PPI values at these sites and the increase of Plant-parasitic Index (PPI) values in the woodchip-vermicompost sites may be attributed to an increase in the availability of nutrients released during the decomposition of the woodchip-vermicompost. The decrease in PPI values at the inorganic fertilised sites can also be attributed to the decrease in vegetation cover, which in turn causes a decrease in host plants for plant-parasitic nematodes. The woodchip-vermicompost

sites NR_N, NR_1 and, NR_2 showed a higher overall vegetation cover (Table 5.5) than the inorganic fertilised sites, which could further explain the increase in PPI values at these sites. Higher plant cover increases the available plant material by increasing root growth as well as above ground plant material.

Both the inorganic fertilised sites Old_N and Old_O and the woodchip-vermicompost sites NR_N, NR_1and, NR_2 showed a PPI/MI ratio higher than 1 (Table 5.4). A PPI/MI ratio higher than 1.6 is indicative of nutrient enriched ecosystems, under utilisation of nutrients by plants (Bongers et al., 1997). The woodchip-vermicompost sites showed an increase in the PPI/MI ratio indicating that these sites have become more enriched. This enrichment may be attributed, as mentioned previously, to the slow release of nutrients from organic matter. The decrease in PPI/MI ratio at the inorganic fertilised sites may indicate that nutrient sources at these sites are sufficiently utilised by the vegetation.

The natural veldt showed an increased in PPI and PPI/MI values in November 2004, whereafter these values decrease. The increase in values is due to the increase in the number of plant-parasitic and bacterivorous nematodes. The natural veldt shows a lower PPI and PPI/MI value than the treated sites during April 2004 and April 2005. According to the PPI/MI ratio, the natural veldt can be classified as slightly nutrient enriched. It can be concluded that the addition of fertilisers will result in a decrease in the MI and an increase in the PPI and PPI/MI ratio, due to an equilibrium shift between colonisers and persisters as a result of a high turnover owing to surplus nutrients in the food web (Bongers et al.,1997).

5.4 Conclusion

Fertilisation causes a disturbance of the soil ecosystem, which results in the increase of bacterial activity. Opportunistic enrichment nematodes react to the increase in bacterial activity by increasing in abundance. The increase in abundance of these nematodes causes a decrease in the Maturity Index and an increase in the Plant-parasitic Index and PPI/MI ratio. The addition of woodchip-vermicompost to three of the sites has caused these sites to become more enriched. However, the inorganic

fertilised sites where no organic material was added also showed enrichment. It can therefore be concluded that both the addition of woodchip-vermicompost and inorganic fertilisers to platinum mine tailings will cause the system to become enriched, favouring enrichment opportunists. It would seem that both the woodchip-vermicompost and inorganic fertilised sites were at the same level of recovery, both with surplus nutrients. Further studies need to be conducted to monitor the long-term sustainability of the ecological processes active at the woodchip-vermicompost sites, in order to construct a model of the correlation between the Maturity Index (MI) and Plant-parasitic Index (PPI). Such a model could be used as a predictive tool to estimate the time scale and success of treated platinum mine tailings.

Table 5.1 Nematode taxa present at each site with their specific coloniser-persister values.

		New Tailings Dam					
Trophic groups	**cp-value**	**NR_N**			**Old_N**		
		Apr_04	**Nov_04**	**Apr_05**	**Apr_04**	**Nov_04**	**Apr_05**
Plant parasites							
Paratylenchus spp	3	80.67 ± 74.74	-	-	-	-	-
Helicotylenchus spp	3	460.33 ± 319.28	-	-	2.00 ± 2.00	-	-
Meloidogyne spp	3	7.67 ± 7.67	-	8.33 ± 41.80	-	-	-
Criconema spp	3	69.33 ± 52.81	7.33 ± 7.33	-	-	7.67 ± 7.67	3.67 ± 3.67
Rotylenchus spp	3	-	-	-	1.33 ± 1.33	-	-
Mesodorylaimus spp	4	-	8.00 ± 8.00	-	2.00 ± 2.00	-	-
Trichodorus spp	4	-	-	-	-	-	-
Tylenchorhynchus	2	-	-	-	-	-	4.00 ± 4.00
Anguina spp	2	-	-	-	-	-	-
Hoplolaimidae spp	3	-	50.00 ± 25.24	108.00 ± 15.53	-	-	58.00 ± 8.00
Dorylaimida spp	4	-	-	25.00 ± 12.50	-	-	-
Fungal feeders							
Tylenchus spp	2	-	23.33 ± 23.33	-	0.33 ± 0.33	-	-
Aphelenchus spp	2	-	-	-	-	-	-
Paraphelenchus spp	3	-	-	-	-	-	-
Bacterial feeders							
Plectus spp	2	15.33 ± 15.33	-	-	57.33 ± 57.33	-	-
Rhabditidae	1	57.67 ± 41.83	483.00 ± 228.65	233.67 ± 61.33	14.00 ± 8.39	288.00 ± 270.02	262.67 ± 206.92
Predators							
Makatinus spp	4	-	-	-	11.33 ± 11.33	-	-

Coloniser-persister (cp) values as ascribed to each taxa by Bongers (1990).

Values of each specific taxon at each site given as mean ± SEM.

Table 5.2 Nematode taxa present at each site with their specific coloniser-persister values.

		Old Tailings Dam					
Trophic groups	**cp-value**	**NR_1**			**NR_2**		
		Apr_04	**Nov_04**	**Apr_05**	**Apr_04**	**Nov_04**	**Apr_05**
Plant parasites							
Paratylenchus spp	3	61.67 ±10.27	7.67 ± 7.67	-	-	-	29.67 ± 29.67
Helicotylenchus spp	3	21.67 ± 10.93	-	-	61.67 ± 55.29	-	-
Meloidogyne spp	3	-	-	-	0.33 ± 0.33	-	-
Criconema spp	3	-	30.00 ± 8.08	21.00 ± 21.00	-	4.00 ± 4.00	-
Rotylenchus spp	3	33.67 ± 17.70	-	-	-	-	-
Mesodorylaimus	4	-	-	-	-	19.33 ± 19.33	-
Trichodorus spp	4	-	-	-	-	-	-
Tylenchorhynchus	2	7.67 ± 7.67	-	245.33 ± 185.92	-	-	-
Anguina spp	2	-	-	-	-	2.00 ± 2.00	-
Hoplolaimidae spp	3	-	23.33 ± 13.57	72.00 ± 58.27	-	8.00 ± 8.00	12.00 ± 7.23
Dorylaimida spp	4	-	-	4.00 ± 4.00	-	-	3.33 ± 3.33
Fungal feeders							
Tylenchus spp	2	-	-	-	10.00 ± 10.00	-	-
Aphelenchus spp	2	-	8.00 ± 8.00	-	-	-	-
Paraphelenchus spp	3	-	3.67 ± 3.67	-	-	7.67 ± 7.67	-
Bacterial feeders							
Plectus spp	2	30.67 ± 30.67	-	-	-	-	-
Rhabditidae	1	22.67 ± 11.33	744.00 ± 80.21	326.67 ± 164.83	65.33 ± 26.81	156.33 ± 59.50	291.67 ± 112.42
Predators							
Makatinus spp	4	-	-	-	-	-	-

Coloniser-persister (cp) values as ascribed to each taxa by Bongers (1990).

Values of each specific taxon at each site given as mean ± SEM.

Table 5.3 Nematode taxa present at each site with their specific coloniser-persister values.

		Old Tailings Dam			Natural veldt		
Trophic groups	**cp-value**	**Old_O**			**Nat**		
		Apr_04	**Nov_04**	**Apr_05**	**Apr_04**	**Nov_04**	**Apr_05**
Plant parasites							
Partylenchus spp	3	4.33 ± 4.33	-	-	-	-	-
Helicotylenchus spp	3	188.00 ± 10.02	-	-	-	-	-
Meloidogyne spp	3	11.67 ± 11.67	3.33 ± 3.33	-	-	4.00 ± 4.00	-
Criconema spp	3	64.33 ± 61.36	16.00 ± 16.00	54.67 ± 25.18	-	3.67 ± 3.67	4.33 ± 4.33
Rotylenchus spp	3	-	-	-	-		-
Mesodorylaimus spp	4	19.33 ± 13.78	-	-	-	28.00 ± 24.98	-
Trichodorus spp	4	-	-	-	-	-	-
Tylenchorhynchus	2	0.67 ± 0.67	1.00 ± 0.58	99.00 ± 49.51	-	-	-
Anguina spp	2	-	1.67 ± 1.67	-	-	-	-
Hoplolaimidae spp	3	-	31.33 ± 25.57	154.00 ± 86.16	-	71.00 ± 6.93	-
Dorylaimida spp	4	-	-	-	-	28.00 ± 28.00	-
Fungal feeders							
Tylenchus spp	2	-	-	-	11.67 ± 6.64	-	-
Aphelenchus spp	2	-	-	-	-	-	-
Paraphelenchus spp	3	-	-	-	-	-	-
Bacterial feeders							
Plectus spp	2	75.00 ± 75.00	-	-	-	-	-
Rhabditidae	1	61.33 ± 40.57	120.00 ± 60.40	754.33 ± 86.05	91.33 ± 42.86	264.00 ± 99.29	36.00 ± 8.14
Predators							
Makatinus spp	*4*	-	-	-	-	-	-

Coloniser-persister (cp) values as ascribed to each taxa by Bongers (1990).

Values of each specific taxon at each site given as mean ± SEM.

Table 5.4. Calculated indexes for each treatment on each tailings dam.

		Tailings dam		Tailings dump			Natural veldt
Apr_04		**NR_N**	**Old_N**	**NR_1**	**NR_2**	**Old_O**	**Nat**
	PPI	2.68	3.29	2.94	2.86	3.07	2.00
	MI	1.21	0.72	1.58	1.00	1.55	1.00
	MMI	2.81	2.52	2.53	1.98	2.58	1.11
	PPI/MI	2.22	4.56	1.87	2.86	1.98	2.00
Nov_04							
	PPI	2.91	3.00	2.55	3.42	2.95	3.41
	MI	1.00	1.00	1.00	1.00	1.00	1.00
	MMI	1.67	1.05	1.17	1.50	1.60	1.82
	PPI/MI	2.91	3.00	2.55	3.42	2.95	3.41
Apr_05							
	PPI	3.19	2.94	2.30	3.07	2.68	1.00
	MI	1.00	1.00	1.00	1.00	1.00	1.00
	MMI	1.68	1.39	1.66	1.28	1.49	1.21
	PPI/Mi	3.19	2.94	2.30	3.07	2.68	1.00

PPI: Plant-parasitic Index, calculated for the plant-parasitic nematodes

MI: Maturity Index contains all free living nematodes and excludes all plant-parasitic nematodes

MMI: Modified Maturity Index includes both plant-parasitic and free living nematodes

PPI/MI: Plant-parasitic to Maturity Index ratio.

Table 5.5 Crown cover of vegetation for each site.

	New Tailings Dam				Old Tailings Dam					
	NR_N		Old_N		NR_1		NR_2		Old_O	
	Apr_04	**Apr_05**	**Apr_04**	**Apr_05**	**Apr_04**	**Apr_05**	**Apr_04**	**Apr_05**	**Apr_04**	**Apr_05**
Vegetation Cover (%)	64	64	34	26	72	55	66	53	38	23
Bare ground (%)	37	37	66	74	28	45	34	47	62	78

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CHAPTER 6

SOIL INVERTEBRATES (MICRO-ARTHROPODS) AS INDICATOR OF ECOSYSTEM DEVELOPMENT DURING PLATINUM MINE TAILINGS REHABILITATION

ABSTRACT

The use of woodchip-vermicompost as an alternative amendment to inorganic fertilisers during the rehabilitation of platinum mine tailings, was evaluated using micro-arthropod community composition. All four Acari groups namely, Prostigmata, Cryptostigmata, Mesostigmata and Astigmata were present in the woodchip-vermicompost sites, as well as other micro-arthropods with Collembola being abundant. Prostigmata dominated in the inorganic fertilised sites, whereas little or no of the other micro-arthropod groups occurred on these sites. The woodchip-vermicompost sites showed a higher abundance of Acari families and mites present, with the bacterivorous and nematovorous mites dominating these sites. The presence of all Acari groups, as well as other micro-arthropods indicated that the addition of the woodchip-vermicompost might have contributed to the acceleration of the colonisation of these groups, by providing sufficient microhabitats for the establishment of sufficient prey such as bacteria and nematodes that typically increase in numbers after the addition of organic material. Fluctuation in family composition and number of mites present in the wood chip-vermicompost sites was observed from April 2004 to April 2005, with the family composition and number of mites in April 2005, relatively returning to that observed during the April 2004. The inorganic fertilised sites showed a decrease in micro-arthropod families and species during the study period, with no indication of recovery. Therefore, the woodchip-vermicompost sites seemed to be a more functional ecosystem in terms of both r-strategist and K-strategist being present in these sites whereas the inorganic fertilised sites seemed to be a less preferred microhabitat for the establishment of predatory micro-arthropods of the K-strategists group.

Acari groups, Collembola, Inorganic fertiliser, Platinum mine tailings, Rehabilitation, Woodchip-vermicompost

6.1 Introduction

Mining activities cause a great deal of disturbance to the surrounding environment due to the depositing of mine spoil in this areas (Wong, 2003). One of the main aims of rehabilitating these mine tailings is to create a suitable environment to stimulate biological activity, which contributes to the soil formation process and establishment of a self-sustaining vegetation cover on mine tailings (Wanner & Dunger, 2002). These newly created platinum mine tailings environments may be colonised by soil biota and plants depending on the environmental conditions, including soil physical and chemical properties and microclimates (Koehler, 2000). Succession starts shortly after disturbance with species diversity being low at the beginning, while specialised pioneer species show a high abundance (Koehler, 1999). Diversity can be viewed as a key component of ecosystem resilience (Bengtsson, 2002), since the occurrence of diversity among the soil mesofauna indicates that species are available for the re-organisation of ecosystem function as conditions change (Bengtsson, 1998).

Soil mesofauna are abundant and play a well-recognised role in the ecosystem (Coleman et al., 2004). Their life history portrays the condition of the soil ecosystem, with several species being recognised as potential indicators of soil quality (Parisi et al., 2005). Soil mesofauna community composition changes during the rehabilitation process as well as with age of the rehabilitated sites (Kumssa et al., 2004). Relative few studies have considered using micro-arthropods as indicators of ecosystem recovery during the rehabilitation of mine areas regardless of the important role they play (Koehler, 2000). Micro-arthropods are an important linkage in the food web and play an important role in the decomposition. The Acariformes of the micro-arthropods are related to spiders and are the most abundant micro-arthropods in the soil (Coleman et al., 2004). Micro-arthropods can be divided into Acariformes, which includes the Astigmata, Prostigmata and Cryptostigmata as well as the Parasitiformes, consisting of Mesostigmata (Koehler, 1997). These suborders play important roles in ecosystems and may be indicative of certain ecosystem conditions, i.e. Cryptostigmata that contribute to the comminuting of organic matter and distribution of bacteria and fungi (Behan-Pelletier, 1999) and are

associated with nitrogen rich sources. Prostigmata contain a wide variety of feeding groups and may grow rapidly in population size (Coleman et al., 2004). Mesostigmata are indicative of environments with decaying organic material containing abundant nematodes and are predominantly predators, which feed on small soil fauna (Koehler, 1997). Other mesofauna playing a role in the ecosystem includes Collembola, which has been found to contribute to mobilisation of nitrogen (Mebes & Filser, 1998) and Enchytraeids found to have a positive effect on the mineralisation of both inorganic and organic nitrogen (N) and phosphorous (P) (Briones et al., 1998).

The aim of this study was to use soil mesofauna as indicators to evaluate the effectiveness of woodchip-vermicompost as rehabilitation method over the use of inorganic fertilisers, in establishment of vegetation cover on platinum mine tailings dams in a semi-arid region of the North-West province of South Africa.

6.2 Materials and Methods

6.2.1 Experimental design

A total of five experimental sites were identified on two tailings dams on the premises of Impala Platinum mine, Rustenburg, South Africa. Three sites were located on an old tailings dam, which has been dormant since 1976. Two of the three sites measuring 30m x 10m in size, were treated with the woodchip-vermicompost, while the other site, of the same size, was treated with inorganic fertilisers. Two sites measuring 30m x 30m in size were located on the second tailings dam, which was still under production. One of the two sites was treated with the woodchip-vermicompost, while the other site was treated with inorganic fertilisers. The platinum mine tailings were previously analysed to determine the fertilisation rate for optimum growth conditions. Woodchip-vermicompost was produced out of woodchips obtained from mine blasting, separated from waste rock during the screening process and mixed with sewage sludge and water (Van Rensburg & Morgenthal, 2004). The woodchip mixture was decomposed using the earthworm species *Eisenia fetida*. A total of 50 tons per hectare was manually applied to the tailings and

worked in to a depth of 30 cm. The aspects of all experimental sites were in a northerly direction. Sites were sown with a mixture of 12 different grass species. Sites were not irrigated and were subjected to rainfall. Sites were surveyed during April 2004, November 2004 and, April 2005 during which the experimental site received an average of 474.4 mm of rain. A natural veldt in the vicinity of the mine was selected to serve as a reference area as no suitable rehabilitated platinum mine tailings dam could be obtained to serve as reference area.

6.2.2 Soil sampling

Before soil was sampled, the organic matter was removed from the soil surface. Soil samples were then obtained from the first 10 – 15 cm of the soil surface. Three samples were taken for each sample site (n = 18). Soil samples were handled carefully to avoid unnecessary pressure that might lead to crushing of soil organisms (Koehler, 1997). The extraction of the soil samples took place within days of the actual sampling.

6.2.3 Vegetation cover

Vegetation cover was expressed as the percentage vegetation cover to bare ground. A total of twenty $1m^2$ quadrants were placed at random locations on each site. The mean representative value of the vegetation cover and bare ground of the twenty quadrants served as the percentage vegetation cover of the whole site. (Kent & Coker, 1997).

6.2.4 Analyses

6.2.4.1 Extraction of soil invertebrates

Soil micro-arthropods were extracted using a modified Berlese-Tullgren funnel as described in Krantz (1978). The soil core was spread out on a sieve (2 mm) fitted over a funnel. The funnel with the soil core was left undisturbed for a period of 7 days. A 60 W incandescent bulb was use as heating source at the top of the funnel. The increase in brightness and temperature causes the micro-arthropods to move to the bottom of the soil

core. Soil animals fell via the funnel into a container containing 75% ethanol. The content of the containers were sorted and identified under a stereoscope.

6.2.5 Statistical analyses

Statistical analysis was preformed on all data sets using STATISTICA 7 (StatSoft, Inc ©). Parametric and non-parametric statistical analyses were performed on all data sets. The Shapiro-Wilk's test was used to test for normality. In the case of data being parametric (normally distributed), a one-way breakdown ANOVA was performed. If data were found to be non-parametric, non-parametric data analyses were preformed and the Kruskal-Wallis ANOVA and Median test was used to determine statistical significant differences. If no difference was observed between the parametric and non-parametric analyses, parametric analyses were used to discuss relevant statistics (Claassens et al., 2005).

6.3 Results and Discussion

6.3.1 Experimental sites

6.3.1.1 Vegetation cover

A summary of the percentage vegetation cover to bare ground obtained during the study is presented in Table 6.1. Higher vegetation to bare ground cover was observed in the woodchip-vermicompost sites than in the sites treated with inorganic fertilisers. Vegetation cover to bare ground ratios of the woodchip-vermicompost sites were as follows: NR_N (64%:37%), NR_1 (72%:28%) and NR_2 (66%:34%), whereas the inorganic fertilised sites had a vegetation cover of 34%:66% for Old_N and 38%:62% for Old_O. A decrease in vegetation cover was observed at all sites except in NR_N. The decrease in vegetation cover may be attributed to the decrease in rainfall received from the first growth season during November 2003 to February 2004, which received 719.1 mm of rain to a decrease of 413.5 mm received during November 2004 to February 2005. Species that might have contributed to the higher vegetation cover at the woodchip-vermicompost sites were all part of the sown-in grass seed mixture and include the

perennial species *Cenchrus ciliaris* (L.) var. *ciliaris*, *Chloris gayana* Kunth, *Melinis repens* (Wild) Zizka and *Heteropogon contortus* (L.) Beauv and the stoloniferous species *Cynodon dactylon* (L.) Pers., *Digitaria eriantha* Steud.

6.3.1.2 Micro-arthropod community structure

Mesofauna consists of nematodes that feed on microorganisms as well as the litter transformers such as micro-arthropods, enchytraeids and other small arthropods, which, contributes to cycling of essential nutrients and soil organic decomposition (Ekschmitt et al., 2005). The free-living mites (Acari) of the micro-arthropods represented the majority of the mesofauna population in both treatments. A summary of the mesofauna observed in the different treatments during the study is presented in Tables 6.2.1. to 6.2.3. The Prostigmata group of the Acari were the most abundant with the most families present. Prostigmata mites consist of a large group of predators and can be classified as r-strategists (Coleman et al., 2004; Koehler, 1998). Agroecosystem activities such as drainage, irrigation and the application of fertilisers have been shown to increase the reproduction of Prostigmata (Behan-Pelletier, 1999). Twenty-one families of Prostigmata were identified and none of these occurred at all treatments. Eight of the twenty-one families occurred in the woodchip-vermicompost site NR_N, while the woodchip-vermicompost sites NR_1 and NR_2, had seven and four families present during April 2004, which may be attributed to the addition of the woodchip-vermicompost. Tydeidae was abundant in both the woodchip-vermicompost sites NR_N and NR_2, while Pygmephoridae occurred in all woodchip-vermicompost sites and was the most abundant Prostigmata family in these sites during April 2004 (Table 6.2.1. to 6.2.3.). The family, Tydeidae plays an important role in regulating nematode populations (Whitford & Santos, 1980). *Coccotydeus* was the dominant species in the family of Tydeidae, with *Bakerdania* being the dominant species of the family Pygmephoridae. Four families of Prostigmata occurred in the inorganic fertilised sites (Old_N and Old_O) during April 2004. Tydeidae and Nanorchestidae were the most abundant of the four families in Old_N and Old_O, respectively. Nanorchestidae belongs to the fungivorous mites (Coleman et al., 2004), with *Speleorchestes* being the dominant species in this family.

Nematodes are fast colonisers, especially bacterivorous nematodes and are quickly followed by their predators (Bulluck et al., 2002). The addition of the woodchip-vermicompost may have provided an ideal habitat for the establishment of a sufficient food source of nematodes, therefore contributing to the increase in nematodes and nematode predators in these sites. From April 2004 to November 2004, a decrease in the number of Prostigmata families as well as number of mites present was observed in both treatments. The woodchip-vermicompost site NR_N showed a decrease in the number of Prostigmata mites and families, with only five families observed. The dominant Prostigmata family shifted from Tydeidae (nematovorous mites) to Eupodidae (fungivorous mites) in November 2004, with *Eupodes* spp. being the dominant species of the family Eupodidae. Eupodidae are general opportunist with a fast reproduction rate and reproduce rapidly after disturbance or when a sudden shift in resource availability occurs (Coleman et al., 2004). The woodchip-vermicompost sites NR_1 and NR_2 showed a decrease in number of Prostigmata mites as well as number of families with only three species observed within these sites during November 2004. Eupodidae also dominated in NR_1, while the nematovorous mites, Tydeidae still dominated in NR_2. The abundance of Eupodidae in NR_N and NR_1 may indicate a change in resource availability in these sites, which may have favoured fungal communities and therefore may have contributed to the increase in these fungivorous mites. Nearly no Prostigmata mites were observed in the inorganic fertilised site Old_N during November 2004, indicating lower occurrence of nutrient resources for the micro-arthropods, causing the decrease in Prostigmata. In the inorganic fertilised site Old_O, four families of Prostigmata mites was observed with Tydeidae being dominant during November 2004. During the last sampling period in April 2005, an increase in the number of Prostigmata families but a decrease in number of Prostigmata mites was observed in the woodchip-vermicompost sites. The woodchip-vermicompost site NR_N was once again dominated by the nematovorous mites, Tydeidae, (*Coccotydeus* spp.), but showed a considerable decrease in the number of mites from April 2004 to April 2005. Both the woodchip-vermicompost sites NR_1 and NR_2 showed a decrease in number of Prostigmata mites from April 2004 to April 2005, with Tydeidae dominating the Prostigmata families in

April 2005. A slight increase in the number of Prostigmata families was observed in the inorganic fertilised site Old_N, but the number of mites was still considerably low. The slow colonisation of this site may be attributed to plant litter contributing to organic material in this site, which may have lead to the slow introduction of suitable prey for these Prostigmata mites. Nanorchestidae (*Speleorchestes* spp.), dominated the two families of Prostigmata observed during April 2005 in the Old_O.

The Acari group, Cryptostigmata (Oribatida), was the second most abundant group in the treated sites, with twelve families identified, which was especially dominant in the woodchip-vermicompost sites. Cryptostigmata differ from other mite groups by having sclerotised, often calcareous exoskeletons (Coleman et al., 2004). They play an active role in the decomposition of organic materials, as well as in the dispersal of bacteria and fungi within the soil ecosystem and can be classified as K-strategists (Behan-Pelletier, 1999). The recovery of microbial communities and the resilience of the decomposer subsystem can be accelerated by the presence of Cryptostigmata (Maraun et al., 1998). Low numbers and diversity of these Acari group may indicate unfavourable soil condition, due to low vegetation biomass production potential (Minor et al., 2004). Five families of Cryptostigmata were observed in the woodchip-vermicompost site NR_N, while four families were observed in NR_1 and NR_2, during April 2004. Oridatulidae was the most abundant family in all the woodchip-vermicompost sites during this period, with *Scheloribates* being the dominant species. Hypochthoniidae was the only family observed in the inorganic fertilised site Old_N, whereas no Cryptostigmata families were observed in Old_O during April 2004. Oridatulidae are part of the secondary decomposers and feed predominantly on fungi, while Hypochthoniidae can be classified as carnivores, scavengers and omnivores, feeding on living and dead Collembola, nematodes and fungi (Schmeider et al., 2004). It can therefore be expected that the woodchip-vermicompost have entered the stage of secondary decomposition, since Cryptostigmata contributes to the acceleration of the decomposition process. An increase in Cryptostigmata mites was observed in NR_N, while it decreased in NR_1 and NR_2 from April 2004 to November 2004. No Cryptostigmata mites were observed in NR_2

during this period. Brachychthoniidae was the dominant family in both NR_N and NR_1 and is an indicator of recent disturbance (Behan-Pelletier, 1999). The indication of recent disturbance in these sites may be attributed to a shift in resource availability, since, Eupodidae (Prostigmata), which typically increased after disturbance as well as with a shift in resource availability, also showed an increase during this period. No Cryptostigmata mites were observed in the inorganic fertilised sites for the remainder of the study. Oridatulidae dominated in NR_N during April 2005, with a higher number of mites than that observed in the first sampling period in April 2004. The woodchip-vermicompost sites NR_1 and NR_2 showed a recovery in Cryptostigmata mites from November 2004, but numbers was still lower than that observed during April 2004. Four families of Cryptostigmata were observed in NR_1 and NR_2, with Brachychthoniidae and Oridatulidae dominating. The occurrence of Cryptostigmata on the woodchip-vermicompost sites may therefore indicate that the vegetation established on the sites may have a higher biomass production potential than the inorganic fertilised sites.

Mesostigmata was the third group of Acari observed. This group contains less soil dwellers than the Prostigmata and Cryptostigmata groups and are all predators (Coleman et al., 2004). Six families of Mesostigmata were identified in this study. Uropodina and Gamasina form part of the Parasitiformes of the group Mesostigmata (Koehler, 1999). The subgroup Uropodina typically occurs in areas, which have been composted, manured, or treated with old sewage sludge. Their distribution is also specific to the habitat quality (Koehler, 1997). High numbers of Gamasina occurs in disturbed ecosystems or in ecosystems in early stages of succession (Koehler & Weidemann, 1995). Ascidae and Parasitidae (Gamasina) typically hunts on the soil surface for Collembola, while Macrochelidae prefers compost and feeds on insect eggs. Another family, the Rhodacaridae are soil dwellers, feeding on nematodes and are dependent on the density of the prey in an area. They are also capable to survive disturbance and are found in sandy soils with low organic material (Koehler, 1997). Mesostigmata increase in ecosystems where r-strategists show a decrease due to a decrease in suitable food sources and can therefore be classified as K-strategists (Koehler, 1998). Three of the six families

of Mesostigmata mites were observed in NR_N, while NR_1 and NR_2 had four each. No Mesostigmata families were observed in the inorganic fertilised sites, which may indicate that these sites have not yet reached early stages of succession. Rhodacaridae was the dominant family in NR_N, while Laelapidae dominated in both NR_1 and NR_2 during April 2004. A decrease in organic material may therefore be expected in NR_N, since Rhodacaridae typically occurs in sandy environments with low organic material. The families of Mesostigmata mites decreased in the woodchip-vermicompost sites from April 2004 to November 2004, with Uropodidae being the only family observed in NR_N during this period, while no families were observed in NR_1 and NR_2. The decrease in Mesostigmata families in the woodchip-vermicompost sites may have indicated a decrease in organic material since they are typically associated with decaying organic materials. Three families of Mesostigmata mites were observed in NR_N during April 2005, with Rhodacaridae once again dominating, indicating a further possible decrease in organic material. Laelapidae was the only family observed in NR_1, whereas no families were observed in NR_2. Laelapidae are voracious predators and prey on red spider mites and false spider mites (Coleman et al., 2004). An increase in these mites within NR_1 might have cause the increase of the Laelapidae family. Overall, the occurrence of the Mesostigmata mites in the woodchip-vermicompost sites may be attributed to the addition of the organic matter and the higher vegetation cover, which stimulated the increase in coloniser species on which Mesostigmata preys. The decrease in the availability of this organic matter may therefore have lead to the decrease in Mesostigmata mites and the occurrences of the K-strategist Mesostigmata mite, Rhodoacaridae.

Astigmata are the least common of the Acari, favouring ecosystems high in moisture and organic material and typically feeds on bacteria (Coleman et al., 2004). This group are predominantly obligated parasites of birds and vertebrates and only contain a small group of soil dwellers, which typically inhabit grasslands (Behan-Pelletier, 1999). Only two families of Astigmata were observed during the study, with low numbers present in the treated sites. Acaridae dominated both NR_N and NR_1 during the study, while

Pediculochelidae only occurred in NR_2 during November 2004. The presence of these Astigmata mites may be attributed to the added woodchip-vermicompost, which stimulated the increase in microbes, on which the Astigmata mites feed. The woodchip-vermicompost may further have attributed to some retention of soil moisture in these sites.

Other micro-arthropods, observed include Araneae, Collembola, Hemiptera, Thysanoptera and Coleoptera, which contributed to the smallest part of the mesofaunal community. Of these groups, the Collembola was the most abundant on the treated sites, especially on the woodchip-vermicompost sites. Collembola feeds on soil microorganisms and are therefore expected to occur in sites with high occurrence of microorganisms (Axelsen & Kristensen, 2000). The woodchip-vermicompost contributed to the increase in microorganisms, thus the increase in Collembola could be attributed to the addition of the woodchip-vermicompost. In a study conducted by Filser (1995), a considerable high abundance of Collembola was found in systems treated with green manure, while systems treated with mineral fertilisers showed considerable lower abundance of Collembola. This was also observed in this study, as the woodchip-vermicompost sites showed higher abundance of Collembola than the sites treated with inorganic fertilisers.

6.3.2 Reference area

6.3.2.1 Micro-arthropod community structure

The natural veldt showed a higher abundant of Acari present than the treated sites. This was expected since higher micro-arthropod densities are often observed within native ecosystems, especially during the early growing season accompanied by an increase in nitrogen availability to plants (Doles et al., 2001). Seventeen of the twenty-one Prostigmata groups were observed in the natural veldt. The family Tydeidae was the most abundant family of Prostigmata mites present during April 2004 to April 2005, with an increase in this family observed in November 2004. The families of Prostigmata mites

decreased from seventeen families in April 2004 to eight families in November 2004. Eight of the twelve Cryptostigmata mites were observed during the study in this site, with Oridatulidae dominating in April 2004 and again in April 2005, whereas Cosmochthoniidae dominated in November 2004. An increase in the number of Cryptostigmata families was observed from April 2004 to November 2004. The family Rhodacaridae dominated the Mesostigmata during April 2004 and November 2004. In April 2005, Ascidae dominated the Mesostigmata families. A decrease in the abundance of Mesostigmata mites were observed from April 2004 to April 2005, with exception of Ascidae, which showed an increase. The other mesofaunal groups observed also showed considerable higher abundance than that observed in the treated sites. Although the woodchip-vermicompost sites showed lower abundance of mites present in their ecosystems, it followed the same patrons of dominant families as that observed in the natural veldt.

6.4 Conclusion

The woodchip-vermicompost sites contained all four groups of Acari and might therefore indicate that the addition of the woodchip-vermicompost has stimulated the faster colonisation of the different Acari groups. The above ground vegetation affects the abundance of mesofauna as well as the diversity (Koehler, 1997). The higher vegetation cover on the woodchip-vermicompost sites may have contributed to the establishment of micro-arthropods by providing specific microhabitats, which favours the establishment of microfauna and thus increasing the availability of food for mesofauna (Meuller et al., 1990). The decrease in Acari observed in the woodchip-vermicompost sites NR_1 and NR_2 from April 2004 to November 2004, may be attributed to the burning of vegetation, causing a decrease in microhabitats and so a decrease in available food sources. Inorganic fertilised sites contained a relative lower vegetation cover than the woodchip-vermicompost sites. The lower vegetation cover may have contributed to less favourable micro-condition in these sites. The low abundance of Acari on these sites may also be attributed to lower microfaunal activity, which indicates a lower occurrence of food source for the Acari. It can therefore be concluded that the addition of woodchip-

vermicompost may have attributed to the faster colonisation of mesofaunal groups of different trophic groups, by creating a more favourable micro-habitat. The inorganic fertilised sites were dominated by the presence of opportunistic mites, which may indicate that these sites do not have a sufficient trophic structure, which may lead to lower quality of soil cycling processes. Therefore, the woodchip-vermicompost sites in terms of micro-arthropod communities, seems to develop toward the micro-arthropod communities observed in the reference site. However, long -term monitoring is a necessity to determine the effects of the woodchip-vermicompost in providing a micro-habitat with sufficient nutrient supply to sustain the micro-arthropods in these systems.

Table 6.1. Summary of vegetation crown cover of the different treatments.

	New Tailings Dam				Old Tailings Dam					
	NR_N		Old_N		NR_1		NR_2		Old_O	
	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05	Apr_04	Apr_05
Vegetation Cover (%)	64	64	34	26	72	55	66	53	38	23
Bare ground (%)	37	37	66	74	28	45	34	47	62	78

Table 6.2.1. Mesofaunal community composition

	New Tailings Dam					
	NR_N			Old_N		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Prostigmata						
Nanorchestidae	26.00 ± 11.68	-	4.67 ± 4.17	11.33 ± 6.12	-	-
Pachygnathidae	-	-	-	-	-	-
Nematalycidae	-	-	-	2.67 ± 2.19	-	-
Eupodidae	15.33 ± 7.26	76.33 ± 22.36	3.33 ± 2.40	-	-	1.00 ± 1.00
Rhagidiidae	-	-	-	-	-	-
Paratydeidae	-	-	-	-	-	-
Tydeidae	153.33 ± 86.32	46.67 ± 24.76	34.67 ± 26.36	65.33 ± 33.39	-	2.00 ± 2.00
Tarsonemidae	-	-	-	-	-	-
Scutacaridae	-	-	-	-	-	-
Pygmephoridae	124.33 ± 67.87	-	1.67 ± 0.33	8.67 ± 8.67	-	-
Cunaxidae	19.33 ± 15.51	5.33 ± 5.33	5.67 ± 5.67	-	-	-
Bdellidae	6.67 ± 4.70	4.33 ± 4.33	10.67 ± 5.61	-	-	-
Tetranychidae	-	-	-	-	-	-
Stigmaeidae	0.67 ± 067	-	3.33 ± 3.33	-	-	-
Caeculidae	-	-	-	-	-	-
Cheyletidae	-	-	-	-	-	-
Pseudochelidae	-	1.00 ± 1.00	1.00 ± 1.00	-	-	-
Anystidae	-	-	-	-	-	-
Trombidiidae	-	-	-	-	-	-
Erythraeidae	0.33 ± 0.33	-	-	-	0.33 ± 0.33	-
Barbutidae	-	-	-	-	-	1.33 ± 1.33
Mesostigmata						
Laelapidae	16.67 ± 16.67	-	6.67 ± 4.06	-	-	-
Macrorchelidae	-	-	-	-	-	-
Ascidae	2.00 ± 2.00	-	1.00 ± 1.00	-	-	-
Rhodacaridae	82.00 ± 68.48	-	62.00 ± 59.02	-	-	-
Uropodidae	-	4.33 ± 4.33	-	-	-	-
Phytoseiitdae	-	-	-	-	-	-

Table 6.2.1. Mesofaunal community composition

Cryptostigmata				-		
Hypochthoniidae	-	2.00 ± 2.00	-	70.00 ± 70.00	-	-
Brachychthoniidae	16.33 ± 15.34	40.00 ± 40.00	26.67 ± 21.69	-	-	-
Cosmochthoniidae	0.33 ± 0.33	2.33 ± 2.33	2.33 ± 2.33	-	-	-
Nothridae	0.33 ± 0.33	1.00 ± 1.00	-	-	-	-
Camisiidae	-	-	0.33 ± 0.33	-	-	-
Gymnodamaeidae	-	-	-	-	-	-
Oppiidae	-	2.00 ± 2.00	4.33 ± 4.33	-	-	-
Oripodidae	-	-	-	-	-	-
Oridatulidae	46.00 ± 24.02	27.67 ± 27.67	52.67 ± 50.17	-	-	-
Galumnidae	19.67 ± 9.87	-	2.33 ± 2.33	-	-	-
Phthiracadidae	-	-	-	-	-	-
Epilohmanhidae	-	-	-	-	-	-
Astigmata						
Acaridae	0.33 ± 0.33	3.33 ± 3.33	-	-	-	1.00 ± 1.00
Pediculochelidae	-	-	-	-	-	-
ARANEAE						
Lycosidae	-	-	-	-	-	-
Gnaphosidae	-	-	-	-	-	-
Salticidae	0.33 ± 0.33	-	0.33 ± 0.33	-	-	-
COLLEMBOLA						
Entomobryidae	9.00 ± 7.09	-	-	-	1.33 ± 1.33	-
Tomoceridae	4.67 ± 3.29	-	-	-	-	-
Isotomidae	92.67 ± 66.99	-	5.67 ± 3.87	-	-	-
Poduridae	-	17.67 ± 10.39	5.67 ± 5.67	0.67 ± 0.67	-	-
Sminthuridae	-	103.33 ± 16.41	1.33 ± 1.33	-	-	-
HEMIPTERA						
Aphididae	-	-	-	-	-	-
Coreidae	-	-	-	-	-	-
Coccidae	0.33 ± 0.33	-	-	-	-	-
THYSANOPTERA						
Thripidae	3.00 ± 3.00	-	1.33 ± 1.33	0.67 ± 0.67	-	-
Aelothripidae	-	-	-	-	-	-

Table 6.2.1. Mesofaunal community composition

COLEOPTERA						
Staphylinidae	-	1.33 ± 0.67	-	-	-	-
Pselaphidae		-	-	-	-	-
Elateridae (larwes)	4.00 ± 1.00	1.67 ± 0.88	0.33 ± 0.33	0.33 ± 0.33	-	-
Carabidae	-	-	-	-	-	-
Scarabidae	-	-	-	-	-	-
Tingidae	-	-	-	-	-	-
Tenebrionidae	-	-	-	-	-	-
Bostrichidae	-	-	-	-	-	-

Values given as mean ± SEM

Table 6.2.2. Mesofaunal community composition

	Old Tailings Dam					
	NR 1			NR 2		
	Apr 04	Nov 04	Apr 05	Apr 04	Nov 04	Apr 05
Prostigmata						
Nanorchestidae	6.33 ± 2.03	-	2.33 ± 2.33	29.33 ± 7.84	-	3.67 ± 2.72
Pachygnathidae	-	-	-	-	-	-
Nematalycidae	-	-	-	-	-	-
Eupodidae	2.00 ±2.00	10.00 ± 10.00	0.67 ± 0.67	56.67 ± 56.67	13.33 ± 13.33	0.67 ± 0.67
Rhagidiidae	-	-	-	-	-	-
Paratydeidae	-	-	-	-	-	-
Tydeidae	3.00 ± 0.32	-	3.33 ± 0.33	95.67 ± 89.67	22.67 ± 12.80	11.67 ± 9.67
Tarsonemidae	-	-	-	-	-	2.33 ± 2.33
Scutacaridae	-	-	-	-	-	-
Pygmephoridae	75.33 ± 62.78	-	2.00 ± 0.58	316.00 ± 125.80	-	4.00 ± 1.73
Cunaxidae	-	1.00 ± 1.00	-	1.00 ± 1.00	1.00 ± 1.00	-
Bdellidae	-	2.33 ± 1.45	-	2.67 ± 2.67	-	-
Tetranychidae	-	-	-	-	-	1.00 ± 1.00
Stigmaeidae	-	-	-	-	-	-
Caeculidae	-	-	-	-	-	-
Cheyletidae	-	-	-	-	-	-
Pseudochelidae	-	-	-	-	-	-
Anystidae	-	-	-	-	-	-
Trombidiidae	-	-	-	-	-	-
Erythraeidae	-	-	0.33 ± 0.33	0.67 ± 0.67	-	-
Barbutidae	-	-	-	-	-	-
Mesostigmata						
Laelapidae	17.33 ± 11.46	-	1.00 ± 1.00	87.00 ± 40.45	-	-
Macrorchelidae	-	-	-	-	-	-
Ascidae	2.67 ± 1.67	-	-	2.33 ± 2.33	-	-
Rhodacaridae	3.67 ± 2.73	-	-	19.00 ± 17.04	-	-
Uropodidae	0.33 ± 0.33	-	-	-	-	-
Phytoseiitdae	-	-	-	1.33 ± 1.33	-	-

Table 6.2.2. Mesofaunal community composition

Cryptostigmata				-		
Hypochthoniidae	-	-	-	-	-	-
Brachychthoniidae	5.00 ± 2.08	23.33 ± 12.02	1.67 ± 0.88	74.33 ± 58.21	-	7.67 ± 7.17
Cosmochthoniidae	3.67 ± 2.73	-	-	90.67 ± 80.39	-	-
Nothridae	-	-	-	-	-	-
Camisiidae	7.00 ± 4.73	-	-	-	-	1.00 ± 1.00
Gymnodamaeidae	-	-	-	3.33 ± 3.33	-	-
Oppiidae	-	-	0.33 ± 0.33	8.33 ± 5.23	-	1.00 ± 1.00
Oripodidae	0.33 ± 0.33	-	0.33 ± 0.33	-	-	-
Oridatulidae	17.33 ± 2.97	4.33 ± 2.96	1.67 ± 0.76	247.67 ± 209.43	-	24.67 ± 22.18
Galumnidae	-	-	-	100.67 ± 79.68	-	-
Phthiracadidae	-	-	-	-	-	-
Epilohmanhidae	-	-	-	-	-	-
Astigmata						
Acaridae	-	5.00 ± 5.00	0.67 ± 0.33	-	-	-
Pediculochelidae	-	-	-	-	1.33 ± 1.33	-
ARANEAE						
Lycosidae	-	-	-	-	-	-
Gnaphosidae	-	-	-	-	-	-
Salticidae	-	-	-	-	-	-
COLLEMBOLA						
Entomobryidae	-	-	-	40.67 ± 38.19	-	1.00 ± 1.00
Tomoceridae	-	-	-	-	-	-
Isotomidae	2.33 ± 2.33	2.33 ± 2.33	2.00 ± 1.00	186.67 ± 150.74	-	1.33 ± 1.33
Poduridae	7.00 ± 5.57	-	-	-	-	3.00 ± 3.00
Sminthuridae	-	67.33 ± 31.21	-	-	18.33 ± 18.33	4.67 ± 4.67
HEMIPTERA						
Aphididae	-	-	-	-	-	-
Coreidae	1.00 ± 1.00	-	-	1.33 ± 0.33	-	-
Coccidae	1.00 ± 1.00	-	-	0.67 ± 0.67	-	-
THYSANOPTERA						
Thripidae	4.00 ± 2.08	-	0.33 ± 0.33	2.33 ± 2.33	-	0.67 ± 0.67
Aelothripidae	-	-	-	-	-	-

Table 6.2.2. Mesofaunal community composition

COLEOPTERA						
Staphylinidae	-	-	-	0.33 ± 0.33	1.00 ± 1.00	-
Pselaphidae		-	-	-	-	-
Elateridae (larwes)	1.00 ± 0.58	-	-	0.67 ± 0.67	0.67 ± 0.67	-
Carabidae	-	-	-	-	-	-
Scarabidae	-	-	-	-	0.33 ± 0.33	0.33 ± 0.33
Tingidae	-	1.33 ± 1.33	-	-	-	-
Tenebrionidae	-	-	-	-	0.67 ± 0.67	-
Bostrichidae	-	-	-	-	-	0.33 ± 0.33

Values given as mean ± SEM

Table 6.2.3. Mesofaunal community composition.

	Old Tailings Dam			Natural Veldt		
	Old_O			Nat		
	Apr_04	Nov_04	Apr_05	Apr_04	Nov_04	Apr_05
Prostigmata						
Nanorchestidae	35.67 ± 12.47	3.33 ± 3.33	9.67 ± 1.76	43.67 ± 22.85	15.00 ± 6.81	22.67 ± 8.41
Pachygnathidae	-	-	-	-	-	1.00 ± 1.00
Nematalycidae	-	6.00 ± 6.00	-	-	-	-
Eupodidae	-	-	-	9.67 ± 5.04	7.33 ± 2.60	6.00 ± 1.53
Rhagidiidae	-	-	-	5.00 ± 2.64	3.67 ± 3.67	2.00 ± 1.15
Paratydeidae	-	-	-	6.67 ± 3.38	7.33 ± 7.33	1.67 ± 1.67
Tydeidae	2.00 ± 0.58	25.00 ± 22.55	0.67 ± 0.67	74.33 ± 41.13	227.67 ± 214.31	30.00 ± 13.23
Tarsonemidae	-	-	-	4.67 ± 3.28	20.00 ± 20.00	-
Scutacaridae	-	-	-	5.33 ± 3.53	-	9.67 ± 1.76
Pygmephoridae	3.67 ± 3.67	-	-	13.00 ± 6.80	-	1.67 ± 1.67
Cunaxidae	-	-	-	8.00 ± 4.93	-	2.67 ± 2.19
Bdellidae	-	-	-	2.67 ± 1.76	9.67 ± 4.91	9.67 ± 4.18
Tetranychidae	-	-	-	3.00 ± 2.08	-	1.00 ± 1.00
Stigmaeidae	0.33 ± 0.33	5.00 ± 5.00	-	4.00 ± 4.00	-	13.67 ± 13.67
Caeculidae	-	-	-	1.00 ± 1.00	-	3.00 ± 1.15
Cheyletidae	-	-	-	1.00 ± 1.00	-	-
Pseudochelidae	-	-	-	-	2.00 ± 2.00	3.00 ± 3.00
Anystidae	-	-	-	1.00 ± 0.58	-	-
Trombidiidae	-	-	-	0.67 ± 0.67	-	1.00 ± 1.00
Erythraeidae	-	-	-	2.33 ± 1.86	1.00 ± 1.00	-
Barbutidae	-	-	-	-	-	-
Mesostigmata						
Laelapidae	-	-	-	15.67 ± 8.95	11.33 ± 11.33	6.67 ± 2.96
Macrorchelidae	-	-	-	6.00 ± 4.10	-	2.67 ± 1.33
Ascidae	-	-	-	0.67 ± 0.67	-	19.00 ± 7.58
Rhodacaridae	-	-	-	27.67 ± 13.91	25.67 ± 8.95	3.67 ± 2.03
Uropodidae	-	-	-	5.67 ± 5.67	2.33 ± 2.33	2.00 ± 1.53
Phytoseiitdae	-	-	-	0.33 ± 0.33	-	-

Table 6.2.3. Mesofaunal community composition

Cryptostigmata						
Hypochthoniidae	-	-	-	15.67 ± 8.69	26.67 ± 26.67	8.00 ± 4.95
Brachychthoniidae	-	-	-	-	47.00 ± 13.87	3.00 ± 3.00
Cosmochthoniidae	-	-	-	25.00 ± 16.07	79.67 ± 65.70	1.67 ± 1.67
Nothridae	-	-	-	52.67 ± 28.31	11.00 ± 5.86	15.33 ± 8.41
Camisiidae	-	-	-	10.00 ± 5.03	7.33 ± 4.33	7.00 ± 1.53
Gymnodamaeidae	-	-	-	7.67 ± 4.63	15.67 ± 8.95	-
Oppiidae	-	-	-	-	8.67 ± 5.21	4.67 ± 4.17
Oripodidae	-	-	-	18.33 ± 9.24	6.67 ± 6.67	7.00 ± 3.51
Oridatulidae	-	1.00 ± 1.00	-	74.67 ± 35.48	19.00 ± 10.69	25.00 ± 11.00
Galumnidae	-	-	-	20.33 ± 9.39	1.67 ± 1.67	-
Phthiracadidae	-	-	-	-	6.33 ± 6.33	1.00 ± 1.00
Epilohmanhidae	-	-	-	-	3.67 ± 3.67	1.00 ± 1.00
Astigmata						
Acaridae	-	-	-	4.33 ± 2.19	8.00 ± 4.36	5.00 ± 0.58
Pediculochelidae	-	-	-	-	16.00 ± 8.71	-
ARANEAE						
Lycosidae	-	-	-	-	-	0.33 ± 0.33
Gnaphosidae	-	-	-	1.67 ± 0.88	-	1.33 ± 0.88
Salticidae	-	-	-	-	-	-
COLLEMBOLA						
Entomobryidae	-	-	-	-	4.33 ± 4.33	5.33 ± 2.85
Tomoceridae	-	-	1.67 ± 0.86	-	2.33 ± 2.33	-
Isotomidae	-	-	-	78.33 ± 37.40	10.00 ± 6.81	21.33 ± 7.88
Poduridae	1.00 ± 1.00	-	-		4.33 ± 4.33	-
Sminthuridae	-	-	-	-	-	-
HEMIPTERA						
Aphididae	-	-	-	-	-	-
Coreidae	-	-	-	-	-	-
Coccidae	-	-	-	-	-	6.00 ± 2.52
THYSANOPTERA						
Thripidae	-	-	-	6.67 ± 5.24	4.67 ± 4.67	8.54 ± 4.93
Aelothripidae	-	-	-	0.67 ± 0.00	1.00 ± 1.00	0.33 ± 0.33

Table 6.2.3. Mesofaunal community composition

COLEOPTERA						
Staphylinidae	-	-	-	-	0.67 ± 0.67	0.33 ± 0.33
Pselaphidae	-	-	-	1.33 ± 0.67	-	-
Elateridae (larwes)	-	-	-	1.67 ± 0.88	-	0.33 ± 0.33
Carabidae	-	-	-	1.00 ± 0.58	-	0.67 ± 0.33
Scarabidae	-	-	-	0.33 ± 0.33	-	1.33 ± 0.33
Tingidae	-	-	-	0.33 ± 0.33	-	-
Tenebrionidae	-	-	-	-	-	-
Bostrichidae	-	-	-	0.67 ± 0.67	-	-

Values given as mean ± SEM

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1 Background

Mining industries and the growth of domestic markets and industries in relation to mining activities provide employment and training for the local population (Chamber of Mines, 2003; Milton, 2001). The growth of these industries has however been detrimental to South Africa's sensitive environment. Mining industries and the related industries contributed to 25% of natural environmental lost (DEAT, 1999). The recovery of the loss of natural vegetation and ecological biodiversity within these disturbed areas are limited by local climatic conditions of South Africa (Milton, 2001). The goal of rehabilitation is to restore the biological integrity of these disturbed areas (Mummey et al., 2002). Rehabilitated areas must be monitored and measured against a set goal, such as the rehabilitation of platinum mine tailings to a stable, self-sustaining ecosystem similar to that of the surrounding environment (Bell, 2001). A growing need exists to improve current rehabilitation techniques and to find more economical and practical ways to rehabilitate mine spoil (Tordoff et al., 2000). Rehabilitation techniques currently implemented to mine spoils include the use of inorganic fertilisers and non-indigenous plant species. These techniques have proven unsuccessful due to the need for further fertiliser applications and management (Milton, 2001; Bradshaw, 1997). The addition of organic matter provides continuous release of nutrients, and contributes to the acceleration of recovery of biological activities within mine spoils (Wong, 2003). The ecosystem within mine tailings may never be properly restored to that of the surrounding environment. However, attempts to rehabilitate mine tailings provide one-step closer to establishing a healthy ecosystem within the tailings.

7.2 Discussion

Platinum mine tailings are a biologically sterile medium, with many physical and chemical limitations for the establishment of vegetation cover (Van Rensburg & Morgenthal, 2004). Physical limitations include the high percentage sand and silt, and low clay content, which may lead to easy compaction and erosion of these tailings dams. The addition of woodchip-vermicompost may contribute to the chemical properties of the tailings by increasing the cation exchange capacity (CEC) and so lowering the base saturation. The organic carbon (C) content of the sites treated with woodchip-vermicompost was higher than that of sites treated with inorganic fertilisers, but a fluctuation in organic carbon was observed during the study. The pH of the different sites showed an increase over the study period. The chemical difference between the treatments may have contributed to the differences observed in soil biota in terms of community compositions and activity.

Redundancy analysis (RDA) ordination diagrams were used to determine the relation between microbial enzymatic activity (Chapter 3, Figures 3.4 and 3.5) and the independent chemical properties carbon (C), nitrate (NO_3), ammonium (NH_4) and pH. The relation between microbial community structure (Chapter 4, Figures 4.7 and 4.8) and the independent chemical components carbon (C), nitrate (NO_3), ammonium (NH_4), phosphorous (P) and pH were also determined using RDA ordination diagrams. Dominant independent chemical properties were selected by the forward selection method using CANOCO.

The woodchip-vermicompost sites were characterised by a higher initial estimated viable microbial biomass and enzymatic activity, with estimated viable microbial biomass showing a positive correlation with organic carbon ($r = 0.66$, $p < 0.05$). Both the woodchip-vermicompost sites NR_N and NR_1 were characterised by a higher dehydrogenase and β-glucosidase activity than the woodchip-vermicompost site NR_2. These sites were also characterised by higher mole % mid-branched saturated fatty acids than NR_2 in November 2004 (Chapter 4, Figure 4.4). Mid-branched saturated fatty acids were found to increase in manured sites (Bassio et al., 1998), whereas β-glucosidase activity contributes to carbon cycling (De Mora et al., 2005).

The woodchip-vermicompost sites NR_N and NR_1 may therefore have a higher organic carbon content than NR_2. A correlation existed between percentage carbon and both dehydrogenase activity ($r = 0.37$, $p < 0.05$) and β-glucosidase activity ($r = 0.87$, $p < 0.05$) (Chapter 3, Figure 3.4). The woodchip-vermicompost site NR_2 showed a slight increase in dehydrogenase activity over the study period, but the activity was still lower than that of NR_N and NR_1. An increase in β-glucosidase activity was observed in NR_N and NR_1 from April 2004 to November 2004, with NR_1 showing higher β-glucosidase activity than both NR_N and NR_2. The woodchip-vermicompost site NR_1 was also characterised by higher mole % terminal branched saturated fatty acids than NR_N and NR_2 during this period. Terminal branched saturated fatty acids showed a positive correlation with both nitrate ($r = 0.46$, $p < 0.05$) and phosphorous ($r = 0.66$, $p < 0.05$) (Chapter 4, Figure 4.7). The woodchip-vermicompost site NR_1 might be expected to have a higher organic carbon content than NR_N and NR_2 during this period, due to the higher β-glucosidase activity.

From April 2004 to November 2004, an increase in alkaline phosphatase was observed in NR_N and NR_1, as well as an increase in mole % mid-branched saturated fatty acids. A positive correlation existed between alkaline phosphatase ($r = 0.77$, $p < 0.05$) and phosphorous. The increase in β-glucosidase and alkaline phosphatase activity may be attributed to the increase in terminal branched saturated fatty acids and mid-branched saturated fatty acids. Waldrop et al. (2000) found a correlation between β-glucosidase and both Gram-positive bacteria and Gram-negative bacteria, while Actinomycetes showed a positive correlation with phosphatase. Urease activity correlated with ammonium ($r = 0.46$, $p < 0.05$) and nitrate ($r = 0.70$, $p < 0.05$). An increase in urease activity was observed from April 2004 to November 2004 in all woodchip-vermicompost sites, with a decrease in mole % monounsaturated fatty acids.

Estimated viable microbial biomass was the highest in NR_N and NR_2 than in NR_1 during April 2005 (Chapter 4, Figure 4.6). The β-glucosidase activity decreased in NR_N and NR_1 while it increased in NR_2. The woodchip-vermicompost sites NR_N and NR_2 were characterised by a higher β-glucosidase activity than NR_1.

The mole % terminal branched saturated fatty acids decreased in all woodchip-vermicompost sites. The lowest mole % terminal branched saturated acids was observed in NR_2. The woodchip-vermicompost sites NR_1 and NR_2 showed an increase in mole % monounsaturated fatty acids and higher acid phosphatase activity, during November 2004. The mole % monounsaturated fatty acids was lower in NR_N, while alkaline phosphatase activity was higher during this period. In April 2005, NR_N was characterised by higher urease activity. The woodchip-vermicompost sites showed a fungal-to-bacterial ratio lower than one, indicating that bacteria may contribute more to microbial processes than fungi. Values higher than 0.1 was observed for the cyclopropyl fatty acids to *cis*-monoenoic fatty acids ratio during April 2004, indicating that the microbial community have already entered a stage of stationary growth. This ratio decrease from April 2004 to November 2004, and increase toward April 2005. Nutrient stress was also observed to occur in the woodchip-vermicompost sites by showing *trans/cis* ratios of lower than 0.5.

The woodchip-vermicompost site NR_N was characterised by a higher total number of nematodes, during April 2004. The nematovorous mites (Prostigmata: Tydeidae; Mesostigmata: Rhodacaridae) and fungivorous mites (Cryptostigmata: Oribatulidae) was observed in this site during this period (Chapter 6, Table 6.2.1 to 6.2.3). Bacterivorous nematodes dominated the nematode community composition in NR_1 and NR_2 during April 2004 (Chapter 5, Table 5.1 to 5.3). A higher number of bacterivorous nematodes were observed in NR_1, during November 2004 to April 2005. The number of nematodes and nematovorous mites decreased in NR_N and NR_1, from April 2004 to November 2004. Fungivorous mites (Prostigmata: Eupodidae) increased in these sites during this period. The woodchip-vermicompost site NR_2 was characterised by an increase in bacterivorous nematodes and nematovorous mites (Prostigmata: Tydeidae) from April 2004 to November 2004. This may indicate that the availability of prey (microorganisms) has increased in NR_2, while it decreased in NR_N and NR_1.

A decrease in Cryptostigmata and Mesostigmata was observed in both NR_1 and NR_2 from April 2004 to November 2004. Both NR_N and NR_1 showed higher occurrence of plant-parasitic nematodes during April 2005. The higher Plant-parasitic

Index may be attributed to higher vegetation cover in these sites. Nematode community seemed to follow the fluctuation of their prey (microorganisms), whereas micro-arthropod, which preys on nematodes, followed the fluctuation in nematode communities. The Maturity Index values were low for the all woodchip-vermicompost sites, while the Plant-parasitic Index showed higher values (Chapter 5, Table 5.4), indicating nutrient enriched ecosystems.

Estimated viable microbial biomass increased in the inorganic fertilised sites from April 2004 to November 2004. A decrease in enzymatic activity was observed for this period in Old_N. Both inorganic fertilised sites showed a decrease in mole % monounsaturated fatty acids from April 2004 to November 2004. The inorganic fertilised site Old_O showed an increase in mole % monounsaturated fatty acids while Old_N showed a further decrease from November 2004 to April 2005. An increase in mole % mid-branched saturated fatty acids from April 2004 to April 2005 was observed in Old_O. A decrease in mole % mid-branched saturated fatty acids was observed in Old_N from April 2004 to November 2004, with an increase during November 2004 to April 2005. All enzymatic activities showed an increase from November 2004 to April 2005 in both inorganic fertilised sites. Estimated viable microbial biomass was higher in inorganic fertilised sites during November 2004. A further increase in estimated viable microbial biomass from November 2004 to April 2005, took place in Old_O. Fungal-to-bacterial ratios of the inorganic fertilised sites also indicated that bacteria might have played an important role in microbial processes. The phospholipid fatty acid stress ratio indicated that the microbial community might also experience nutrient stress.

The number of bacterivorous nematodes was higher in the inorganic fertilised sites. Prostigmata mites were the dominant group of Acari in these sites. The inorganic fertilised site Old_O contained a higher total number of nematodes and nematovorous mites (Prostigmata: Tydiedae) than Old_N during April 2004. Bacterivorous mites (Prostigmata: Nanorchestidae) dominated Old_N during April 2004. From April 2004 to November 2004, Old_N showed an increase in number of bacterivorous nematodes and plant-parasitic nematodes. A decrease in number bacterivorous nematodes and plant-parasitic nematodes was observed in Old_O. Micro-arthropods decreased in

Old_N, while Tydeidae still dominated the Prostigamata families in Old_O during this period. The inorganic fertilised site Old_N showed a slight decrease in bacterivorous nematodes from November 2004 to April 2005, while Old_O showed an increase in bacterivorous nematodes, and bacterivorous mites (Prostigmata: Nanorchestidae). Both the inorganic fertilised sites showed an increase in number of plant-parasitic nematodes. The Maturity Index indicated that both the inorganic fertilised sites have become more nutrient enriched over the study period, while the Plant-parasitic Index values decreased from April 2004 to April 2005. The decrease in the Plant-parasitic Index may be attributed to a decrease in nutritional levels (Bongers et al., 1997). No or little micro-arthropods were observed in these sites during the remainder of the study. Fluctuation observed in nematode trophic structures may be attributed to the fluctuation in prey (microorganisms), which in turn influence the fluctuation of their predators (micro-arthropods).

7.3 Conclusion

From the results obtained, it can be concluded that the addition of the woodchip-vermicompost may have contributed to the establishment of soil biotic activity in the nearly biologically sterile medium. The enzymatic activity in the woodchip-vermicompost sites increased with an increase in the presence of K-strategists. Nutrient cycling in these sites seem to rely on the presence of K-strategists rather than r-strategists. In the inorganic fertilised sites, enzymatic activity increased with the presence of r-strategists. The cycling of nutrients in these sites therefore seems to be dominated by the activity of r-strategists. An increase in enzymatic activity in NR_2, was also observed to be associated with the increase in r-strategists.

K-strategists are capable to tolerate lower nutrient resource levels, than r-strategists, and typically out compete r-strategists by increasing in population size (Begon et al., 1996). The fluctuation between r- and K-strategists that took place in the different treatments may therefore be attributed to increase in population size of the K-strategist, due to a possible lower nutrient availability. This may have lead to the decrease in r-strategist. K-strategists are however density dependent. An increase in organic carbon, may have contributed to a rapid increase in mole % monounsaturated

fatty acids (r-strategists). This increase in r-strategists may have contributed to the decrease in mole % terminal branched saturated fatty acids and mid-branched saturated fatty acids (K-strategists), due to the density-dependents of these organisms. Plant-parasitic nematodes were represented by both r- and K-strategists. These plant-parasitic nematodes might have contributed to the decrease in vegetation cover, together with other environmental factors. All the Acari groups, containing both r- and K-strategist, were present in the woodchip-vermicompost sites, whereas the inorganic fertilised site predominantly contained r-strategists.

It can therefore be concluded that the addition of the woodchip-vermicompost may be an essential part of the initial stages of rehabilitation, by contributing to the recolonisation of soil biota. Ekschmitt et al (2005) suggested that the presence of K-strategist is indicative of late stages of decomposition. The added woodchip-vermicompost may therefore be in late stages of decomposition, which may indicate that the ecosystem may begin to rely on other sources of organic carbon to function. Studies should however be conducted on the long-term viability of the woodchip-vermicompost in providing the rehabilitated ecosystem with necessary organic carbon. In doing so, an indication of possible further application can be obtained in order to insure the establishment of a self-sustaining independent food chain system on the tailings dams.

7.4 Recommendations for Future Research

The following points should be considered when further studies are conducted on the use of woodchip-vermicompost as alternative amendment on platinum mine tailings:

- Monitoring soil moisture content of the platinum mine tailings during the study period as this may have an effect on nutrient availability to soil biota and distribution of soil biota,
- The C/N ratio of the applied woodchip-vermicompost, as differences in the C/N ratio of the woodchip-vermicompost may have influenced soil enzymatic activity as well as microbial community structure, which in turn influence the distribution of mesofuana and may have had an effect on vegetation growth and,

- Considering the use of an unrehabilitated site, to show the effect of the addition of woodchip-vermicompost on the re-establishment of soil biota from an untreated site to treated site, as well as the time frame of re-establishment,
- Soil temperature, as this contributes to cell degradation and soil moisture content.

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