

Investigation of the microbial diversity and functionality of soil in fragmented South African grasslands along an urbanization gradient

Jacobus Petrus Jansen van Rensburg (B.Sc. Honn)

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Supervisor : Prof. C.C. Bezuidenhout

Co-supervisor : Prof. S.S. Cilliers

Assistant supervisor : Dr. S. Claassens

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Abstract

The diversity of microorganisms and the influence of their enzymatic activities in soil are critical to the maintenance of good soil health. Changes in these parameters may be the earliest predictors of soil quality changes, potentially indicating anthropogenic influences. The goal of this study was to investigate the soil microbial diversity and function of grasslands along an urbanization gradient. Soil samples were collected in the Potchefstroom municipal area, South Africa, at specific sites. Sampling sites were described as urban, suburban and rural - according to the V-I-S (Vegetation-Impervious surface-Soil) model of Ridd (1995). Soil samples were collected over a warmer, wet season (May) and a colder, dry season (August) over two years (2007 and 2008). Collected soil samples were characterised using certain physical and chemical parameters. Plant species composition and abundance were determined at each site, along with basic site data (soil compaction, percentage ground cover, percentage bare ground, percentage organic material present). The Shannon-Weaver diversity index was used to calculate biodiversity values for all the investigated sites regarding collected plant species composition. The microbial component of the soil was quantified and characterized using culture-dependent and culture-independent techniques. Culture-dependent techniques included the investigation of the aerobic heterotrophic bacteria and fungi. Organisms were plated out on different media, and the bacterial component was broadly grouped using morphology. Dominant organisms were identified by sequencing of PCR amplified 16S ribosomal DNA fragments. Shannon-Weaver index for bacterial diversity was determined for each of the sites. Denaturing gradient gel electrophoresis (DGGE) profiling of selected bacterial communities were also conducted. Microbial community function was determined using enzyme assays of five major groups of enzymes, namely (i) dehydrogenase; (ii) β -glucosidase; (iii) acid phosphatase, (iv) alkaline phosphatase and (v) urease. Plant species results were then brought into context with microbiological diversity and functionality results using multivariate statistics.

Physical and chemical parameters of the collected soil samples revealed patterns present along the urbanization gradient. The pH values were mostly higher in the suburban and urban sites than in the rural sites. Electrical conductivity values were

generally highest in the sub-urban sites. Plant species composition revealed trends along the urbanization gradient. Ordinations clearly grouped the plant species into rural, sub-urban and urban groups regarding plant species composition. Rural sites had the highest number of plant species. Shannon-Weaver values regarding the plant diversity supported the plant species composition data indicating higher plant diversity in the rural areas, followed by the sub-urban and the urban areas. Plant structural data indicated that forbs were most numerous in the rural sites, and less so in the urban sites.

Higher average aerobic heterotrophic bacterial levels were present in the urban soil samples. The bacterial levels were lower in the sub-urban and rural soil samples. Subsequent identification of the dominant bacteria in the soil samples revealed organisms of the genus *Bacillus* dominated the aerobic heterotrophic bacterial communities in the soil samples. *Bacillus* species dominated the soil samples along the urbanization gradient. Shannon-Weaver indices based on culture-dependent methods indicated that urban sites had the highest biodiversity. These results could have been exaggerated, because of an overestimation of the number of bacterial morphotypes present in samples. Fungal levels were higher in the soil from samples collected at the rural samples sites. The culture-independent method (DGGE) was not optimized and inconclusive results were obtained.

Enzyme assays revealed that potential dehydrogenase, β -glucosidase and urease activity followed a trend along the urbanization gradient, with urban samples registering the highest values and rural sites the lowest. Enzymes involved in carbohydrate catabolism (β -glucosidase and dehydrogenase) registered significantly higher potential activity in urban sites than the sub-urban and rural sites. The results could indicate that urban sites have the potential to lose carbon at higher rates than the rural sites. This aspect may need further investigation. Higher potential urease activity could indicate higher N-cycling in the urban soil environment.

Ordination results for soil-, plant- and microbial diversity as well as microbial functionality indicated certain trends along the urbanization gradient. Plant species composition and structure data indicated that urbanization has a definite effect on the plant communities in the urban ecosystem. Results regarding aerobic heterotrophic bacteria populations and potential enzyme activity of the dehydrogenase, β -glucosidase

(both active in the carbon cycle) and urease (active in the nitrogen cycle) illustrated clear trends along the urbanization gradient.

In conclusion, results indicated that urbanization has an effect on plant species composition, and the population and function of aerobic heterotrophic bacteria and the fungal population. Furthermore, this study demonstrated the potential of using microbial diversity and activity as tools to investigate carbon utilization and storage along an urban-rural gradient.

Key words: Urbanization gradient, plant species composition and structure, microbial community composition, microbial functionality, enzymatic assays.

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Declaration

I declare that the dissertation for the degree of Master of Environmental Science (M.Sc.) at the North-West University: Potchefstroom Campus hereby submitted, has not been submitted by me for a degree at this or another university, that it is my own work in design and execution, and that all material contained herein has been duly acknowledged.

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Jacobus Petrus Jansen van Rensburg

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Date

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Abbreviations used in the dissertation.

- ATP = Adenosine triphosphate
- BLASTN database = Basic local alignment search tool for nucleotide databases
- BSA = Bovine albumin serum
- Bp = base pairs
- C = Carbon
- CaCl₂ = Calcium chloride
- CANOCO = Canonical correspondence analysis program
- CCA = Canonical Correspondence Analysis
- CLPP = Community-level physiological profiling
- cfu/g = Colony forming units per gram
- CO₂ = Carbon dioxide
- DCA = Detrended correspondence analysis
- DGGE = Denaturing gradient gel analysis
- dH₂O = Distilled water
- DNA = Deoxyribonucleic acid
- GC clamp = Area on primer that is rich in Guanine-Cytosine bonds
- INF = Iodonitrotetrazolium chloride-formazan
- INT = Iodonitrotetrazolium chloride
- KCl = Potassium chloride
- MUB buffer = Modified universal buffer
- N = Nitrogen
- NaOH = Sodium hydroxide
- NH₃ = Ammonia
- NH₄⁺ = Ammonium
- NO₂⁻ = Nitrite
- NO₃⁻ = Nitrate
- P = Phosphor
- PCA = Principle component analysis
- PCR = Polymerase chain reaction
- PDA = Potato dextrose agar

- pH = The cologarithm of the activity of dissolved hydrogen ions (H^+)
- PLFA = phospholipids fatty acid analysis
- PNG solution = *p*-Nitrophenyl- β -D-glucosidase solution
- PNP solution = *p*-Nitrophenol solution
- RDA = Redundancy analysis
- S = Sulphur
- SSCP = Single-strand conformation polymorphism
- TAE buffer = Tris-acetate-EDTA buffer.
- THAM = Tris(hydroxymethyl)aminomethane buffer
- V-I-S model = The Vegetation-Impervious surface-Soil model
- T-RFLP = Terminal restriction fragment polymorphism
- Tris buffer = Tris(hydroxymethyl)aminomethane buffer

Abbreviations used for sampling sites

- NR = Northern rural
- NSU = Northern sub-urban
- NU = Northern urban
- ER = Eastern rural
- ESU = Eastern sub-urban
- EU = Eastern urban
- SR = Southern rural
- SSU = Southern sub-urban
- SU = Southern urban
- WR = Western rural
- WSU = Western sub-urban
- WU = Western urban

Chapter 1 - Introduction

1.1 Background

The process of urbanization is accelerating rapidly across the world (United Nations, 2005). Urbanization has ecological consequences for the environment, leading to the development of unique urban ecosystems (Kaye *et al.*, 2005). These influences of urbanization include effects on nutrient cycles, soil structure and carbon sequestration. It is important to study urban ecological systems, as these are related to environmental protection, ecological balance and the conservation of biodiversity (Pouyat & Turecheck, 2001; Kaye *et al.*, 2005).

Human activity in urbanization creates urban soils that have different characteristics than natural soils (Craul *et al.*, 1985). Urban areas represent ecosystems with modified biogeochemical cycles. These modifications include fluxes and pools of matter, energy, and organisms which differ greatly from ecosystems not influenced by urbanization (Pouyat *et al.*, 2007). Urbanization also contributes towards unique amendments and contaminants that create problems only found in urban environments and which result in urban soils with some distinct characteristics (Craul *et al.*, 1985). According to McDonnell *et al.* (1997), investigation into the physical and chemical characteristics of soil along the urbanization gradient can give insights into the effects that the urbanization process has on soil.

Plant communities are also influenced by the urbanization process. Due to the clearing of land for housing and infrastructure during the process of urbanization, natural areas in and around cities are severely impacted by a reduction in extent, fragmentation, transformation, dumping and alien species invasions (McConnachie *et al.*, 2008). These urban influences cause plant communities to differ along the urban-rural gradient. Objective and subjective methods and techniques are used to investigate and to quantify basic botanical information along gradients (Kent & Coker, 1992).

The Grassland Biome is considered to have extremely high plant species diversity, and includes many rare and threatened species. However, the Grassland Biome is regarded as the most critically threatened southern African ecosystem (Rutherford & Westfall, 1994). It is thus important to study this threatened biome to improve its conservation.

Land cover data (Fairbanks *et al.*, 2002) indicates that almost 30% of the Grassland Biome of South-Africa has been permanently transformed. This has occurred primarily as a result of cultivation, plantation forestry, urbanization and mining. Other factors that severely degraded the grasslands include erosion and agricultural improvement (Mucina & Rutherford, 2006). Significant parts of the remaining vegetation may be secondary lands, or may be degrading gradually by processes such as woody encroachment. Another concern in this regard is the fact that those areas of grassland that are untransformed are highly fragmented, and that as much as half of the remaining areas of grassland may be composed of grassland fragments (Mucina & Rutherford, 2006). This edge effect contributes towards the invasion of alien plant species (Cilliers *et al.*, 2008).

Measuring the environmental impact of anthropogenic activities on soil microbial processes and diversity is considered of great importance for broadening our knowledge of soil ecosystem functioning and also for assessing human-induced changes in soil properties (Gelsomino *et al.*, 2006.) To gain a more profound understanding of the soil environment in urban settings, soil microbial diversity and functionality can be studied. Soil biota plays a very important role in the maintenance of soil fertility and productivity. Soil microorganisms drive most soil processes, including nutrient availability and retention, decomposition of organic materials, soil organic matter build-up and the stabilization of soil aggregates (Coleman *et al.*, 2004). Microbial mediated processes are very important in terms of the functions that soil performs (Wakelin *et al.*, 2008). The microbial component in soil can be investigated by means of various culture-dependent and culture-independent techniques.

Microbes colonize and chemically break down organic matter by means of enzymes (Nannipieri *et al.*, 2002). Soil enzyme activities are involved in soil nutrient cycling dynamics and can catalyze the conversion of nutrients to forms which are readily available for use by plants and microorganisms (Egamberdiyeva, 2006). Microbial

functionality can be studied by investigating the potential enzymatic activity of critical enzymes in major nutrient cycles. The potential enzymatic activity of a wide range of enzymes can be calculated using specifically designed enzyme assays (Alef & Nannipieri, 1995).

All terrestrial ecosystems consist of aboveground and belowground components that interact to influence community and ecosystem-level processes and properties (Wardle *et al.*, 1999). It is well understood that plants and microbes are interdependent on one another for nutrient supply (Singh *et al.*, 2004). By correlating the information gathered along an urbanization gradient, a clearer understanding of the dynamics of ecosystems undergoing urbanization can be achieved. Patterns observed can serve as a tool that can be used to assess soil quality in grasslands undergoing influences associated with urbanization. Biological properties are highly sensitive to changes. For this reason, they are considered as suitable indicators of environmental impacts for properly complementing physical and chemical properties (Elliott *et al.*, 1996; Bending *et al.*, 2004).

An effective way of studying urbanization is to use the urbanization gradient approach. Information gathered from urbanization gradient studies has given insight into the process dynamics of the urbanization process (Niemelä, 1999). The gradient paradigm is a useful organizing tool for research on the ecological consequences of urbanization (McDonnell *et al.*, 1997). The knowledge gained from such studies contributes towards the effective planning, management and conservation of urban ecosystems (McDonnell & Pickett, 1990). In the face of extensive global environmental change, it is very important to understand the processes associated with urbanization in order to formulate predictions about how these important areas may change in the future (McDonnell *et al.*, 1997).

1.2 Research aim

To investigate the microbial diversity and functionality of soil in fragmented South African grasslands along an urbanization gradient.

1.3 Objectives

The objectives of the study were :

- To investigate certain physical and chemical parameters of soil as well as plant species composition and abundance along a described urbanization gradient.
- To isolate, characterize and quantify the major heterotrophic bacterial species from soil samples collected along the urban-rural gradient.
- To screen bacterial communities from soil samples using PCR-DGGE to assess whether any significant differences regarding community structure can be found along the urban-rural gradient.
- To assess the enzymatic activities of five major groups of enzymes in the collected soil samples along an urbanization gradient.
- To use multivariate statistics to bring basic plant and soil information in context with microbiological results all gathered from soil samples along the urban-rural gradient using multivariate statistics.
- To establish the potential of using the trends observed as a tool to investigate soil quality in fragmented South African grassland along an urbanization gradient.

1.4 Layout of dissertation

- Chapter 1: Here the introduction to this study is offered. This chapter also states the research aim and the objectives of this study.
- Chapter 2: This chapter provides the theoretical framework of the study. The principles and applications of the analytical methods are also presented.
- Chapter 3: This chapter presents the methods of investigation. It includes a description of the area studied, material and methods for sampling practices and the collection of data using specified techniques. Analytical methods are also described in detail.

- Chapter 4: This chapter offers a description of all results collected during this study. Results include: (i) the physical and chemical results of the soil samples; (ii) plant species composition and diversity; (iii) microbial culture-dependent and culture-independent results; (iv) enzyme assays results; (v) integration of all the results presented using ordination techniques.
- Chapter 5: Discussion of the results using a theoretical framework from literature.
- Chapter 6: This chapter examines the extent to which the study objectives and hypothesis have been reached, and state the recommendations for future studies.
- References and appendixes.

Chapter 2 - Literature review

2.1 The impact of urbanization on ecosystems

The twentieth century witnessed a rapid urbanization of the world's population. The global proportion of urban population has increased from a mere 13 % in 1900 to 49 % in 2005 (UN, 2005). Urbanization is projected to continue. About 60 % of the global population is expected to live in cities by 2030. Rising numbers of urban dwellers provide the best indication of the scale of these unprecedented trends: the urban population has increased from 220 million in 1900 to 732 million in 1950, and is estimated to reach 3.2 billion in 2005, thus more than quadrupling since 1950 (United Nations, 2005). According to the United Nations (2005) population projections, 4.9 billion people are expected to be urban dwellers in 2030. This urbanization process which has led to the development of a unique urban ecosystem provides mankind with immense challenges regarding the planning, management and conservation of urban areas (Cilliers *et al.*, 2008).

Urban ecosystems are generally recognized as areas that are under profound and constant human activity, and are characterized as spatially heterogeneous and temporally dynamic areas (McIntyre *et al.*, 2001). According to Kaye *et al.* (2005), urbanization is defined by the demographic dynamic of increasing percentages of the global population living in cities, and by the concurrent land-use dynamic of conversion of unmanaged or agricultural land to urban commercial, industrial, recreational, and residential uses. McIntyre *et al.* (2001) define urban ecosystems as comprising of high-density human habitation, industrial and commercial centers, as well as remnants of indigenous habitat. The urbanization process entails the conversion of indigenous habitats to different forms of anthropogenic land-use forms, the fragmentation and isolation of remaining indigenous habitats, as well as an increase of the local human population (McIntyre *et al.*, 2001; Kaye *et al.*, 2005).

Assessing the ecological consequences of urbanization is very important in order to develop a better understanding of the biology of local and global change related to land use (Kaye *et al.*, 2005). It is now accepted that land-use change can dramatically alter

ecosystem functions. These changes can occur in nutrient cycles, soil structure, and carbon sequestration, and a few studies also suggest that expanding urban land area may be linked to regional nitrogen and carbon cycles (Pouyat & Turacheck, 2001; Pouyat *et al.*, 2002; Kaye *et al.*, 2005).

The study area is situated in an area classified as the Rand Highveld Grassland. It is a highly varied landscape and the vegetation is species-rich (Mucina & Rutherford, 2006). The Grassland Biome is considered to have extremely high plant species diversity, and includes many rare and threatened species. However, the Grassland Biome is regarded as the most critically threatened southern African ecosystem (Rutherford & Westfall, 1994). Large sections of grasslands have been disturbed by past cultivation, livestock grazing and disruption of natural fire cycles - resulting in the severe decrease of species diversity of plants, insects and other animals (Cilliers *et al.*, 2008). Urbanization is also considered to aggravate the loss of natural areas in the biome (Rutherford & Westfall, 1994).

2.2 The importance of urban ecological studies

Cities are dependent for their growth and survival on areas of productive ecosystems of up to three orders of magnitude larger than the geographical area of the city itself. Folke *et al.* (1997) referred to this process as the ecological footprint of the city. The ecological footprint of a city is the total land area required to meet the demands of its population in terms of consumption and waste assimilation (Rees & Wackernagel, 1996). Another important distinguishing characteristic of the urban ecosystem is in terms of energy consumption. The energy consumed per unit area is at least one order of magnitude greater than in other types of ecosystems. Although urban ecosystems cover a relatively small percentage of the earth's surface (<5%), they consume most of the energy available (Odum, 1997). Urbanization is therefore regarded as one of the most severe factors that impact on the environment (Cilliers *et al.*, 2004).

Urban ecosystems differ from undisturbed, rural ecosystems in terms of the presence of impervious surfaces, pollution, different species composition of fauna and flora, and also the local climate (McIntyre *et al.*, 2001). According to Wallbridge (1997), urban

ecosystems differ from rural ecosystems solely with regard to the degree of man's influence. It is a truism that certain processes are more prevalent in urban ecosystems than in rural ones. An example of this is the invasion of alien plant species into urban environments (Niemelä, 1999). Trepl (1995) proposed three main properties that distinguish urban landscapes from rural landscapes, namely: (i) patchiness of urban ecosystems and poor connectivity between them; (ii) succession patterns; (iii) invasion by alien species.

Urban areas represent ecosystems with modified biogeochemical cycles where fluxes and pools of matter, energy, and soil-associated organisms differ greatly from the original ecosystems before urbanization has taken place (Hahs & McDonnell, 2006). Knowledge concerning the magnitude and extent of these biogeochemical changes at local, regional and global scales is insufficient for understanding the nature of global change (Pouyat *et al.*, 2007). Over the past century much of the earth's land surface has been transformed by processes such as the alteration of the climate through carbon dioxide, nitrogen deposition, and land-use change (Wardle, 2002). The significance of these effects on ecosystem functioning is widely recognized (Eviner & Chapin III, 2003), but the mechanisms that drive ecosystem responses are not well understood.

Urban biotopes are important in many ways. They serve as refuges, dispersal centers and corridors for species (Cilliers *et al.*, 2004). These biotopes are important for environmental protection and ecological balance (hydrological cycle, water resources and hygiene, climate regulation, air hygiene, noise protection), for the aesthetic quality of the urban landscape, and as areas for low-key recreation opportunities (Cilliers *et al.*, 2004). Ecologically, urban biotopes can serve a most useful purpose as bioindicators of environmental changes and pollution, and for fundamental research into urban ecology (Starfinger & Sukopp, 1994; Muller, 1997). There is also a growing recognition of the important role that urban environments can play in the conservation of biodiversity (Savard *et al.*, 2000).

2.3 The effect of urbanization on soil

Human activity in urban areas creates urban soils that have characteristics unlike those of their natural counterparts. Modifications include fluxes and pools of matter, energy, and organisms which differ greatly from ecosystems not influenced by urbanization (Pouyat *et al.*, 2007). In a study conducted by Pouyat & McDonnell (1991) on forest soils in New York City, it was found that heavy metals, base cations and acidic deposition all more or less followed a trend along an urbanization gradient, having higher concentrations in the urban than in the rural sites. These factors could have an effect on the composition of microbial communities in soil (Cookson *et al.*, 2007). Under these types of stress conditions, the development and biochemical activities of soil microorganisms may undergo several alterations (Filip, 2002).

Urbanization contributes towards unique amendments and contaminants that create problems unique to urban environments in terms of urban soils (Craul *et al.*, 1985). These problems include (a) great vertical and spatial variability, (b) soil structure modification, leading to compaction, (c) surface crust is present on bare soil, which is usually water-repellent, (d) soil reaction is modified, (e) aeration and water drainage are usually restricted, (f) nutrient cycling is interrupted, and (g) modified soil temperature regimes (Craul *et al.*, 1985).

2.4 The effect of urbanization on biogeochemical cycles

2.4.1 The carbon cycle

Carbon is the single most important element in the biological realm. Carbon dioxide is converted into organic carbon by the action of photoautotrophic organisms. The latter consists of higher plants on land, algae and cyanobacteria in aquatic systems (Wigley & Schimel, 2000). Carbon assimilated by these organisms provides the organic nutrients for heterotrophic animals and non-chlorophyll containing microorganisms. Carbon is constantly being fixed into organic form - and once bound, the carbon becomes unavailable for use in the generation of new plant life. It is thus essential for these

materials to be decomposed and returned to the atmosphere for higher organisms to continue to thrive (Wigley & Schimel, 2000). Microbial decomposition is therefore one of the dominant factors in the carbon cycle (Alexander, 1976).

Heterotrophic microorganisms are the main contributors in the conversion of residues into carbon and the release of carbon dioxide back to the atmosphere during residue decomposition and soil carbon mineralization (Asuming-Brempong *et al.*, 2008). Soil carbon sequestration is an important regulator of the net effect of ecosystems on atmospheric carbon dioxide levels, which influence the atmospheric “greenhouse effect” (Pouyat *et al.*, 2007). Soil carbon sequestration is proposed as one of the major ways of capturing atmospheric carbon for safekeeping, thus reducing the ever-increasing atmospheric carbon loads (Asuming-Brempong *et al.*, 2008). Carbon is primarily stored in soil as soil organic matter (SOM), and can remain in soils for millennia. Quick release back into the atmosphere is possible, depending on soil management (Asuming-Brempong *et al.*, 2008). Urbanization is a major component of environmental change and alterations to the biogeochemical cycles of soil, therefore, have an effect on the carbon pools and fluxes in urbanized environments (Pouyat *et al.*, 2002).

According to Pataki *et al.* (2006) soil organic carbon pools are directly and indirectly affected by urban land-use conversions. Soil carbon pools are primarily a function of the inputs of organic matter to the ecosystem (net primary productivity or NPP) and the average rate of decay within the ecosystem (soil heterotrophic respiration) (Pouyat *et al.*, 2002). Both these inputs are controlled by environmental factors such as soil temperature and moisture. Direct anthropogenic effects on the soil include physical disturbances; burial or coverage of soil by fill material and impervious surfaces; and soil management inputs, such as fertilization and irrigation. These direct effects can alter the amount of carbon stored in urban soil significantly. It appears as if human activities such as lawn maintenance practices are significant controllers of variation in carbon dynamics in urban ecosystems (Pouyat *et al.*, 2002). Indirect anthropogenic effects on the soil involve changes in the abiotic and biotic environment, because when areas are urbanized this can influence soil development (Pouyat *et al.*, 2002). Indirect effects in this regard include the urban heat island effect, soil hydrophobicity,

introductions of exotic plant and animal species, and atmospheric deposition of various pollutants. Urban heat islands occur when vegetation cover is replaced by built structures. These events reduce evapotranspiration rates while the introduction of building materials increases the absorption and storage of solar energy, which is later released as sensible heat (Oke, 1990). Moreover, toxic, sub-lethal or stress effects of the urban environment on soil decomposers and primary producers can affect soil carbon fluxes significantly (Pouyat *et al.*, 2002). It is clear that urbanization may be an important component of regional carbon budget change. However, the current knowledge of urban ecosystem ecology and feedbacks between urbanization and the other components of global change are far behind the knowledge possessed of unmanaged and agricultural systems (Kaye *et al.*, 2005).

Results obtained from a litter decomposition experiment along an urban-rural gradient in American forests by Pouyat *et al.* (1997) indicated that decomposition took place at a higher rate in urban than in rural stands. This would suggest that carbon cycling in urban environments takes place at a higher rate than in the natural rural sites. Litter used from rural settings decomposed faster than litter collected from urban sites, indicating that rural plant litter may in some cases be of a higher quality than that from the urban environment. Pouyat *et al.* (1997) concluded that, with variations in physical, chemical and biotic environments along the study transect, the flow of carbon between plants and microbial communities may differ between urban and in rural environments.

Kaye *et al.* (2005) found that differences in the carbon cycle among land use types had a significant effect on nitrogen cycling and soil microbial biomass, but a less significant effect on the composition of the soil microbial community.

Kaschuk *et al.* (2010) studied soil microbial biomass in Brazilian ecosystems. They found that the soil microbial biomass- carbon ratio and related parameters were useful indicators of soil quality in various Brazilian ecosystems. They concluded, however, that direct relationships between the soil microbial biomass- carbon ratio and nutrient-cycling dynamics, microbial diversity and functionality were still unclear

2.4.2 The nitrogen cycle

Plants need nitrogen in large quantities. Nitrogen is assimilated mostly in inorganic form, as NO_2^- or NH_4^+ . The bulk of nitrogenous materials in soil is organic, and is largely unavailable for consumption by other organisms. The release of the bound element and the mobilization of the organically combined nitrogen are essential to the recycling of the nutrient, and therefore to soil fertility (Alexander, 1976).

Nitrogen mineralization is the sole means of regenerating nitrogen in a form usable for the development of green plants. A consequence of mineralization is that NH_4^+ and NO_3^- are generated and the organic nitrogen disappears. These products constitute two distinct microbiological processes, which are ammonification and nitrification (Alexander, 1976).

Nitrogen is often the limiting factor in ecosystems, making it the primary factor controlling the production of aboveground biomass (Vitousek & Howarth, 1991). In natural ecosystems, carbon sequestration increases with nitrogen additions until nitrogen is no longer limiting (Asner *et al.*, 1997). Nitrogen limitation is determined by biological demand and the ability of soil to accumulate nitrogen, which in turn is attributed partly to site history, climate, soil fertility and vegetation type (Aber *et al.*, 1998). In cultivated ecosystems, nitrogen additions over the long term can increase carbon storage, but due to annual disturbances of soil and the removal of plant biomass, these gains are less than those of natural grassland ecosystems (Pouyat *et al.*, 2007). Under conditions in which nitrogen inputs exceed biological demand, for example the urbanization process, the nitrification process (production of NO_2^-) may increase with the eventual loss of NO_3^- from the system (Vitousek *et al.*, 1982; Robertson, 1982; Aber *et al.*, 1989). Nitrification is thus an important process that predisposes ecosystems to nitrogen loss (Zhu & Carreiro, 1999). According to Pouyat & Turechek (2001), previous studies by Likens *et al.* (1969) and Aber *et al.* (1998) regarding NO_3^- losses suggest that both site history and disturbance of nitrogen-transformations are important when interpreting results of this type of study.

According to Pouyat & Turechek (2001), there are short- and long-term factors that affect nitrogen-mineralization and nitrification in urban environments. Short-term

factors include higher soil temperatures in the urban environment. The higher soil temperatures in urban environments are due to the heat island effect (Oke, 1990). This effect has the potential to increase the nitrogen-transformation rate drastically (Pouyat & Turechek., 2001).

Results from a study investigating long-term effects conducted by Pouyat & Turechek (2001) revealed that the potential net nitrification rates for soils from urban sites were significantly higher than the soils from rural sites. This can be attributed to various factors, including atmospheric deposition of nitrogen and earthworm activity. Earthworms may favour nitrifying bacteria in their competitive interactions with heterotrophic organisms for NH_4^+ -N (Steinberg *et al.*, 1997). The higher nitrification rates suggest that the urban environment has the potential to lose high amounts of nitrogen through soil leaching (Pouyat& Turechek, 2001).

Results also suggest that urban soils mineralize and nitrify nitrogen at a higher rate than rural soils along an urbanization gradient, suggesting that the relatively higher net nitrogen mineralization rate in urban soils is a result of a larger pool of mineralizable nitrogen (Pouyat & Turechek 2001). Larger pools of mineralizable nitrogen may be ascribed to various factors. These include differences in the quality of soil organic matter, earthworm abundance and activity, and nitrogen deposition rates (Groffman *et al.*, 1995; Steinberg *et al.*, 1997; Lovett *et al.*, 2000).

In studies conducted by Pouyat & Carreiro (2003) in urban and rural forests in the United States of America, decay rates of leaf litter collected in urban and rural sites were investigated. Their results indicated that leaf litter collected from rural sites decomposed and mineralized nitrogen more rapidly than leaf litter from urban sites. Leaf litter quantities differ between plant species because of each species' unique biological, biochemical and physiological traits. There are a number of factors that could influence the quality of leaf litter within species. Factors include variation that can occur because of site quality differences that affect plant growth rates and nutrient use efficiencies (Birk & Vitousek, 1986).

There is also growing evidence that atmospheric pollutants can influence litter quality (Findlay *et al.*, 1996) and the rate of litter decay (Inman & Parker, 1978). Most urban

atmospheric pollutants originate from the combustion of fossil fuels as well as industrial emissions. Pollutants include sulphur oxides, heavy metals and nitrogen oxides (Pouyat *et al.*, 2007). High pollutant atmospheric concentrations combined with numerous temperature inversions increase the deposition rates of chemicals into urban areas (Seinfeld, 1989). Urban environments thus receive higher nitrogen depositions than rural environments, and are accompanied by other depositions such as heavy metals.

Normally, poor litter quality input either reduces the rate at which labile carbon mineralises nitrogen, or increases the amount of organic matter transferred to recalcitrant pools (Lamb, 1975). Preliminary measurements of soil carbon pools along an urban-rural transect measured by Pouyat & Carreiro (2003) suggested that recalcitrant carbon pools were higher in urban soils relative to rural soils. These results suggest that urban site factors such as high nitrogen deposition, earthworm abundance and activity, as well as soil temperature tend to accelerate decay and potential nitrogen mineralization rates. Urban litter and quality of soil organic matter tends to reduce the decay rates, and subsequently lower nitrogen availability in urban soils (Pouyat & Carreiro, 2003).

2.4.3 The phosphorous cycle

Phosphomonoesterases have been extensively studied in soil because they catalyze the hydrolysis of organic phosphomonoester into inorganic phosphorous, which can be taken up by plants (Alef & Nannipieri, 1995). These enzymes are classified according to their optimum pH. Acid and alkaline phosphatases play an important role in plant nutrition because their activity in the ectorhizosphere is higher than in bulk soil (Tarafdar & Jungk, 1987), while the content of organic phosphorous shows an opposite trend (Alef & Nannipieri, 1995).

It is hypothesized that the management practices in urban land use might stimulate the phosphatase activity, due to the presence of additional phosphorous sources. Zhang & Wang (2006) studied the effect of regular irrigation of soil on enzyme activity, including phosphatase. They found that regular irrigation stimulated phosphatase

activity, and concluded that higher level of water content favoured an increase in phosphatase activity. According to a study conducted by Hall *et al.* (2009), urbanization may increase the phosphorous demand of microorganisms in urban soils. They found that high phosphatase activity was closely associated with soluble organic matter content.

Clements (1983) found that the invasion of exotic weeds increased with increased soil phosphorous in a study conducted in Northern Sydney, Australia. Work done by Wright (1984), Lambert & Turner (1987) and Leishman (1990) demonstrated the significance of increased soil phosphorous in the decline in native plant communities, and the invasion of exotic species (Riley & Banks, 1996).

2.5 The effect of urbanization on plant communities

Due to the clearing of land for housing and infrastructure during urbanization, natural areas in and around cities are severely impacted through a reduction in extent of natural land, its fragmentation, transformation, dumping and alien species invasions (McConnachie *et al.*, 2008). The process of urbanization thus results in native habitat destruction, and can therefore be considered as a threat to the conservation of biodiversity. Despite this, urban areas often have higher plant species diversity than rural areas due to the invasion of alien species in urban areas (Kuhn & Klotz, 2006) and greater habitat heterogeneity in urban areas (Cilliers *et al.*, 2008). Invasion of plants into new territories may affect the interactions between plant communities and soil organisms, especially when the invading plants have vastly different physiological traits than the native flora (Wardle, 2002). Long-term effects of invader plants may be that quality and quantity of resource inputs to soil can change, and these can have an effect on the decomposer organisms in soil and the processes that they drive (Ehrenfeld, 2003).

The increase of common (alien and native) species, translocation and the decrease in abundance of rare species may eventually lead to biotic homogenization. Factors influencing biotic homogenization are not limited to biological invasions, but may

result from other environmental modifications, including urbanization (Kuhn & Klotz, 2006).

According to Forman (1995) linear grasslands are at great risk of suffering from edge effects that alter vegetation composition by promoting exotic species invasion (Cilliers *et al.*, 2008). Edge effects develop due to changes in the flux of energy, nutrients and organisms across the patch-matrix boundary and can alter the abiotic conditions, species composition, structure and ecological processes of ecosystems on both sides of the boundary (Murcia, 1995).

2.6 The effect of urbanization on soil ecology

Understanding how land use influences soil ecology is a key step towards the development of sustainable landscapes and agricultural systems (Stockdale & Cookson, 2003). Soil biota plays a very important role in the maintenance of soil fertility and productivity (Figure 2.1). Land-use changes can be seen as directly linked to soil use changes. The process of urbanization may lead to the replacement of well-developed soils by functionally altered soils, or even by completely new substrates (Vauramo & Setälä, 2010). This conversion of land and soils to urban use may lead to changes in soil biota. Cultivating practices such as the addition of fertilizers in urban environments have an effect on the microbial component of the soil, and can affect the population, composition, and function of soil microorganisms (Marschner *et al.*, 2003). These alterations have a bearing on the overall functioning of the biotic soil community, and can distort life-supporting ecosystem services, such as the decomposition of organic matter, and the cycling of nutrients (Pouyat *et al.*, 2002; Kaye *et al.*, 2005; Vauramo & Setälä, 2010). To predict the effect of urbanization on the structure and functioning of the entire urban ecosystem, it is important to investigate the effect of anthropogenic influences on entire food webs (Pickett *et al.*, 2001), like the soil food web (Figure 2.1).

The formation of soil and soil organic matter content are heavily influenced by the amount and type of vegetation (Figure 2.1) (Wardle, 2002). The quality and quantity of plant root exudates and aboveground litter have a critical effect on the microbial communities (Marschner *et al.*, 2003; Vauramo & Setälä, 2010). Theoretically plant types that produce readily decomposable, labile litter should favor a bacterial-based

energy channel, with plants producing recalcitrant litter favoring a fungal-based energy channel. It is thus expected that the bacterial-based energy channel will result in a fast energy/material flow, with a slow energy/material flow turnover expected in a fungal-based system (Coleman *et al.*, 1983; Moore & Hunt, 1988; Wardle, 2002; Moore *et al.*, 2005; Rooney *et al.*, 2006; Patterson *et al.*, 2008; Vaurama & Setälä, 2010). Soil microorganisms drive most soil processes, including nutrient availability and retention, decomposition of organic materials, soil organic matter build-up and the stabilization of soil aggregates (Coleman *et al.*, 2004).

The biodegradability of soil organic matter is largely associated with intrinsic organic matter quality factors (e.g. its organic acids, proteins, humic acids, lignin content), soil matrix properties (e.g. pH, oxygen content) and microbial characteristics (e.g. population size, activity, composition) (Cookson *et al.*, 2005). The bioavailability of soil organic matter is related to the spatial and temporal interactions of microorganisms with organic matter. It can be restricted if organic matter is protected in small pores or within soil aggregates not accessible for microorganisms (Cookson *et al.*, 2005). Soil organic matter decomposition will also vary over time. This is due to seasonal changes in organic matter inputs, temperature and moisture availability (Murphy *et al.*, 1998). External factors such as management practices can influence both the biodegradability and bioavailability of soil organic matter. Changes in soil organic matter quantity and quality, pore size distribution, microbial biomass size, activity and composition can all influence processes prevalent in soil (Cookson *et al.*, 2005).

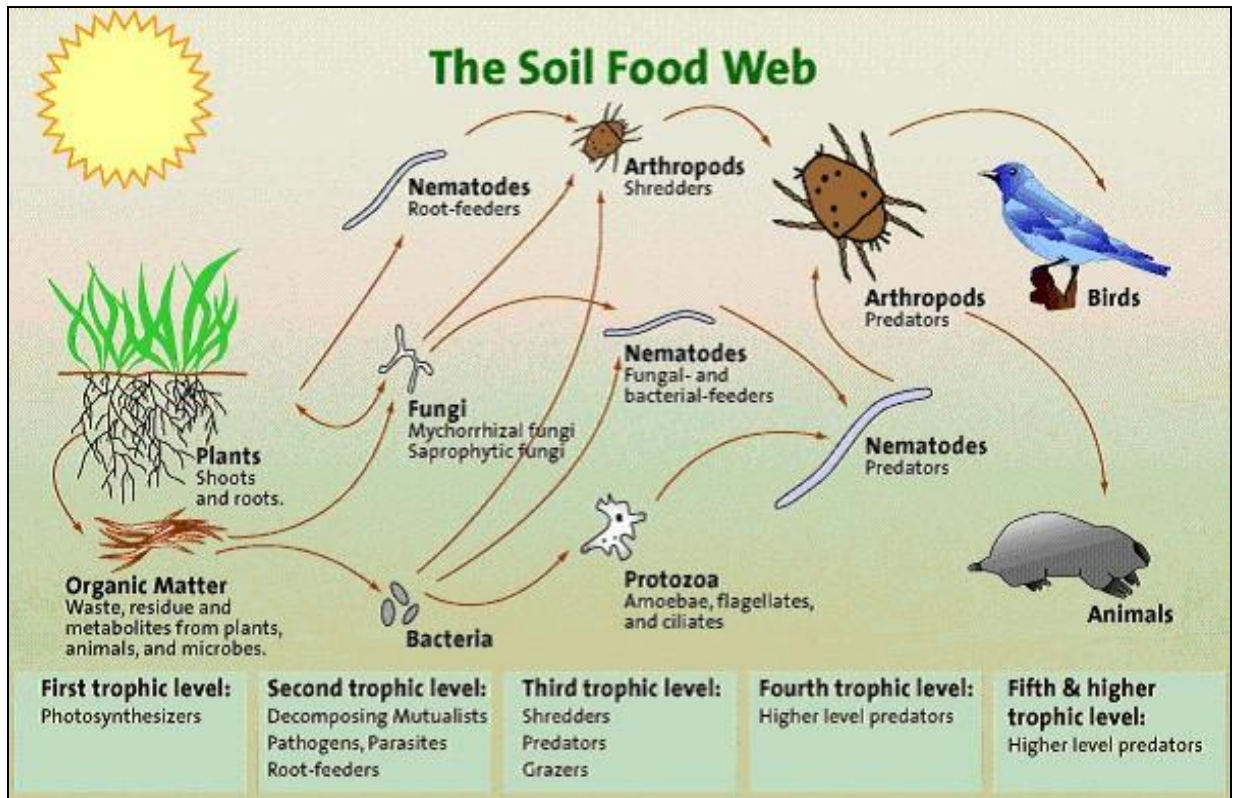


Figure 2.1. Soil food web. The figure indicates the importance of the microbial community in soil food web (Anon, 2008).

Research has reported that microbial community structure is correlated to soil organic-carbon, soil carbon/nitrogen ratio and dissolved organic matter-carbon (Marschner *et al.*, 2003). This finding correlates with the suggestion that changes in microbial community structure are regulated by carbon and nitrogen availability through labile soil organic matter pools (Calderön *et al.*, 2001; Steenwerth *et al.*, 2003). Such a notion would suggest that changes in dissolved organic matter, light fraction organic matter and heavy fragment organic matter pools may possibly influence microbial community structure (Cookson *et al.*, 2005)

Microbes are at the basis of carbon, nitrogen, phosphorous and sulphur cycling in soil. They also contribute towards the decontamination of soil by degrading organic pollutants, or immobilizing heavy metals. Microbial organisms participate in the formation of soil structure, and have positive as well as negative effects on plant growth (Nannipieri *et al.*, 2002). Understanding the key factors that drive microbial species distribution and function in soils is of great importance (Wakelin *et al.*, 2008).

Across different land uses, the range of soil environments is likely to give rise to different microbial communities (Bossio *et al.*, 2005). Previous studies by Priha *et al.* (2001) and Baath & Anderson (2003) indicated that soil pH is a major determinant of soil microbial community composition. The different groups of soil microorganisms have different pH optima, and therefore the soil pH mediates the microbial community composition (Shah *et al.*, 1990). This suggests that vegetation type, management practices and ecological effects of the land use are also important regulators of microbial community composition. A study conducted by Cookson *et al.* (2007) indicated that vegetation type and management practices (which control soil pH and mediate dissolved organic matter availability) are important regulators of gross nitrogen fluxes and microbial community composition. Results obtained by these authors also indicated that changes in microbial activity represented a shift in the composition of the microbial community, suggesting that the microbial composition and ecosystem function are linked (Cookson *et al.*, 2007).

Healthy soil comprises of highly diverse soil biota (Asuming-Brempong *et al.*, 2008). Studies investigating the effects of urbanization on other groups involved in the soil food web have been done. McDonnell *et al.* (1997) studied a forest ecosystem along an urbanization gradient in the U.S.A. They found that the forest at the urban end of the gradient exhibited reduced fungal and microarthropod populations and poorer leaf litter quality than the forest at the rural end of the gradient. However, they found that the possible negative effects on ecosystem functioning, like decomposition and nitrogen-cycling were ameliorated by other anthropogenic influences. These influences were the heat island effect and the introduction and successful colonization of earthworms. They found that decomposition and nitrification rates were in fact higher in the urban areas than the rural areas.

McIntyre *et al.* (2001) studied ground arthropods in urban environments in the U.S.A. They found that although taxonomic richness was comparable across land uses, the community structures differed, and concluded that different habitat structures created by urbanization influenced the arthropod communities.

Jonas (2007) studied epigeal arthropods assemblages along the same urbanization gradient as used in this study. He found that urbanization had a defined impact on the

epigeal arthropod diversity along the urbanization gradient. Of the arthropods found in the study area, ants were the dominant group collected. The species found in urban areas were generalist and opportunistic species that thrive in altered or disturbed habitats. He concluded that the ant group was the most suitable group to serve as a biological indicator in future disturbance studies in grasslands in South Africa when studying epigeal arthropods

2.7. The effect of urbanization on enzyme activities

Microbes colonize and chemically break down organic matter through the utilization of enzymes (Nannipieri *et al.*, 2002). Soil enzyme activities are involved in soil nutrient cycling dynamics and can catalyze the conversion of nutrients to forms readily available for use by plants and microorganisms (Egamberdiyeva, 2006). It is reasonable to suggest that enzyme activity in soil may be used as a research tool in order to assess microbial functional diversity, to study biochemical processes, to investigate microbial ecology, and to provide indicators of soil quality (Zhang & Wang, 2006).

Soil enzyme assays are process-level indicators and are presented as a means of determining the potential of soil to degrade or transform substrates. This can be very useful to indicate the effectiveness of soil to carry out important steps in nutrient cycling, as well as other processes (Dick, 1994), and can be used as an index of microbial functional diversity (Nannipieri *et al.*, 2002). It is important, however, to choose the correct enzymes in the context of the soil parameters to be investigated, and to interpret the results correctly (Zhang & Wang, 2006).

The conceptual rationale for soil enzyme activity as a soil quality indicator is extensive. Concepts include that enzymatic activities are often related to important soil quality parameters. These parameters include organic matter, microbial activity or biomass, and soil physical properties, and that enzymatic activities can change sooner than other properties (e.g. soil, organic carbon). Enzyme activity can provide an early indication of the effect that certain soil management practices have on the quality of the soil. Therefore, it can also serve as a biological index of past soil management (Dick, 1994).

Soil enzymes are believed to be mostly of microbial origin, and are associated with viable cells. There are, however, many enzymes that remain catalytic in cell debris, in soil solution or complexed with clay or organic colloids. The ecological role of these enzymes has not yet been determined, but Burns (1982) has hypothesized that humic-enzyme complexes may benefit some organisms by hydrolyzing substrates that are too large or insoluble for microbial uptake.

The conversion of rural lands into urban areas affects the local air quality and energy balance significantly. Cities are large consumers of fossil fuels. This state of affairs results in urban areas being responsible for the largest proportion of anthropogenic emissions such as carbon dioxide and nitrous oxides (George *et al.*, 2007). The effects relating to this loading on equilibrium levels on soil organic matter are of major interest (Tate, 2002). Generally, it is noted that the increasing amount of carbon dioxide available to plants for photosynthesis increases biomass production (Rosenzweig & Hillel, 1998). Any increase in plant biomass production is likely to stimulate soil microbial activity. The increased availability of energy and carbon will stimulate carbon and nitrogen cycling enzymes. Enzyme activities associated with carbon and nitrogen transformations are commonly linked to the levels of microbial activity. This highlights the potential to use the duration and/or magnitude of change in the enzyme activities in order to predict positive and negative changes in the system - in terms of soil quality, ecosystem stability and function, and environmental risk (Tate, 2002).

2.8 Aboveground and belowground interactions

All terrestrial ecosystems consist of aboveground and belowground components that interact to influence community and ecosystem-level processes and properties (Wardle *et al.*, 2004). It is well understood that plants and microbes are interdependent on each other for nutrient supply (Singh *et al.*, 2004). Plants provide organic carbon required for the functioning of the decomposer subsystem, as well as the carbon resources needed for obligate root-associated organisms. The decomposer subsystem in turn breaks down dead plant material and indirectly regulates plant growth and community composition by determining the supply of available soil nutrients. Root-associated organisms influence plants more directly. They also have a bearing on the quality, direction, and

flow of energy and nutrients between plants and decomposers (Wardle *et al.*, 2004). The mechanistic basis by which primary productivity is regulated by belowground interactions is understood (Wardle, 2002). This influences plant communities and plant function, directly or indirectly. Direct influences can have a negative feedback effect on plant growth where parasites, pathogens and root herbivores directly remove carbon in the rhizosphere of the plant (Bever *et al.*, 1997). Positive feedback occurs when mutual symbionts such as mycorrhizal fungi enhance access to limiting nutrients for the plants, stimulating plant growth (Smith & Read, 1997). An example of indirect influences is when the decomposer community liberates nutrients locked up in dead organic material, and thus increases plant productivity (Setälä & Huhta, 1991; Moore *et al.*, 2003).

It has been recognized that soil organisms are responsive to the nature of organic matter that enters the decomposer subsystem (Swift *et al.*, 1979). Plant species differ both in terms of the quantity and quality of resources that they return to the soil. Individual plant species may have important effects on components of the soil biota and the processes that they regulate (Wardle *et al.*, 2004). Effects include differences in microbial community diversity and biomass, altered nutrient cycling and varying soil enzyme activity (Dornbush, 2007). Plant community composition has a significant influence on the community composition of root-associated organisms (Yeates, 1999; Tscherko *et al.*, 2005). The effects of plant community composition on the decomposer communities are fairly context-dependent (Mikola *et al.*, 2002; Wardle *et al.*, 2004). Intimate interactions between plants and the microbial community may be direct drivers of plant community diversity (Van der Putten, 2003). However, the effects of the biodiversity of these soil organisms have rarely been studied (Wardle *et al.*, 2004). Evidence available indicates that the effect of soil biodiversity on aboveground attributes can range from positive to negative, depending on the context (Wardle, 2002; Mikola *et al.*, 2002).

A conceptual framework for predicting how the diversity of decomposer organisms may influence plant diversity is presented in Figure 2.2. When decomposers influence nutrient availability, the response curve regarding plant species diversity to site fertility changes. If soil decomposers enhance nutrient availability, the plant diversity is easier

to be established even though the site fertility is low and vice versa (Wardle *et al.*, 2004).

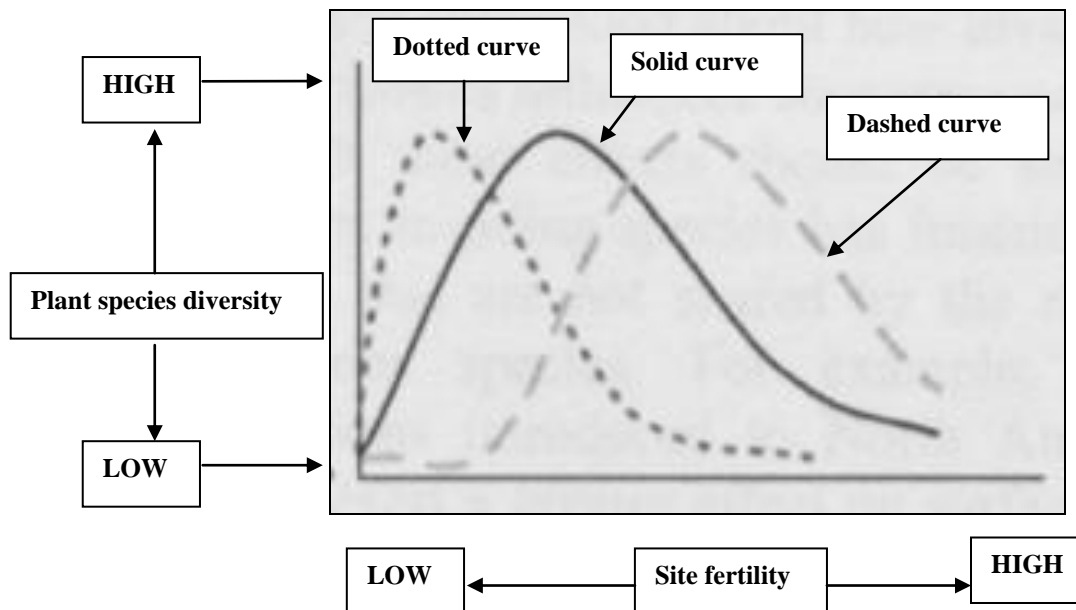


Figure 2.2. Conceptual framework illustrating the relationship between underlying site fertility and plant species diversity, in which diversity is maximized by intermediate fertility (Al-Mufti *et al.*, 1976).

This results in plant diversity being maximized in less fertile sites. Alternatively, when decomposers reduce nutrient availability, local plant diversity shifts from the solid curve to the dashed curve, indicating maximum fertility in the more fertile sites (Figure 2.2). The framework suggests that the aboveground consequences of soil biodiversity are strongly dependent on context. Specialist soil organisms (organisms intimately associated with plant roots) are predicted to have stronger effects on the aboveground diversity than low-specificity organisms such as the decomposer organisms (Wardle *et al.*, 2004). The effects of decomposer diversity may be unpredictable (Lawton, 1994), because diversity may enhance or reduce the availability of nutrients to plants. Such effects depend on the initial site fertility. In practice, net primary productivity is probably relatively insensitive to decomposer diversity because of the generalist feeding behavior of most consumers in the soil subsystem (Setälä *et al.*, 2005).

It is important to understand aboveground-belowground interactions in ecosystems. Insights gained in this way can be used to improve our predictions of the effects of human-induced environmental changes on biodiversity and ecosystem properties. This, in turn, can enhance the efficiency of human interventions in restorations and conservation efforts (Wardle *et al.*, 2004).

2.9. Analytical techniques

2.9.1 Urban-rural gradient studies

Using the urbanization gradient approach can contribute towards the understanding of anthropogenic influence on ecosystems in the urban-rural environment. According to McDonnell and Pickett (1990), the gradient paradigm can be summarized as the view that environmental variation is ordered in space. The spatial environment patterns govern the corresponding structure and function of ecological systems. These could be populations, communities or ecosystems.

A conceptual framework is presented where anthropogenic and biophysical ecosystem characteristics form a continuum (Figure 2.3). The framework incorporates the importance of built structures and the effect of impervious surfaces on ecosystem processing. At the urban core, engineered flow paths (c and d) disconnect material inputs and flows (a) from natural processing that occurs in rural ecosystems (Figure 2.3). In these areas, the connectivity between the engineered and biophysical templates is low (b). In residential areas, the connectivity between engineered and biophysical templates can be relatively high, depending on the spatial relationship of impervious and pervious surfaces. Management and environmental inputs in residential areas can be high (a and e) on a per-unit pervious area (Figure 2.3). Depending on site history, soil type, and the concentration of flows, these areas can have surprisingly high cycling rates (F) for processing or storing these inputs (Kaye *et al.*, 2006).

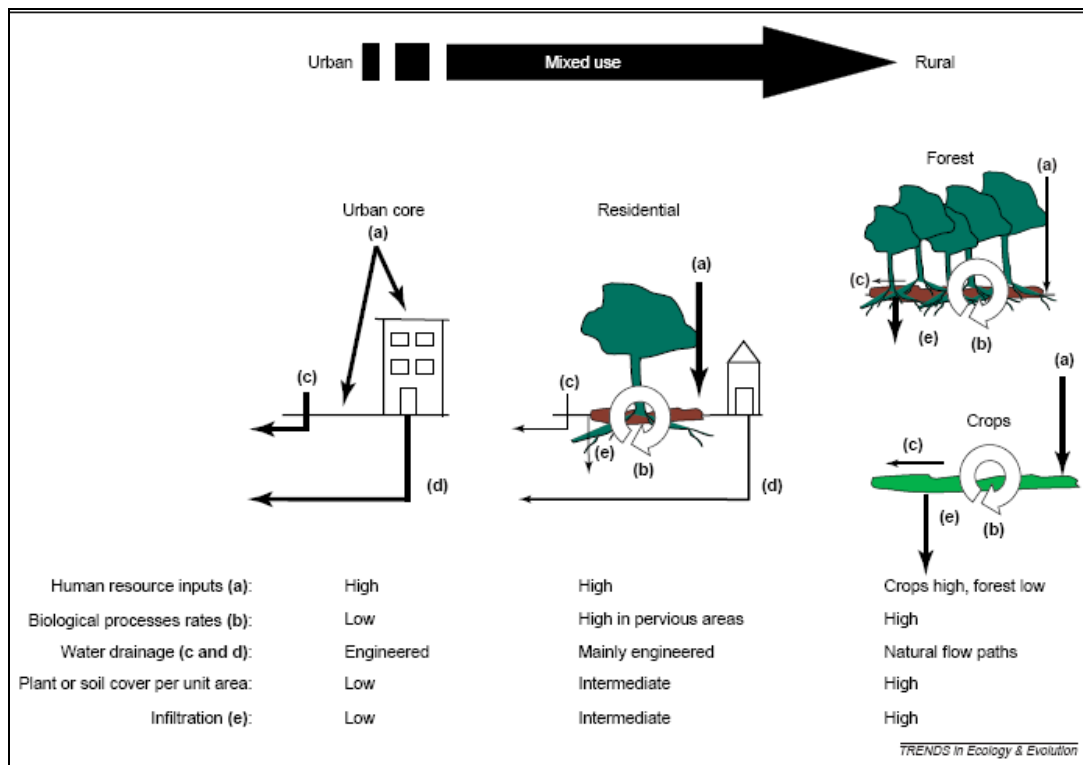


Figure 2.3. Conceptual framework illustrating the gradient from urban to rural environments (Kaye *et al.*, 2006).

Figure 2.3 clearly illustrates the spatial environmental variation in the different settings, ranging from urban to rural. It also illustrates the effects that these spatial variations have on the environment, and the effect they have on an ecosystem. The urban-rural gradient can thus be used to assess how urbanization is changing ecological patterns and processes across the landscape. According to Niemelä (1999), examining the differences in ecological processes between urban and rural environments is an especially fruitful approach for urban ecological research. A disturbance gradient from urban through sub-urban to rural environments is an effective tool for studying the effects of urbanization on ecological studies (McDonnell & Pickett, 1990).

Many studies have been conducted using the urban-rural gradient, including studies of arthropods (Niemelä *et al.*, 2002), birds (Simon *et al.*, 2007), plants (McDonnell *et al.*, 1997), amphibians and reptiles (Gibbs, 1997), leaf litter decomposition and nutrient cycling (Pouyat *et al.*, 1997), pollution (Wong *et al.*, 2004), water quality (Pantshwa, 2006), and the structure of landscape elements (Medley *et al.*, 1995). Studies regarding

microorganisms have also been done (Pouyat *et al.*, 1994; Vilisics *et al.*, 2007), but more investigation is necessary (McDonnell & Hahs, 2008).

Soil microbial communities are very important in normal ecosystem functioning, and have important interactions with the different biogeochemical cycles. Although the biogeochemical effects of agricultural land conversions and their recovery after abandonment have been relatively well studied, conversions to urban land uses have not been as thoroughly investigated (Pouyat *et al.*, 2002). Studying microbial parameters will assist towards a more profound understanding of the effects of urbanization on ecosystems. For this reason, it is important to investigate the microbial component of ecosystems along an urbanization gradient, and to investigate how it correlates with plant community data. This may yield a measure of insight into the microbial community structure and function, and the effect that the microbial and the plant communities, respectively, have on each other along an urbanization gradient.

2.9.2 The V-I-S model

In order to study ecosystems along the urban-rural gradient, the urban-rural gradient must be quantified. The Vegetation-Impervious surface-Soil (V-I-S) model of Ridd (1995) quantifies the urban-rural gradient. This approach has been successfully employed in various other studies, including the study of epigeal arthropods (Jonas, 2007), vegetation cover (Buyantuyev *et al.*, 2007), and faecal pollution and occurrence of antibiotic resistant bacteria in water (Pantswa, 2006).

The Vegetation-Impervious surface-Soil model (Ridd, 1995) proposed that the combination of impervious surface material, green material and exposed soil are the most fundamental components of the urban ecosystem, in terms of contrast to the surrounding environment as well as contrast within the city (Ridd, 1995). The Vegetation-Impervious surface-Soil model (Figure 2.4) consists of the S-V axis, the V-I axis, and the I-S axis. The S-V axis represents land that is not yet urbanized or land that is undergoing a number of changes. There are few to no impervious surfaces in non-urban/pre-urban environments. The V-I axis represents a typical residential sequence, and the I-S axis represents the traditional commercial and industrial areas.

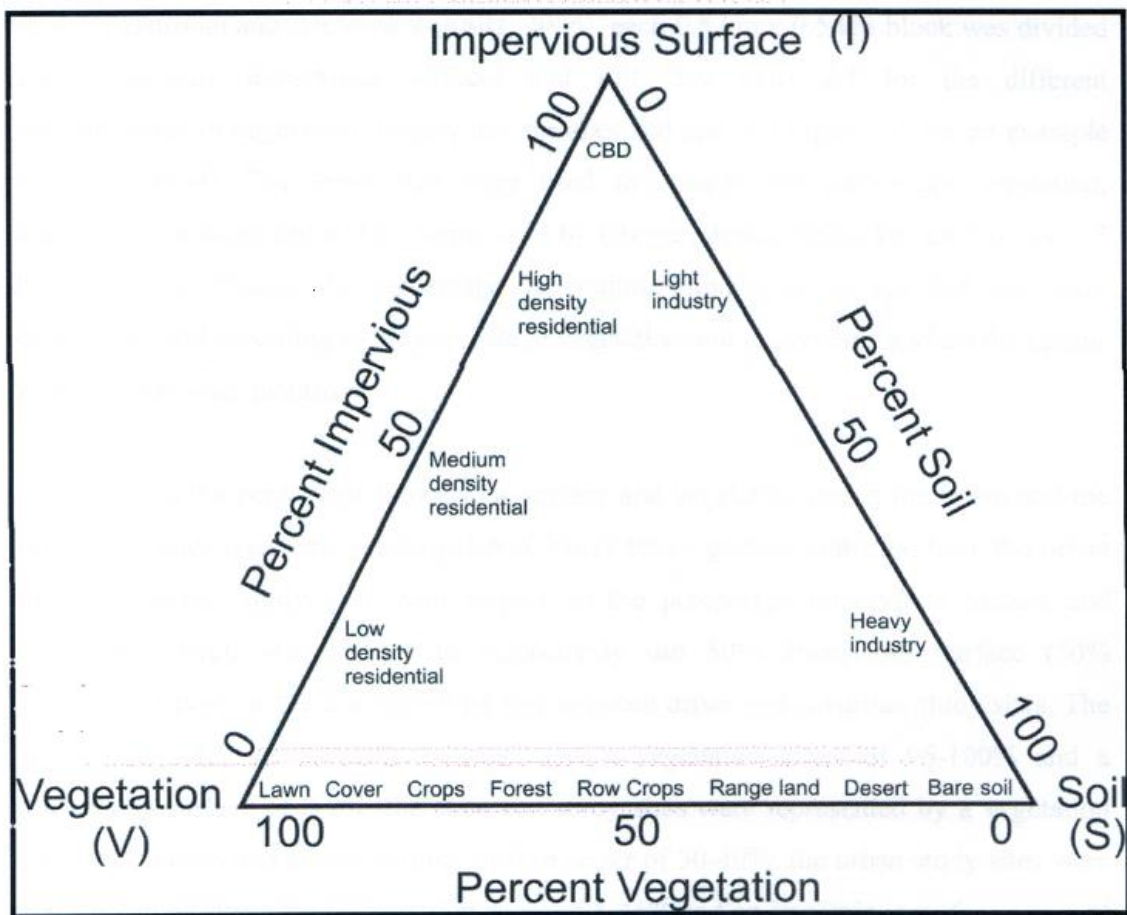


Figure 2.4. V-I-S model of Ridd (1995) used to quantify the urbanization gradient.

The objective of this model is to identify and characterize variable land cover patterns, and not to identify land use. For this method of quantification, remote sensing is used to classify different study sites into urban or rural areas. Remote sensing divides the earth's surface into remote sensing lineage pixels. By classifying the image pixels into vegetation, impervious surface or soil, the degree of urbanization could be determined (Ridd, 1995).

2.10 Vegetation analysis techniques

The methods selected for investigating vegetation varies depending on a number of factors. Factors influencing the selection of analysis techniques include the purpose, scale, overall habitat type and available resources of the study. Techniques of

vegetation description can be based on physiognomy and structure of the vegetation, habitat classification or floristics. These various techniques can either yield qualitative or subjective data (Kent & Coker, 1992).

Information gathered regarding the vegetation of a study area can be very useful. Information on vegetation may help to solve ecological problems, and such information is important for biological conservation and management purposes. It also constitutes an essential input towards environmental impact statements and with a view to monitor management practices. Information gathered in this manner can also be used as a basis for predicting possible future changes (Kent & Coker, 1992).

2.11 Microbial analysis techniques

2.11.1 Microbial population study in soils using culture-dependent techniques

Different techniques can be used to assess microbiological parameters. Culture-dependent techniques rely on the cultivation of the microorganisms on selected media by growing the colonies on the media in a controlled environment. To investigate viable heterotrophic microorganisms, various non-selective media can be used. Samples are suspended in a suitable dilutant, for example 0.8% saline solution. This is diluted and spread-plate or pour-plate inoculated.

The plates are incubated according to protocol. Colonies formed can be counted in order to obtain the number of viable bacterial cells (colony forming units). Taking the dilution factor into account, the bacterial levels are expressed as colony forming units per gram soil (cfu/g soil). Colony characteristics can be used to classify the colonies according to colony morphology. The streak-plate technique and the spread-plate technique are widely used to isolate and purify colonies from samples containing multiple organisms (Olsen & Bakken, 1987).

The cfu/g soil is an indicator of the abundance of heterotrophic bacterial cells and fungal cells capable of replicating in a specific media, and is a useful comparative

assessment of different samples. Heterotrophic plate counts have been used successfully in a number of studies. Olsen & Bakken (1987) optimized the plate count method for soil studies. They compared plate count results with total microscopic counts, and concluded that the plate counts were representative of about 2-4% of the total microscopic counts. Although this represents a small percentage, the technique is useful in comparing culturable organisms to each other in soil studies. Li *et al.* (2008) used the plate count method to assess the effect of certain pesticides on the fungi in the soil. Different concentrations of the pesticide were used as test treatment on the fungi, and the plate count method was used successfully to assess the effects on the fungal cfu/g soil.

Culture-dependent techniques have limitations, due to the fact that only viable cells can be accounted for. Culture-based approaches can be very useful for understanding the physiological potential of isolated organisms. However, they do not necessarily provide comprehensive information on the composition of microbial communities (Amman *et al.*, 1995; Orphan *et al.*, 2000). It is widely recognized that microbial diversity is underestimated when using culture-dependent approaches (Hori *et al.*, 2006). Due to documented imbalances between cultivatable and *in situ* diversity, it is often difficult to assess the significance of cultured members in resident microbial communities (Orphan *et al.*, 2000). Ward *et al.* (1990) employed culture-independent molecular methods to show that cultivated microorganisms from both temperate and extreme environments often may represent very minor components of the microbial community as a whole. Many of the recent studies regarding microbial diversity include culture-dependent and culture-independent methods to complement each other (Aminin *et al.*, 2008; Zhang *et al.*, 2009).

Purified cultures representing different phenotypes from the culture-dependent methods can be identified by means of molecular methods. DNA is extracted by means of techniques like a CTAB-chloroform method, or it can be extracted through commercial DNA extraction kits. PCR is performed on the extracted DNA using an appropriate primer to amplify, for example, 16S rRNA gene fragments. After this process, the PCR product is sequenced. Results are compared to data in GeneBank (for example using the BLASTN database), and isolates are identified accordingly. Identification of organisms by sequence results has been achieved successfully in previous studies. Smit *et al.*

(2001) investigated the diversity and seasonal fluctuations of the dominant members of the soil bacterial community. Soil samples were collected in a wheat field in the Netherlands. Dominant bacterial species were isolated, and DNA was extracted for 16S rDNA PCR. The PCR products were sequenced, and identification was done by screening sequencing results against GeneBank using BLAST. They found that there was an even distribution among five main divisions, *Acidobacterium*, *Proteobacteria*, *Nitrospira*, cyanobacteria, and green sulphur bacteria in the bacterial soil community. Statistical analysis of the banding patterns of the DGGE gel revealed significant differences between samples taken in different seasons. Xu *et al.* (2008) used bacterial isolates that were hard to identify phenotypically. These isolates were obtained from a clinical laboratory environment. DNA was isolated, and 16S rDNA PCR was done. The PCR products were subsequently sequenced and identified by screening sequencing results against GeneBank using BLAST.

2.11.2 Microbial population study in soils using culture-independent techniques

Most soil organisms cannot be cultured in laboratory environments. Culture-independent techniques do not rely on the cultivation of microorganisms on media in a controlled environment. Therefore, it is a more reliable way of investigating the microbial component in soil environments (Felske *et al.*, 1998).

To assess the microbial community structure, various molecular techniques can be used. Extracting microbial community DNA with commercially available kits constitute the first step. Using the PCR technique, extracted DNA can be amplified using specific primers to fill all needs for downstream applications. It is very important, though, to have high quality DNA for further downstream PCR applications. Thakuria *et al.* (2008) demonstrated that multiple steps of DNA purification should be included in the extraction process to optimize DNA quality. Results from the study indicated that it is essential to extract high quality DNA. They also stated that there is probably not one specific DNA extraction method that yields high quality DNA for all soil types, but quality assurance should help optimize the DNA extraction method.

Many other culture-independent methods have been used for the investigation of microbial community characteristics. These techniques include community-level physiological profiling (CLPP), phospholipids fatty acid analysis (PFLA), and nucleic acid examination such as terminal restriction fragment polymorphism (T-RFLP), denaturing gradient gel analysis (DGGE), and single-strand conformation polymorphism (SSCP) (Kowalchuck *et al.*, 2004). A short description of the nucleic acid examination technique DGGE is presented in paragraph 2.11.2.1.

Using fingerprinting techniques have advantages. Fingerprinting techniques may be less selective than culture-dependent techniques, and provide a more representative view of a microbial community structure (Reysenbach *et al.*, 1992). Large numbers of samples can be analysed and compared, making them the ideal tool for ecological studies (Smalla *et al.*, 2007). Potential limitations of fingerprinting techniques include that biases may be introduced during sample collection, and that selection of certain DNAs may occur during cloning steps (Reysenbach *et al.*, 1992). Factors may be present that influence the resolution of the fingerprinting technique, masking some of the information (Smalla *et al.*, 2007).

2.11.2.1 Denaturing Gradient Gel Electrophoresis (DGGE)

The DGGE technique is a PCR-based fingerprinting technique. DGGE separation of the PCR amplicons depends on the denaturation of double-stranded DNA in the gel containing DNA denaturants (Muyzer *et al.*, 1993). DGGE analysis is used for the separation of double-stranded DNA fragments that are identical in length, but differ in sequence. The technique exploits the difference in stability of G-C pairing as opposed to A-T pairing. DGGE implies that a mixture of different DNA fragments be electrophorized in an acrylamide gel containing a gradient of increasing DNA denaturants. Typically, fragments richer in GC will be more stable, and will remain double-stranded until reaching higher denaturant concentrations. Double-stranded DNA fragments migrate faster through the acrylamide gel, while denatured DNA becomes larger and stop in the gel. By means of this mechanism, DNA with differing sequence can be separated in acrylamide gel (Green, 2005). Bands can be excised from the

DGGE gels, and sequenced. The DGGE process can thus provide diversity data as well as identifications of individual species.

The technique has a variety of applications, including soil studies. Nakatsu *et al.* (2000) used the technique to investigate microbial community composition in bulk soil samples collected in agroecosystems in Norway and the U.S.A. Campbell *et al.* (2009) used the PCR-DGGE technique to compare bacterial community structure of fresh and archived soil samples collected along a Chihuahuan desert elevation gradient.

2.12. Enzyme assays

The assay of a number of specific enzymes is a useful culture-independent technique to assess microbial community properties. Soil enzyme activities reflect potential activity rather than actual *in situ* activity. This is due to some important factors. These factors include the contrasting conditions of the assay relative to the sampled site, the various enzyme sources, and the possible confounding chemical reactions that affect the measured activity (Nannipieri *et al.*, 2002). An example is dehydrogenase. This enzyme is considered an intracellular enzyme. Some dehydrogenase enzymes may be located extracellular in soil due to cell lyses, and may also be associated with organic matter or soil colloidal surfaces (Nannipieri *et al.*, 2002). These factors must be considered in the interpretation of results and with the assumption that results are relevant in characterizing the functional activity of the microbial community present in the soil on the sampling date (Udawatta *et al.*, 2009). The advantage of measuring potential enzymatic activity through the use of buffered and optimized methods have the advantage of standardising environmental factors, which allows for comparison of soils from different geographical locations and environmental conditions (Schloter *et al.*, 2003). Enzyme activities often correlate with other indicators of activity such as soil respiration, ATP content and microbial biomass (Dick, 1997). Soil enzyme activities can also provide a measure of insight into the metabolic capabilities of soil microbial communities by assessing the potential for transformation of specific sources of energy or nutrients (Shaw & Burns, 2006). By performing this, an indication of the relative availability or limitation of particular energy or nutrient sources in the environment can be achieved. The activity of carbon and nitrogen cycle enzymes in soil has been used to

assess soil ecosystem adaptation to anthropogenic intervention and the effects of reclamation management decisions (Tate, 2002).

The interpretation of enzyme assays should be done with caution. When interpreting the results proper consideration needs to be made, because soil enzyme assays are usually done under optimised conditions. Measured activity includes the integrated activity of enzymes from both living and dead organisms, as well as stabilized extracellular enzymes (Burns, 1978).

Enzymes that are useful to study include:

- **Dehydrogenase:** Dehydrogenase is an important enzyme in the carbon cycle, and is present in all viable organisms. For this reason the assays of dehydrogenase activity are considered an accurate measure of the soil microbial oxidative capacity (Dick, 1994). Therefore dehydrogenase activity in soil has been used to measure for overall microbial activity (Alef & Nannipieri, 1995). Dehydrogenase assays are commonly used in studies to assess microbial function in soils. The Iodonitrotetrazolium chloride (INT) method used in this study is based on the incubation of soil with the substrate INT at 40°C for two hours, followed by the colorimetric estimation of the reaction product Iodonitrotetrazolium chloride-formazan (INF) (Von Mersi & Schinner, 1991). This method has been used successfully in previous studies. Trevors (1984) used the INT method to investigate dehydrogenase activity in sandy loam under various incubation conditions. He found that there was a high positive correlation between dehydrogenase activity and substrate concentration, incubation temperature, and soil pH. He also found that dehydrogenase activity displayed a high negative correlation with O₂ concentrations. Hopkins *et al.* (2008) investigated enzymatic activities in Antarctic dry valley soil. They used the INT method to investigate the potential dehydrogenase activity, and found that the potential activity remained unchanged when large amounts of carbon and nitrogen detritus were added to the soil samples. Potential dehydrogenase activity were, however, unchanged or diminished by the addition of only nitrogen detritus, or when a large amount of nitrogen detritus were added with a

smaller amount of carbon detritus. They concluded that in the presence of large amounts of nitrogen, the carbon supply for biosynthesis was limited in their study.

- **β -glucosidase:** β -glucosidase is also an important enzyme in the carbon cycle. It is the rate limiting enzyme in microbial degradation of cellulose to glucose (Alef & Nannipieri, 1995). β -glucosidase activity has been found to be sensitive to soil management in various studies, and has been proposed as a soil quality indicator (Ndiaye *et al.*, 2000). The method used in this study is based on the colorimetric determination of the *p*-nitrophenol released by β -glucosidase when soil is incubated with buffered (pH 6.0) *p*-nitrophenyl- β -D-glucosidase. The *p*-nitrophenol released is extracted by filtration and determined colorimetrically (Dick *et al.*, 1996). This method has been used successfully in previous studies. Monkiedje *et al.* (2006) investigated the effects of land use on soil health indicators in peri-urban agriculture in the humid forest zone of southern Cameroon. They found that the potential β -glucosidase activity were higher in the peri-urban samples, and concluded that the land use types and associated practices were responsible for the higher potential β -glucosidase activity. Hall *et al.* (2009) investigated the effects of urbanization on soil microbial functioning in the Sonaran desert. Their investigation included β -glucosidase assays. They found that potential β -glucosidase activity was higher under plants than in the inter-plants spaces in outlying deserts. They concluded that patch type was more important in the urban environment in explaining the enzyme activity.
- **Alkaline- and Acid Phosphatases:** Phosphatases catalyze the hydrolysis of phosphorous esters and are enzymes with relatively broad specificity (Alef & Nannipieri, 1995). Alkaline and acidic phosphatases are so called because of the optimum pH range at which each enzyme functions. Phosphomonoesterases have been extensively studied because they catalyze the hydrolysis of organic phosphomonoester to inorganic phosphorous, which can be taken up by plants (Alef & Nannipieri, 1995). Phosphatase activity procedures used in this study are based on a similar principle as the β -glucosidase assay in that hydrolysis of *p*-nitrophenyl phosphate releases *p*-nitrophenol, which is extracted quantitatively from soil and measured colorimetrically (Dick, 1996). Hall *et al.*

(2009) investigated the effect of urbanization on soil microbial function in the Sonoran desert. They found that phosphatase activity were higher in urban soils samples than in all desert soil samples in their study. They concluded that microbial processes in arid soils are responsive to human activities through creation of managed urban landscapes, and through certain factors associated with the urban environment that increase carbon and nitrogen supply. Garcia-Ruiz *et al.* (2008) investigated the growing interest in the application of soil enzymes, including acid- and alkaline phosphatases, as early indicators of soil quality change. This was done under contrasting agricultural management practices. They found that organic management resulted in significantly higher potential enzyme activities, and that tillage intensity slowed down the improvement of soil quality in organic crops.

- **Urease:** Urease catalyses the hydrolysis of urea to CO₂ and NH₃, and is widely distributed in nature. This process is important for plant nutrition. It is present in microbial, plant and animal cells (Alef & Nannipieri., 1995). The urease assay used in this study is based on the colorimetric determination of released ammonia after the incubation of soil with a urea solution for two hours at 37°C (Kandeler & Gerber, 1988). Roscoe *et al.* (2000) investigated the relationship between urease activity, soil organic matter, microbial biomass, nitrogen content, and urea-nitrogen fertilizer assimilation. The study was done on maize in Dark Red Latosol that was cultivated for nine years under no-tillage, tillage with a disc plough, and tillage with a moldboard plough. Two depths were sampled at 4 different times during the crop cycle. Urea was applied four times during the study, at different rates. They found that the fertilizer did not affect potential urease activity. Potential urease activity was significantly higher in the no-tillage system. They concluded that the tillage treatment of the soil influenced the urease activity the most. Lorenz & Kandeler (2005) biochemically characterized the urban soil profiles from Stuttgart, Germany. They found the urease activity decreased with depth, although in three profiles the potentially mineralizable nitrogen was higher in the deeper layers of the soil than the overlaying soil layers. They concluded that the processes involved in urbanization affected the urease activity.

2.13. Statistic analysis tools and techniques

2.13.1 Diversity indices

Various indices are used to interpret data in ecological research. Indices include the Simpson index and the Shannon-Weaver index. The Simpson index (Simpson, 1949) is a measurement that accounts for the species richness and the percentage of each species from a biodiversity sample. The Simpson's index is published in three different ways in ecological research. In all three variations, the first step is to calculate P_i , which is the number of a given species divided by the total number of organisms observed.

The Shannon-Weaver index (Shannon & Weaver, 1949) is similar to the Simpson index, but this measurement takes into account species richness and the proportion of each species within the community. The formula is:

$$\text{Shannon-Weaver index (H)} = - \sum (P_i \ln [P_i])$$

H = Index value

P_i = The number of a given species divided by the total number of organisms observed.

The Shannon-Weaver index has been used in numerous studies in order to determine diversity in a wide range of scientific fields. Zilli *et al.* (2004) investigated cowpea rhizobium diversity in Brazil. Morphological traits were used to classify the different types of rhizobium. The data gathered were used to determine Shannon-Weaver diversity indices for the rhizobium. Zul *et al.* (2007) investigated the effects of plant biomass, plant diversity, and water content on bacterial communities in soil lysimeters. They used the PCR-DGGE technique to analyze the bacterial communities present in the soil samples. They used ONE-D scan electrophoresis analysis software (Scanalytics, Billerica, MA) to determine the Shannon-Weaver diversity indices from the DGGE image, using the relative abundance (intensity) of the bands of the gel. Romane *et al.* (2001) studied plant species diversity of chestnut ecosystems in France. They used the plant species diversity data obtained from quadrats in their study area to estimate Shannon-Weaver diversity indices.

Information gathered from molecular techniques can be used to determine diversity using the Shannon-Weaver index. Relative fluorescence (staining intensity of the bands) is measured using specialized software by plotting the pixel density along the molecular profile. Results indicated a peak pattern of which individual peaks and the baseline have to be determined. Subsequently relative fluorescence values can be obtained for individual bands. These molecular technique-derived values for genetic richness and abundance (defined as relative fluorescence of bands on the molecular profile) can be used to calculate diversity indices, such as the Shannon-Weaver index (Paul, 2001).

2.13.2 Multivariate analysis

Data can be analyzed through the CANOCO-program using several ordination techniques (Ter Braak, 1992). The CANOCO-program allows for quick determinations of how community composition varies with the environment using correlation coefficients to analyze the data (Ter Braak & Smilauer, 1998).

Ordination is the arrangement of all the data in relation to each other in terms of their similarity in species composition and their associated environmental controls (Kent & Coker, 1992). For this reason, ordination can be used in gradient analysis. There are many types of ordinations, including detrended correspondence analysis (DCA), principle component analysis (PCA), canonical correspondence analysis (CCA), and redundancy analysis (RDA).

PCA ordinations are widely used in order to synthesize environmental data. According to Kent and Coker (1992), the PCA ordination is one of the best methods for analysis in this regard. PCA ordination consists of two variables that are ordinated together (Bi-plots), where CCA ordinations consist of three variables that are ordinated for comparison (Tri-plots). According to Ter Braak and Verdonschot (1995), CCA ordination triplot diagrams can consist of points representing species and sampling sites, and arrows for quantitative environmental variables. Each species point in the diagram is positioned to represent the average of the sampling site points in which it occurs. Individual arrows represent the quantitative value for each environmental

variable. The length of each arrow indicates the correlation of the variables with the ordination axes. Four possible alternatives exist that explain the type of abundances that can occur in a CCA diagram (Ter Braak & Verdonschot, 1995).

1. Centroid principle: This entails that species are at the centre of their niche habitat and therefore sampling sites that contain a particular species are scattered around that specific point. This also means that sampling sites close to the species point tend to have a higher abundance of that species than sites further away from that species point.
2. Distance rule: The abundance of a species is highest when the sampling site point coincides with the species point and decreases in all directions from the site point.
3. Biplot rule: This rule states that an arrow representing an environmental variable points in the direction of maximum change for the associated variable, and that the arrow length is proportional to this maximum rate of change. Variables with short arrows do not vary much across the diagram. The variable does not change in value in a perpendicular direction. The origin (0.0) indicates the mean of the variable when considering the ranking of the projected points. For environmental variables, the origin also indicates a zero correlation.
4. Sign of correlation coefficients: This represents an alternative qualitative rule of interpretation between variables. The importance of defined gradients can be interpreted by the magnitude of the eigenvalue (eigenvalue > 0.3 indicates a strong gradient).

RDA is a multivariate direct gradient analysis method in which species are presumed to have linear relationships to environmental gradients. Like CCA, the results of RDA analysis can be expressed in a triplot, i.e. a plot of sample scores, species scores, and environmental arrows. Unlike CCA, the species scores in RDA are most accurately represented by arrows (the direction in which that species is increasing in abundance).

2.14 Summary of literature review

The literature presented, illustrated the rapid urbanization of the population of the world. Furthermore it demonstrated that urbanization led to the development of unique urban ecosystems, and that the management and conservation of urban areas pose immense challenges. Ecological footprints made by urban environments imply that urban ecosystems differ from undisturbed ecosystems. Urban ecological research may give insight into environmental changes caused by the urbanization process.

Literature presented illustrated that the urbanization process influences soils in the urban environment. Urban soils tend to have characteristics that are different from soils found in undisturbed environments. Furthermore, it was also demonstrated that urbanization influences the biogeochemical cycles present in soils found in the urban environment. The presented literature illustrated that the urbanization process has distinct effects on the carbon-, nitrogen- and phosphorous cycles.

Urbanization heavily influences plant communities. Literature presented illustrated that plant species composition along with plant diversity is altered in the urban environment.

Changes in land use influences soil ecology. Examples presented in the literature indicated that the different organisms involved in soil food webs are impacted by urbanization, and the associated anthropogenic practices.

A review of the analytical techniques used for the investigation of urbanization is presented in the literature review. The urban-rural gradient as research tool is discussed, and the quantification of the urbanization gradient using the V-I-S model is illustrated. Vegetation analysis techniques are reviewed in the text. The different approaches that can be used to investigate vegetation are discussed. Importance and applications of microbial analysis techniques were discussed under culture-dependent and culture-independent techniques. Enzyme assays are discussed, with the relevant principles of the different assays used for the individual enzyme activity that was investigated. Literature presented illustrated the importance of enzyme activities in assessing soil quality. It demonstrated that soil in the urban environment tend to have altered enzyme

activities compared to soils found in undisturbed environments. Furthermore, literature illustrated that the aboveground and belowground interactions are altered in urban ecosystems.

Statistical analysis tools and techniques were discussed. Literature presented illustrated the relevance of Shannon-Weaver diversity indices in ecological studies, and how multivariate statistical analysis can contribute to the interpretation of the results obtained.

Chapter 3 – Materials and Methods

3.1 Study area

3.1.1 Location – Potchefstroom municipal area

Potchefstroom is situated in the North-West Province, one of the nine provinces in South Africa (Figure 3.1). The city is situated between 27°00' and 27°07' E longitude and 26°40' and 26°44' S latitude at an altitude of 1350 m above sea level (Figure 3.2).



Figure 3.1. Map of the nine provinces of South Africa. The North-West province is indicated by the red square (Anon, 2007).

Potchefstroom is regarded as the first settlement north of the Vaal River. It was founded in 1838 on the banks of the Mooi River. The city has a population of approximately 400 000 inhabitants, and is made up of Potchefstroom City, Ikageng, Promosa, Mohadin and a number of informal settlements (Prinsloo, 1988).



Figure 3.2. Map of the North-West province, South Africa. Potchefstroom is indicated in the red square (Anon, 2009).

3.1.2 Grassland characteristics of the study area in context of the grassland biome of South Africa

The study area is situated in an area classified as the Rand Highveld Grassland. It is a highly varied landscape, and the vegetation is species-rich, with grasses from the genera *Themeda*, *Eragrostis*, *Heteropogon* and *Elionurus* being the most common (Mucina & Rutherford, 2006). A high diversity of herbs is also a typical feature, and contributes to species richness. The canopy cover of grassland is moisture-dependent,

and decreases with lower rainfall. Other factors influencing the canopy cover are frost, grazing (amount and type) and the presence of fire. Trees are absent, except in a few localized habitats (Mucina & Rutherford, 2006).

The Grassland Biome is considered to have extremely high plant species diversity, and includes many rare and threatened species. However, the Grassland Biome is regarded as the most critically threatened southern African ecosystem (Rutherford & Westfall, 1994). Large sections of grasslands have been disturbed by past cultivation, livestock grazing and disruption of natural fire cycles - resulting in the severe decrease of species diversity of plants, insects and other animals (Cilliers *et al.*, 2008). Urbanization is also considered to aggravate the loss of natural areas in the biome (Rutherford & Westfall, 1994).

Land cover data (Fairbanks *et al.*, 2002) indicates that almost 30% of the Grassland Biome of South-Africa has been permanently transformed. This has occurred primarily as a result of cultivation, plantation forestry, urbanization and mining. Other factors that severely degraded the grasslands include erosion and agricultural improvement (Mucina & Rutherford, 2006). Significant parts of the remaining vegetation may be secondary lands, or may be degrading gradually by processes such as woody encroachment. Another concern in this regard is the fact that those areas of grassland that are untransformed are highly fragmented, and that as much as half of the remaining areas of grassland may be composed of grassland fragments (Mucina & Rutherford, 2006). This edge effect contributes towards the invasion of alien plant species (Cilliers *et al.*, 2008).

3.1.3 Geology and soils of the study area

The geology of this area consists of quartzite ridges of the Witwatersrand Supergroup and the Pretoria Group, as well as the Selons River Formation of the Rooiberg Group (last two are of the Transvaal Supergroup). The geology supports soils of various quality (shallow Glenrosa and Mispah forms especially on rocky ridges), typical of Ba, Bc, Bb, and Fb land types (Mucina & Rutherford, 2006).

3.1.4 Topography and climate of the study area

The Potchefstroom area forms part of the Central Interior Plain. The landscape varies from flat to undulating plains (Kruger, 1983).

The area is situated in a strongly seasonal summer rainfall, warm-temperate region and winters are very dry. The mean annual precipitation (MAP) is 654 mm, ranging between 570 mm and 730 mm. Frost occurs during the cold winters (Mucina & Rutherford, 2006).

3.2 Sampling sites and – period

Twelve sites were examined in the Potchefstroom municipal area. Sampling sites were allocated in the Northern, Southern, Eastern, and Western areas in and around Potchefstroom (Figure 3.3). Selected sampling plots by Jonas (2007) were used in this study, as the plot classification has already been done using the Vegetation-Impervious surface-Soil (V-I-S) model on the Potchefstroom municipal area.

Aerial photographs (Figure 3.4) were used in this study to determine the degree of urbanness of the study sites (Phinn *et al.*, 2002). An area of 0.5 km x 0.5 km around each study site was used to determine urbanness.

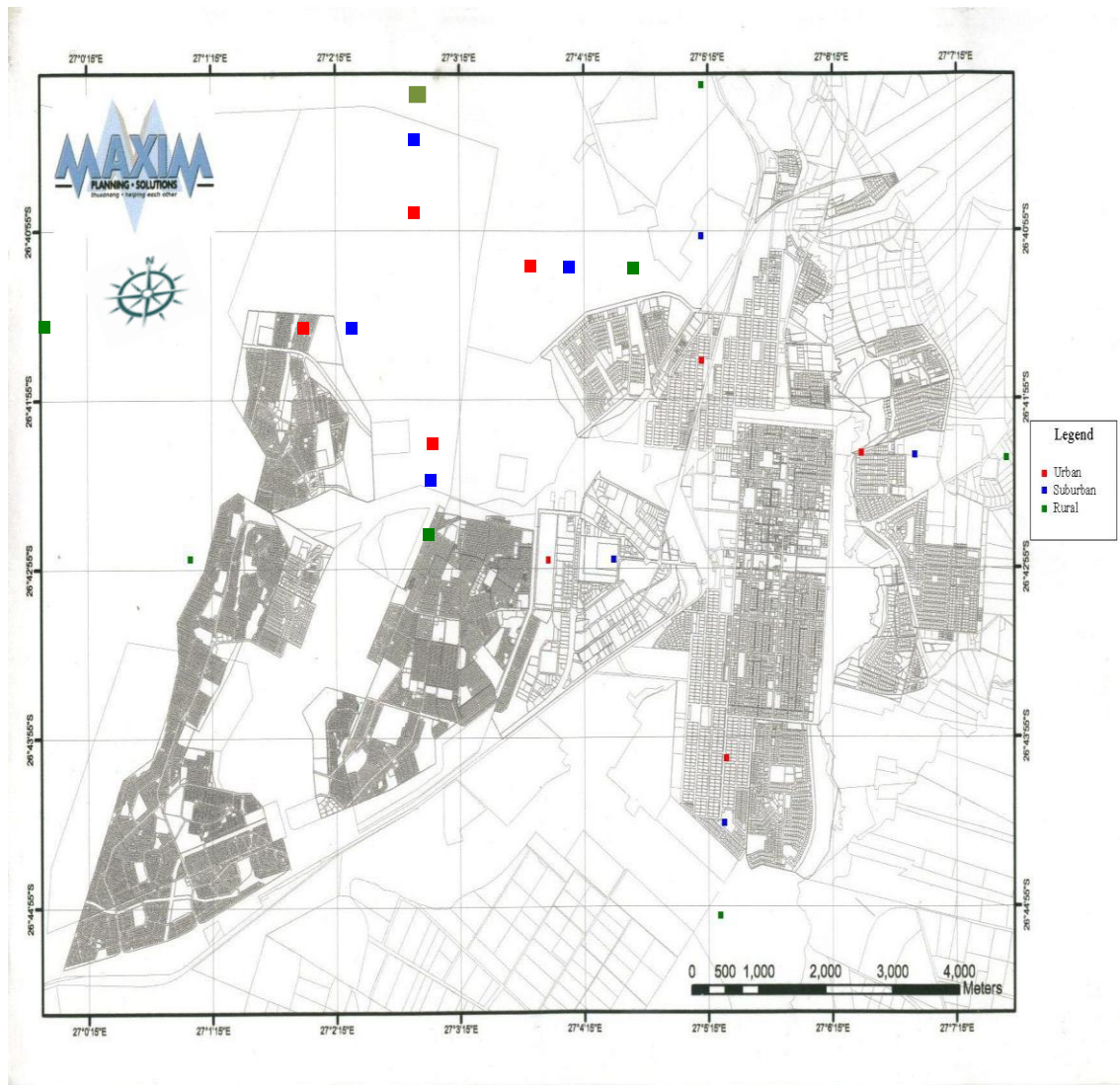


Figure 3.3. Map of Potchefstroom, South Africa, indicating sample locations. Locations selected along an urbanization gradient. (Red = Urban, Blue = Sub-urban, Green = Rural).

Using the aerial photograph of Potchefstroom and ArcView 9 (ESRI, 2006), each 0.25 km² block was divided into vegetation, impervious surfaces and soil (Figure 3.4). This information was used to quantify the urban-rural gradient according to the percentage vegetation and impervious surfaces. The data are presented in Table 3.1.

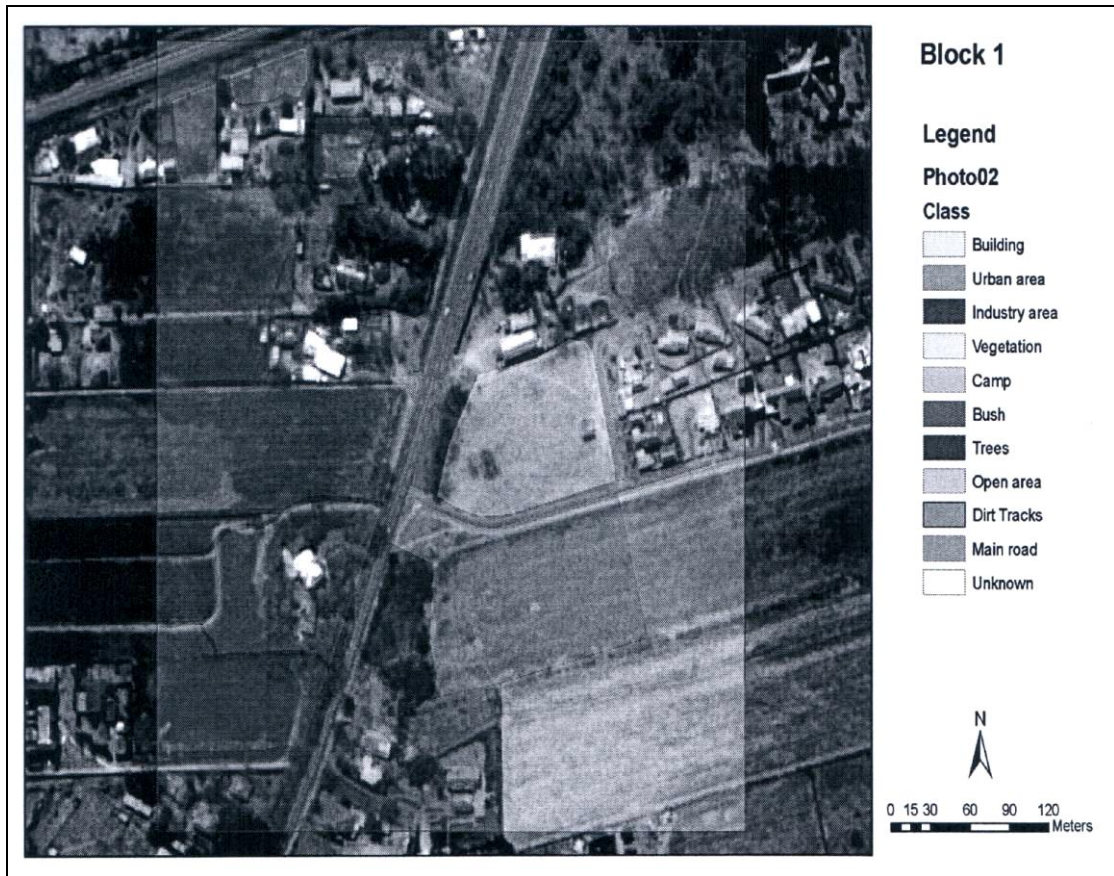


Figure 3.4. Surface classification of vegetation, impervious surfaces and soil according to ArcView for the Potchefstroom municipal area.

Sites were chosen to represent rural, sub-urban and urban grasslands. Sampling took place during the warmer, wet season (May), and also during the colder, dry season (August) in 2007 and 2008. During 2007 and 2008 rainfall continued into May, making it viable to classify the sampling season wet. The August sampling did not receive any rainfall, making it viable to classify the season as dry. Temperatures in May were higher than the temperatures in August, classifying May as the warmer sampling period and August as the colder sampling period. Three quadrats (each approximately 1m x 4m) were marked out at each sampling site, with a soil sample taken in each quadrat. In

total 36 sampling plots (Table 3.1) were studied over two seasons in each of the two years (2007 and 2008).

Table 3.1. Site characteristics of the different sampling localities of grasslands along an urbanization gradient in the Potchefstroom municipal area.

Sampling Area	Locality	Sampling Plot Abbreviation	Coordinates		% Vegetation	% Impervious Surface	% Soil
Northern Sampling Sites	Rural	NR 1	26°35'32 S	027°07'15 E	96	2	2
		NR 2	26°35'32 S	027°07'14 E			
		NR 3	26°35'33 S	027°07'14 E			
	Sub-urban	NSU 1	26°40'57 S	027°06'41 E	50	49	1
		NSU 2	26°40'57 S	027°06'40 E			
		NSU 3	26°40'57 S	027°06'40 E			
	Urban	NU 1	26°41'57 S	027°05'48 E	36	61	3
		NU 2	26°41'57 S	027°05'45 E			
		NU 3	26°41'56 S	027°05'48 E			
Eastern Sampling Sites	Rural	ER 1	26°40'05 S	027°11'33 E	100	0	0
		ER 2	26°40'02 S	027°11'32 E			
		ER 3	26°40'02 S	027°11'31 E			
	Sub-urban	ESU 1	26°42'17 S	027°06'18 E	52	46	2
		ESU 2	26°42'17 S	027°06'18 E			
		ESU 3	26°42'17 S	027°06'19 E			
	Urban	EU 1	26°42'17 S	027°05'52 E	33	65	2
		EU 2	26°42'17 S	027°05'54 E			
		EU 3	26°42'17 S	027°05'55 E			
Southern Sampling Sites	Rural	SR 1	26°46'39 S	027°06'14 E	100	0	0
		SR 2	26°46'38 S	027°06'12 E			
		SR 3	26°46'38 S	027°06'11 E			
	Sub-urban	SSU 1	26°44'49 S	027°05'43 E	47	48	5
		SSU 2	26°44'49 S	027°05'44 E			
		SSU 3	26°44'49 S	027°05'45 E			
	Urban	SU 1	26°43'57 S	027°05'57 E	5	95	0
		SU 2	26°43'57 S	027°05'56 E			
		SU 3	26°44'01 S	027°05'56 E			
Western Sampling Sites	Rural	WR 1	26°41'52 S	027°01'04 E	100	0	0
		WR 2	26°41'52 S	027°01'05 E			
		WR 3	26°41'53 S	027°01'05 E			
	Sub-urban	WSU 1	26°43'11 S	027°04'20 E	48	48	4
		WSU 2	26°43'10 S	027°04'19 E			
		WSU 3	26°43'09 S	027°04'20 E			
	Urban	WU 1	26°42'59 S	027°04'00 E	32	67	1
		WU 2	26°43'01 S	027°04'00 E			
		WU 3	26°43'03 S	027°04'01 E			

3.3 Physical and chemical characteristics of the soil in the sampling sites – Soil structure, composition and pH.

Physical and chemical analyses of soil samples collected (500 g samples collected at 300 mm depth) in the sampling sites were conducted, with emphasis on certain environmental variables (Table 3.2). The soil analysis method described by Van Rensburg *et al.* (1998) was used. This information was gathered at only one of the plots per site for the urban, sub-urban and rural sites. In total twelve soil samples were analyzed.

Table 3.2. Environmental variables used in the soil survey of soil samples collected in grasslands along an urbanization gradient in the Potchefstroom municipal area.

Environmental variables	Types
Physical attributes	pH, electrical conductivity, cation exchange
Metals	Fe
Heavy metals	Pb, Cr, Cd, As, Se, B, Mn, Cu, Zn
Particle size distribution	Sand, Silt, Clay

3.4 Basic information regarding the sampling sites

3.4.1 Soil compaction

Soil compaction was measured with a hand soil test penetrometer. Values were determined by measuring the penetration of a steel cylinder into the soil dropped from a certain height. At each plot 10 readings were taken and recorded during each sampling season. The average value of the 10 readings was then calculated for each of the 36 plots in 2007 and 2008. Average values for each site is presented in the results section.

3.4.2 Gravimetical water measurement

The weight of the soil samples was determined in its field-moist state, and then again after oven drying. The difference in weight is attributed to the loss of water. This value is related to the soil moisture weight, and the gravimetical water content can be calculated (Foster, 1995). This was performed for each soil sample of the 36 plots taken in each sampling season in 2007 and 2008. Average values for each site is presented in the results section.

3.4.3 Percentage vegetation cover

Kent and Coker (1992) describe cover as the area of ground within a sampling quadrat that is occupied by the aboveground parts of each plant species viewed from above. Estimation was done by eye, which entails that this method is subjective. This was performed at each of the 36 plots over the sampling seasons in 2007 and 2008. Average values for each site is presented in the results section.

3.4.4 Percentage dry organic matter

Estimation of the dry organic matter present on the soil surface was performed. Estimation was done by eye, also implying that this method is subjective. This was performed at each of the 36 plots over the sampling seasons in 2007 and 2008. Average values for each site is presented in the results section.

3.4.5 Percentage bare ground

Estimation of the bare soil patches present on the soil surface was done. Estimation was carried out by the eye, which means that this method is also subjective. This was performed at each of the 36 plots over the sampling seasons in 2007 and 2008. Average values for each site is presented in the results section.

3.5 Plant species data

Species present in the study were identified and the abundance of each species in terms of percentage canopy cover was recorded. Plant species names were in accordance with Germishuizen *et al.* (2006). This was done using quadrats of 4m x 1m. The purpose of the quadrat is to establish a standard area for examining the vegetation (Kent & Coker, 1992). A quadrat was placed out in each plot from all the different sampling sites (urban, sub-urban and rural) in the study area (Northern, Eastern, Southern and Western). Thirty-six data sets (for each plot a data set) were collected during the warmer/wet season (May) in 2007. Plant species richness was determined as average number of species per sample plot.

3.5.1 Diversity index for floristic data

The Shannon-Weaver index (Shannon & Weaver, 1949) was used to assess the plant species diversity present according to results obtained from the floristic analysis of the sampling sites. Diversity was estimated using data rendered regarding the number of plant species encountered at each sampling site, and the abundance of each individual species. The diversity indices were calculated from data collected at the 36 sampling plots. An average value was calculated for the sampling sites (urban, sub-urban and rural) in the study area (Northern, Eastern, Southern and Western) for the warmer/wet season (May) in 2007. Twelve average values were calculated.

The formula used to determine the Shannon-Weaver index was:

$$\text{Shannon-Weaver index (H)} = - \sum (P_i \ln [P_i])$$

H = Index value

P_i = The number of a given species divided by the total number of organisms observed.

3.6 Microbial analysis

3.6.1 Culture-dependent techniques

A soil sample was collected at each of the three plots from all the different sampling sites (urban, sub-urban and rural) in the study area (Northern, Eastern, Southern and Western). Thirty-six soil samples were collected during each seasonal sampling period in 2007 and 2008. Average values from the triplicate results for each sampling site were calculated and presented. Samples collected consist of the upper 10 cm of the soil. These samples were placed in Zip-lock bags, and stored in a cooler box. At the laboratory the samples were stored at 4° C. A portion of the soil sample was immediately frozen at -60° C, and was used for the molecular profiling of the soil microbial community. Samples were analyzed for heterotrophic bacteria and fungi levels within six hours of collection. Soil samples collected were sieved using a 2 mm sieve to remove large particles and organic matter. One gram of soil from each transect was added to 9 ml of 0.8 % saline solution, and mixed. This soil buffer solution was used to make dilution series from 10^{-1} to 10^{-5} . One hundred microlitres of each dilution was aseptically transferred to nutrient agar (Merck, Germany) and half-strength potato dextrose agar (PDA) (Merck, Germany). The media was inoculated using the spread-plate technique. Nutrient agar was used to assess the soil bacterial component, and half-strength PDA agar was used to assess the fungal component. These plates were incubated at room temperature (25° C). After 72 hours of incubation, the bacterial cfu/g was determined by counting the bacterial colonies on the nutrient agar plates. Basic colony morphology and pigmentation were used to differentiate between different bacterial morphotypes. Only bacterial colonies were purified. Fungal cfu/g soil was determined by counting the fungal colonies on the half-strength PDA plates.

3.6.1.1 Shannon-Weaver index data for bacteria

The Shannon-Weaver index was used to assess the bacterial biodiversity. To determine this, bacterial plate counts (cfu/g soil) data for the different morphotypes on the nutrient agar plates were used. See 2.13.1 and 3.5.1 for details.

The diversity indices were calculated from data collected at the 36 sampling plots. An average value was calculated for the sampling sites (urban, sub-urban and rural) in the study area (Northern, Eastern, Southern and Western) for the warmer/wet season (May) and the colder/dry season (August) in 2007. Twelve values were calculated for each season.

3.6.1.2 Identification of morphotypes

Representatives of the various bacterial morphotypes were purified from nutrient agar plates that were used to determine the cfu/g soil for the two seasons in 2007. DNA was extracted from pure cultures using a commercial bacterial DNA extraction kit (PEQ Lab, Germany). The protocol was provided by manufacturer. PCR amplification was conducted using an ICycler (Bio-Rad, UK). The 16S primers GM5F and 907R were used to amplify a 550 bp fragments (Table 3.3).

Table 3.3. Primers used to amplify genomic DNA extracted from heterotrophic bacterial isolates collected from soil samples in the Potchefstroom municipal area along an urbanization gradient.

Primer	Primer sequence	Reference
GM5F	5'-CCTACGGGAGGGAGCAG-3'	Muyzer <i>et al.</i> , 1993.
907R	5'-CCGTCAATTCCTTTGAGTTT- 3'	Muyzer <i>et al.</i> , 1993.

Conditions for DNA amplification using the primer set in Table 3.3 were as follows : 12.5 µl of double concentration PCR Master Mix (0,05 U/µl *Taq* DNA polymerase in reaction buffer, 4mM MgCl₂, 0.4mM of each dNTP) from Fermentas (US), 2 µl DNA template (100 ng), 1 µl BSA (100 ng), additional 1 µl MgCl₂ (25mM), 0.5 µl primer mix (50 pmol each) (Table 3.3), and 8 µl PCR-quality dH₂O (nuclease free), to give a final volume of 25 µl. Cycling conditions consisted of an initial denaturing step at 95 °C for three minutes, followed by further DNA denaturation (cycle x1) at 95 °C for five minutes. Cycle 2 (x35) consisted of DNA denaturation at 95 °C for 30 seconds, primer

annealing at 58 °C for 30 seconds, and extension at 72 °C for 1 minute. Final extension (cycle x1) was at 72 °C for 10 minutes (Muyzer *et al.*, 1993).

PCR amplification was confirmed by 1.5% (w/v) agarose gel in 1 x TAE buffer (40 mM Tris, 20 mM Acetic acid, 1 mM EDTA, pH 8.0). Ethidium bromide (1 µg/ml) was added for visualization under UV light. Five microlitre PCR product was mixed with 5 µl loading buffer (6x Orange Loading Dye, Fermentas Life Sciences, US) and loaded into a gel slot. Three microlitres of 100 bp molecular marker (O'GeneRuler, Fermentas Life Sciences, US) was also loaded into each gel to confirm the sizes of the fragments. Electrophoresis was performed for 45-60 minutes at 80 V using 1 x TAE buffer (Muyzer *et al.*, 1993). Gel images were captured using a Gene Bio Imaging System (Syngene, Synoptics, UK) and Genesnap (version 6.00.22) software. The sequences of the 550 bp fragment were determined by Inqaba Biotec (RSA) and were analyzed using BlastN searches in GeneBank (<http://www.ncbi.nlm.nih.gov/BLAST>).

3.6.2 PCR-DGGE

3.6.2.1 Soil community DNA extraction, PCR and DGGE

The bacterial community structure of the soil samples collected from the 36 sampling plots in the warmer, wet season (May) of 2007 was investigated using PCR-DGGE. Composite soil samples were made to represent the different sampling sites. To produce a composite sample, one gram of soil from each sampling plot was combined in a sterile flask (for example, one gram soil from sampling plot NR 1, one gram of soil from sampling plot NR 2, and one gram of soil from sampling plot NR 3 would be combined to create a composite sample for the sampling site NR). Twelve composite samples were thus analyzed. Community DNA was extracted using commercial DNA extraction kits from Zymo Research (US). Nanodrop spectrophotometer system was used to characterize the isolated community DNA (Table A9).

PCR amplification was conducted using a PCR master mixture from Fermentas (US) and the 16S primers PRBA338F with a 40 bp GC clamp region and PRUN518R (Table

3.4). Primers used (PRBA338F and PRUN518R) were synthesized by Inqaba Biotech. The primer combination amplifies a 200bp fragment.

Table 3.4. Primers used to amplify bacterial community DNA extracted from soil samples collected from soil samples in the Potchefstroom municipal area along an urbanization gradient.

Primer	Primer sequence	Reference
PRBA338F	5'-ACTCCTACGGGAGGCAGCAG- 3'	Lane, 1991
PRUN518R	5'-ATTACCGCGGCTGCTGG-3'	Muyzer <i>et al.</i> , 1993

In order to attain the best PCR product for DGGE analysis, many different compositions of the reaction mixture were attempted. Template DNA was varied, ranging from 1-150 ng/reaction mixture. Different primer concentrations per reaction mixture were also tested, and in some cases *Taq* polymerase were added to the reaction mixture.

The most successful PCR reaction mixture contained 12.5 µl double concentration PCR Master Mix (0,05 U/µl *Taq* DNA polymerase in reaction buffer, 4mM MgCl₂, 0.4mM of each dNTP) from Fermentas (US), 1 ng DNA template, 1 µl BSA (100 ng), additional 1 µl MgCl₂ (1 mM), 0.5 µl primer mix (50 pmol each) (Table 3.4), and PCR-quality dH₂O (nuclease free), to yield a final volume of 25 µl.

PCR protocol consisted of initial DNA denaturation (cycle x1) at 95 °C for five minutes. This was followed by 35 cycles consisting of DNA denaturation at 95 °C for 30 seconds, primer annealing at 65 °C for 30 seconds, and extension at 72 °C for one minute. The final extension was at 72 °C for 10 minutes.

PCR amplification was confirmed by 1.5% (W/V) agarose gel in 1 x TAE buffer (40 mM Tris, 20 mM Acetic acid, 1 mM EDTA, pH 8.0). EtBr (1µg/ml) was added for visualization under UV light. Five microlitre PCR product was mixed with 5 µl loading buffer (6x Orange Loading Dye, Fermentas Life Sciences, US) and loaded into a gel

slot. Three microlitres of 100 bp molecular marker (O'GeneRuler, Fermentas Life Sciences, US) was also loaded into each gel to confirm the sizes of the fragments. Electrophoresis was performed for 45-60 minutes at 80 V using 1 x TAE buffer. Gel images were captured using a Gene Bio Imaging System (Syngene, Synoptics, UK) and Genesnap (version 6.00.22) software.

A D-Code Universal Mutation Detection System (Bio-Rad, UK) was used for DGGE analysis. Electrophoresis was conducted on a 6% (w/v) acrylamide gel for 16 hours at 80 V in 1 x TAE buffer. A 40-60% acrylamide gradient was used for the analysis. EtBr (1µl/ml) stained gels were visualized using a Gene Genius Bio Imaging System (Syngene, Synoptics, UK) and Genesnap (version 6.00.22) software.

3.7 Enzyme assays

Three soil samples were collected at each of the 12 sampling sites (thus one soil sample per sampling plot). Average values were determined for the urban, sub-urban and rural sites from all the sampling areas (Northern, Eastern, Southern and Western). In total twelve average assay values were determined for each seasonal sampling season (May and August) in 2007 and 2008.

3.7.1 Dehydrogenase assay

The INT method was used to determine the potential dehydrogenase activity for each soil sample (Von Mersi & Schinner, 1991). Field-moist (1g) soil was weighed into 100 ml Schott bottles, and mixed with 1.5 ml Tris buffer and 2 ml INT solution. The bottles were capped, and incubated at 40 °C in the dark for two hours. The controls were prepared exactly the same, except autoclaved soil were used instead of field-moist soil. Each analysis was carried out in triplicate. After the incubation period, each sample was mixed with 10 ml of the extraction solution and kept in the dark for one hour. The samples were shaken vigorously every 20 minutes. The resulting suspension was then filtered, and the developed INF was measured spectrophotometrically at 464 nm against a prepared blank.

A calibration curve was prepared using known amounts of INF solution and extracting solution. Dehydrogenase activity was calculated as $\mu\text{g INF g}^{-1} \text{dwt } 2 \text{ h}^{-1}$, using the following equation:

$$INF (\mu\text{g g}^{-1} \text{dwt } 2 \text{ h}^{-1}) = \frac{S_1 - S_0}{dwt}$$

where S_1 is the INF (micrograms) of the test, S_0 is the INF (micrograms) of the control and dwt is the dry weight of 1 g moist soil.

3.7.2 β -glucosidase

The following method was used to determine the potential β -glucosidase activity in each soil sample: Air-dried (1g) soil was weighed into 100 ml Schott bottles, and 0.25 ml toluene, 4 ml of MUB pH 6 and 1 ml *p*-Nitrophenyl- β -D-glucosidase (PNG) solution were added. The bottles were then capped, and swirled for a few seconds. The samples were then incubated for one hour at 37 °C. After the incubation, 1 ml 0.5M CaCl_2 and 4 ml of 0.1M THAM buffer pH 12 were added, and the suspension was swirled for a few seconds. The suspensions were then filtrated, and the *p*-nitrophenol was measured spectrophotometrically at 410 nm against a prepared blank. The controls were prepared in the same way, but the addition of 1 ml PNG solution took place after the addition of the 0.5M CaCl_2 and the 4 ml of 0.1M THAM buffer pH 12, and immediately before the filtration of the soil suspension. Each analysis was carried out in triplicate (Dick *et al.*, 1996).

The calibration curve was prepared using known amounts of standard *p*-nitrophenol solution. β -glucosidase activity was calculated as *p*-nitrophenol ($\mu\text{g g}^{-1} \text{dwt h}^{-1}$) using the following equation:

$$p\text{-nitrophenol } (\mu\text{g g}^{-1} \text{dwt h}^{-1}) = \frac{C \times v}{dwt \times SW \times t}$$

where C is the measured concentration of the *p*-nitrophenol ($\mu\text{g ml}^{-1}$ filtrate), dwt is the dry weight of 1 g soil, v is the total volume of the soil suspension in millilitres, SW is the weight of the soil sample used and t is the incubation time in hours (Tabatabai & Bremner, 1969; Eivazi & Tabatabai, 1977) .

3.7.3 Acid- and alkaline phosphatase

The following method was used to determine the potential acid and alkaline phosphatase activity in each soil sample: Air-dried (1g) soil was weighed into 100 ml Schott bottles, and 0.25 ml toluene, 4 ml of MUB (for acid phosphatase: pH 6.5; for alkaline phosphatase: pH 11) and 1 ml *p*-Nitrophenol (PNP) solution were added. The bottles were then capped, and swirled for a few seconds. The samples were then incubated for one hour at 37 °C. After the incubation, we added 1 ml 0.5M CaCl₂ and 4 ml 0.5M NaOH, and the suspension was swirled for a few seconds. The suspensions were then filtrated, and the *p*-nitrophenol was measured spectrophotometrically at 410 nm against a prepared blank. The controls are prepared the same way, but the addition of 1 ml PNP solution took place after the addition of the 0.5M CaCl₂ and the 4 ml 0.5M NaOH, and immediately before the filtration of the soil suspension. Each analysis was carried out in triplicate (Dick *et al.*, 1996).

The calibration curve was prepared using known amounts of standard *p*-nitrophenol solution. Phosphatase activity was calculated as *p*-nitrophenol ($\mu\text{g g}^{-1} \text{dwt h}^{-1}$) using the following equation:

$$p\text{-nitrophenol } (\mu\text{g g}^{-1} \text{dwt h}^{-1}) = \frac{C \times v}{dwt \times SW \times t}$$

where C is the measured concentration of the *p*-nitrophenol ($\mu\text{g ml}^{-1}$ filtrate), dwt is the dry weight of 1 g soil, v is the total volume of the soil suspension in millilitres, SW is the weight of the soil sample used and t is the incubation time in hours (Tabatabai & Bremner, 1969; Eivazi & Tabatabai, 1977).

Results gathered during the wet and dry sampling periods in 2007 indicated that no significant patterns could be observed regarding the phosphatase results. It was decided that the assays would not be repeated during the 2008 season, due to the lack of significant results.

3.7.4 Urease assay

The non-buffered method of Kandeler and Gerber (1988) was used in order to estimate the urease activity. Five grams of moist soil was weighed in 100 ml Schott bottles, and 2.5 ml of urea solution was added. The bottles were capped, and incubated for two hours at 37 °C. After the incubation period, 50 ml of KCl solution was added to each sample, and the samples were shaken for 30 minutes in a bench shaker at 100 rpm. The resulting suspension was filtered, and the filtrate was analyzed for ammonium content. Blanks were prepared in the same manner as samples, but 2.5 ml distilled water was used instead of 2.5 ml urea solution. The urea solution was added after the incubation period, immediately before the addition of the KCl solution. Each analysis was carried out in triplicate.

To determine the ammonium concentration, 1 ml of each of the samples was taken and added to a clean 100 ml Schott bottle. Nine millilitres of distilled water, 5 ml sodium salicylate/NaOH solution, and 2 ml of sodium dichloroisocyanide solution were added. The samples were allowed to stand at room temperature for 30 minutes. After the waiting period, the optical density can be measured spectrophotometrically at 690 nm (Kandeler and Gerber 1988).

The calibration curve was prepared using known amounts of ammonium solution, and determining the concentrations as described above. Results had to be corrected to the blanks, and then urease activity was calculated as $\mu\text{g NH}_4\text{-N g}^{-1} \text{dwt } 2 \text{ h}^{-1}$, using the following equation:

$$\text{Ammonium } (\mu\text{g NH}_4\text{-N g}^{-1} \text{dwt } 2 \text{ h}^{-1}) = \frac{\mu\text{g NH}_4\text{-N ml}^{-1} \times V \times 10}{\text{dwt} \times 5}$$

where dwt is the dry weight of 1 g moist soil, V is the total volume of the extract (52.5 ml), 10 is the dilution factor and 5 is the weight of the soil used in the assay (Kandeler and Gerber 1988).

3.8 Statistical analysis

Parametric and non-parametric statistical analyses were performed on all data obtained using Statistica 7 (Stasoft, Inc.). ANOVA and Tukey's honest significant difference (HSD) were used on the parametric or non-parametric data with a view to determine statistical significance between the various samples. CANOCO (Ter Braak, 1992) was used to create ordinations, indicating the correlations between the microbial and botanical information.

Chapter 4 - Results

The results generated during the study period are presented in this chapter. Results regarding soil characteristics, plants and basic sampling site characteristics, and microbial investigations along the urbanization gradient are evaluated and relevant trends are highlighted. The integration of the different data sets concludes this chapter. For sampling site identification of all the figures, refer to the sampling site abbreviation list on page xviii and Table 3.1 on p. 45. All statistical significance results are presented in the appendix.

4.1 Chemical and physical analysis of the collected soil samples

Results from the physical and chemical analyses are presented in this section. In particular, the results regarding the physical attributes of the soil (percentage sand, silt and clay) are presented in Figure 4.1.

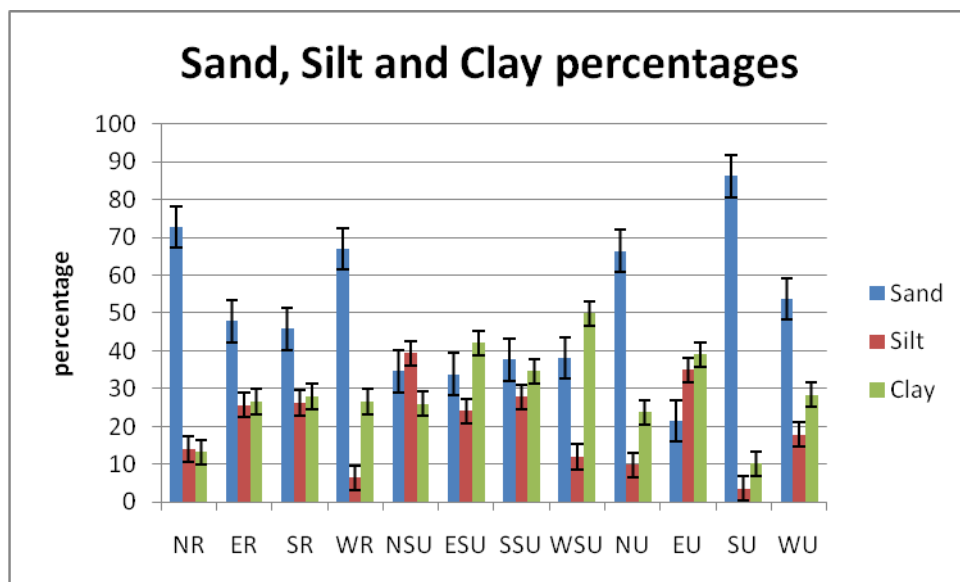


Figure 4.1. Average sand, silt and clay concentrations of soil samples collected at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during 2007.

Soil particle distribution results indicated that the soil is mostly sandy in nature (Figure 4.1). Silt and clay contents are generally higher in the sub-urban and urban sites, but no clear trend could be observed in terms of the urbanization gradient (Figure 4.1).

Soil samples collected at all the sampling sites were analyzed to determine the pH. These results are presented in Figure 4.2.

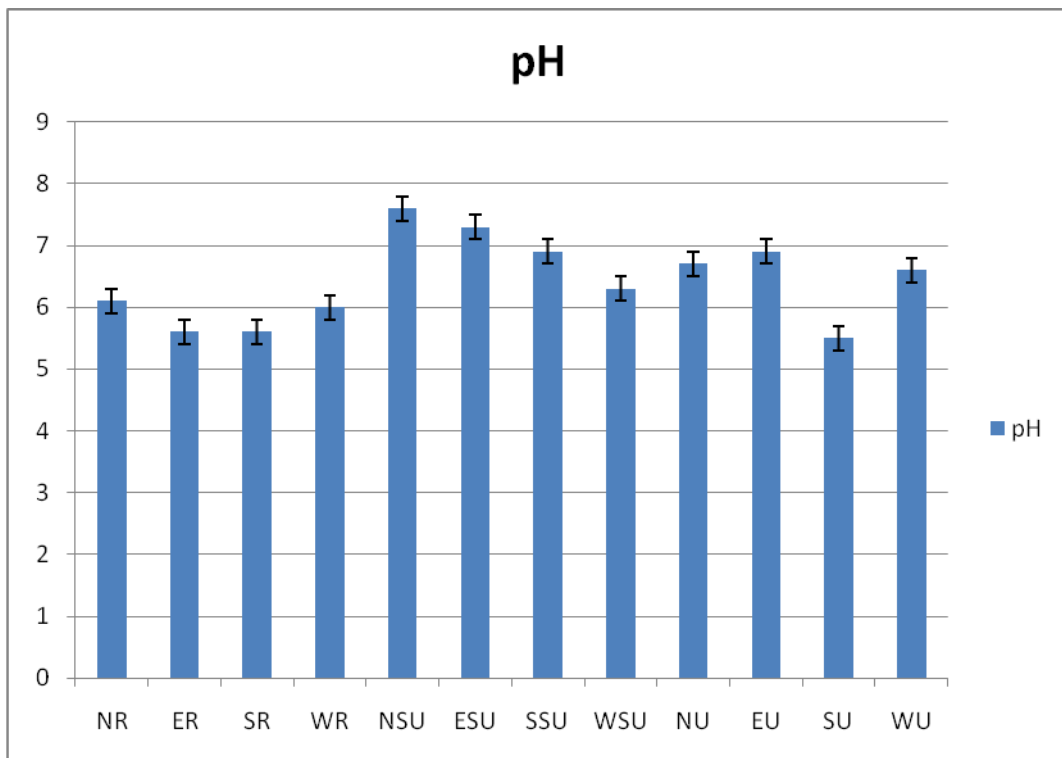


Figure 4.2. Average pH of soil samples collected at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during 2007.

Soil pH ranged from neutral to slightly acidic (Figure 4.2). The highest pH value (7.6) was registered at the northern sub-urban site. The most acidic pH value (5.5) was registered at the southern urban site. The sub-urban sites generally registered the highest pH values - this was the case at the northern, eastern and southern sampling sites. Only at the western sampling sites was the urban site value the highest. Collectively the rural sites had the lowest pH values. pH values of the sub-urban sites were statistically significantly different from the rural site values ($P < 0.05$) (Figure 4,2). Soil samples collected at all the sampling sites were analyzed in order to determine the electrical conductivity. These results are presented in Figure 4.3.

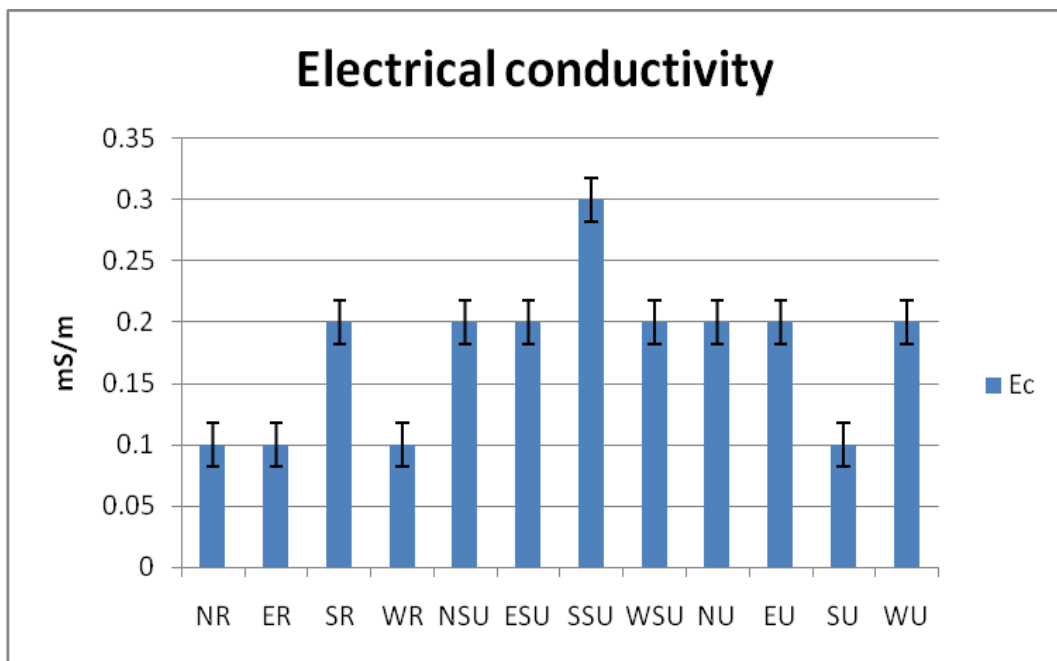


Figure 4.3. Average electrical conductivity (Ec) of soil samples collected at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during 2007.

The results indicate that the electrical conductivity was generally higher in soils from sub-urban and urban sites. Only the southern rural site value was similar to the values collected from the sub-urban and urban sites. The southern urban site value was the lowest of all the urban and sub-urban sites. No statistically significant differences were observed ($P > 0.05$).

Soil samples collected at all the sampling sites were analyzed to determine the cation exchange potential. These results are presented in Figure 4.4.

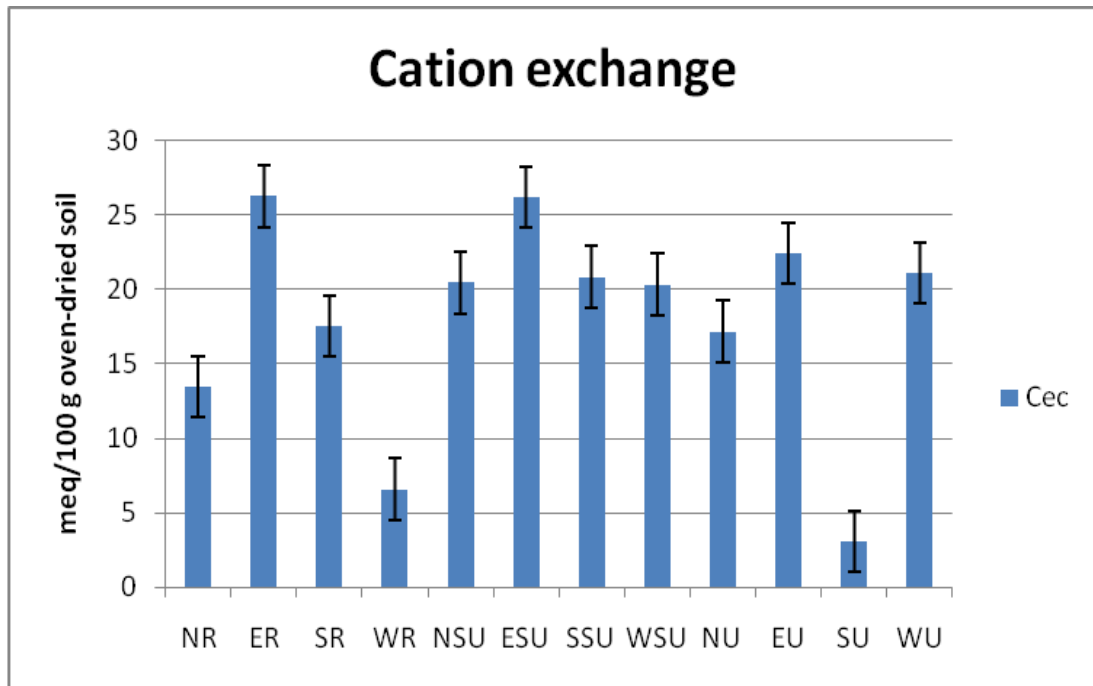


Figure 4.4. Average cation exchange capacity (Cec) of soil samples collected at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during 2007.

Results indicate that the cation exchange potential is generally high at the sub-urban sites. However, no clear trend could however be established. No statistically significant differences were observed ($P > 0.05$).

Soil samples collected at all the sampling sites were analyzed with a view to determine the concentrations of certain metals and heavy metals. These results are presented in Table 4.1.

The results indicate no clear trend regarding the urbanization gradient. Values were higher at the northern rural site for almost all the analyzed metals (except Fe and B). Fe, Mn and Se values were in some cases higher at the rural sites than the sub-urban and urban sites, but the differences were not statistically significant ($P > 0.05$).

Table 4.1. Chemical parameters analysis data (presented in parts per million (ppm)) of soil samples collected at the different sampling sites during the study in the Potchefstroom municipal area along an urbanization gradient.

Site	Cu	Fe	Mn	Zn	B	Pb	Cr	Cd	As	Se
NR	1.57	78	4.23	2.32	0.5	8.8	740	0.08	5.5	7.4
ER	0.51	80	1.52	0.41	10.3	0.61	34	0.01	0.01	5.9
SR	0.01	3.3	0.14	0.02	0.05	0.01	0.01	0.01	0.01	6.66
WR	0.01	5.4	0.08	0.03	0.01	0.01	0.07	0.01	0.09	3.97
NSU	0.01	0.6	0.01	0.01	0.01	0.01	0.04	0.01	3.14	4.35
ESU	0.025	6	0.02	0.01	0.07	0.01	0.05	0.01	1.24	3.26
SSU	0.085	6	0.11	0.18	0.12	0.045	0.34	0.01	0.01	1.77
WSU	0.02	2.5	0.1	0.03	0.18	0.01	0.01	0.01	0.01	1.37
NU	0.025	3.7	0.08	0.015	0.01	0.01	0.07	0.01	1.91	1.2
EU	0.03	5.3	0.02	0.015	0.07	0.01	0.02	0.01	0.01	2.61
SU	0.01	5.2	0.05	0.01	0.01	0.01	0.01	0.01	0.01	5.44
WU	0.02	6.8	0.05	0.01	0.01	0.01	0.09	0.01	1.66	2.02

4.2 Basic sampling site information

4.2.1 Average soil compaction

The average soil compaction was measured at all the sampling locations across the different seasons during the study period. These results are presented in Figure 4.5.

Results indicated that average soil compaction was generally higher during the August (colder, dry) sampling periods. Soil compaction was generally higher in the sub-urban and urban sites. All the results were, however, mostly statistically insignificant ($P > 0.05$).

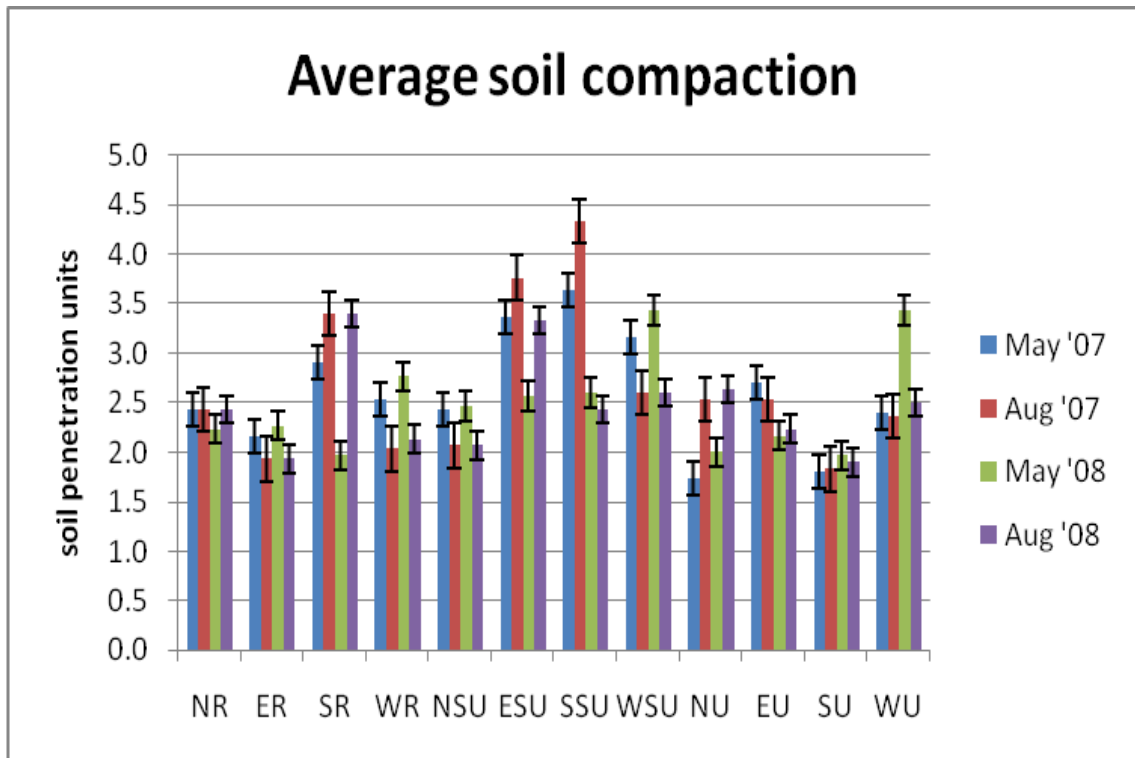


Figure 4.5. Average soil compaction data collected at the different sampling locations over 4 sampling seasons in the Potchefstroom municipal area along an urbanization gradient.

4.2.2 Average gravimetric water measurement

The average gravimetric water content was measured for the collected soil samples from all the sampling locations across the different seasons during the study period. These results are presented in Figure 4.6.

No clear trend was observed regarding the average gravimetric water results. It was assumed that the May (warmer, wet) season samples would contain significantly more water than the August (colder, dry) samples, but the data did not clearly illustrate this assumption. The spike observed at the NU site in August '08 could be attributed to irrigation. During the specific sampling period the area was under intense watering practices.

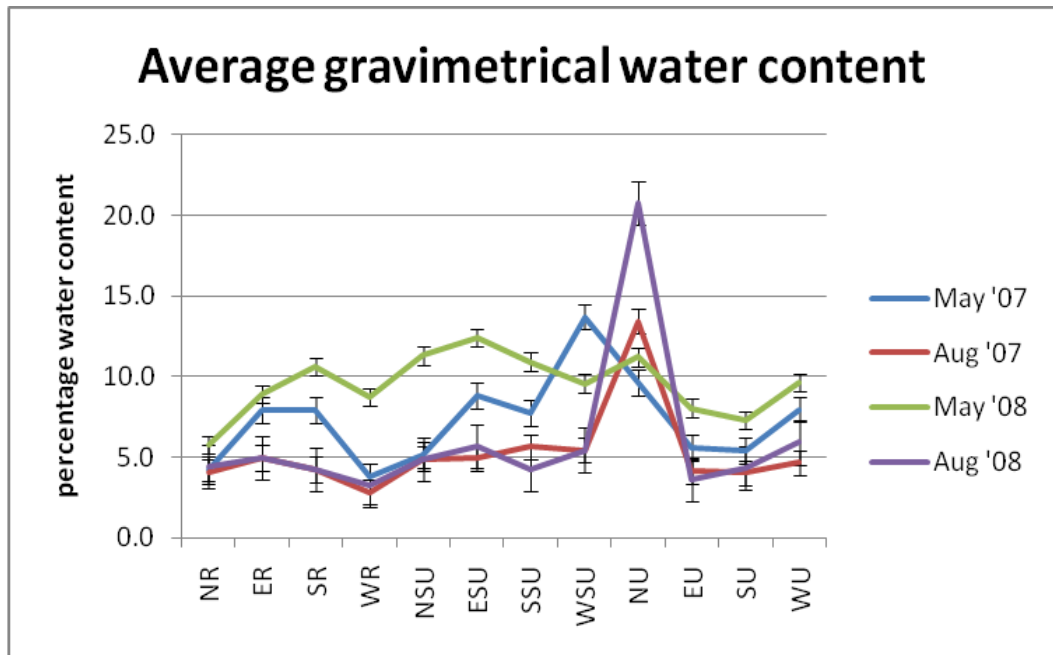


Figure 4.6. Average percentage gravimetrical water measured in the samples collected at the different sampling locations over four sampling seasons in the Potchefstroom municipal area along the urbanization gradient.

4.2.3 Average percentage vegetation cover

The average percentage vegetation cover was estimated at all the sampling plots across the different seasons during the study period. These results are presented in Figure 4.7.

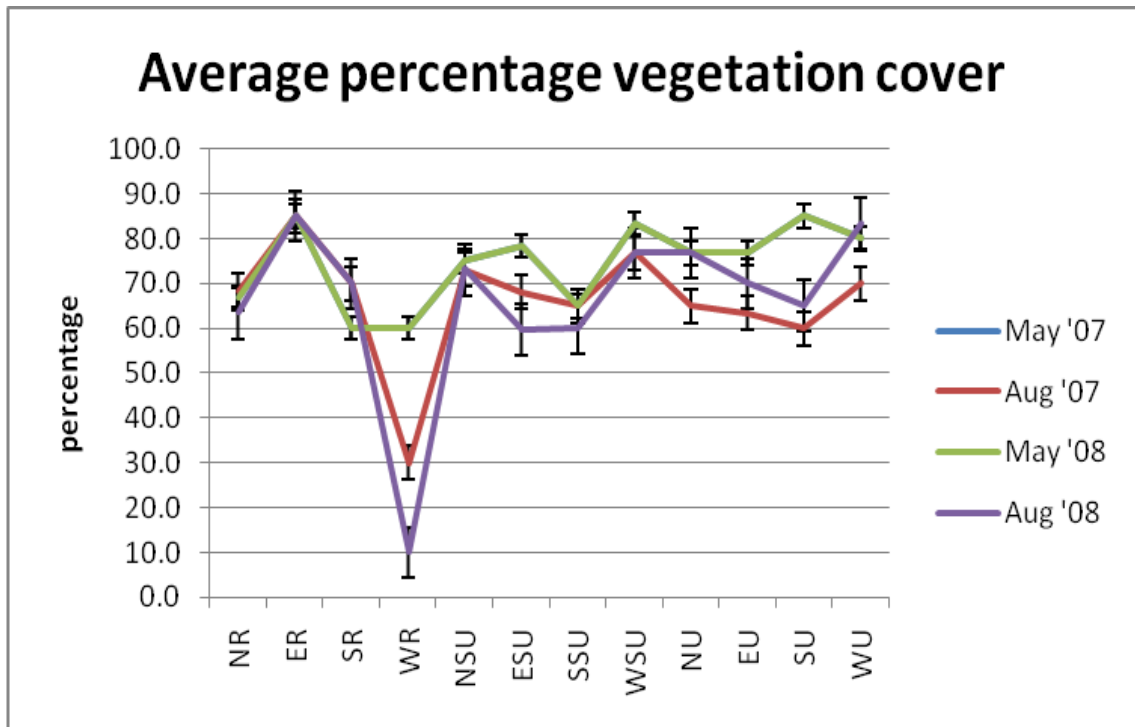


Figure 4.7. Average percentage vegetation cover data collected at the different sampling locations over four sampling seasons in the Potchefstroom municipal area along an urbanization gradient.

The average percentage vegetation cover results obtained showed that vegetation cover was highest during the May (warmer, wet) sampling period. This was expected because of the summer rainfall and higher temperatures. The August (colder, dry) estimates for the western rural sites were considerably lower due to fires in the area.

It was generally noted that average percentage cover estimates were higher for urban and sub-urban sites than the rural sites.

4.2.4 Average percentage dry organic matter

The average percentage dry organic matter was estimated at all the sampling plots across the different seasons during the study period. These results are presented in Figure 4.8.

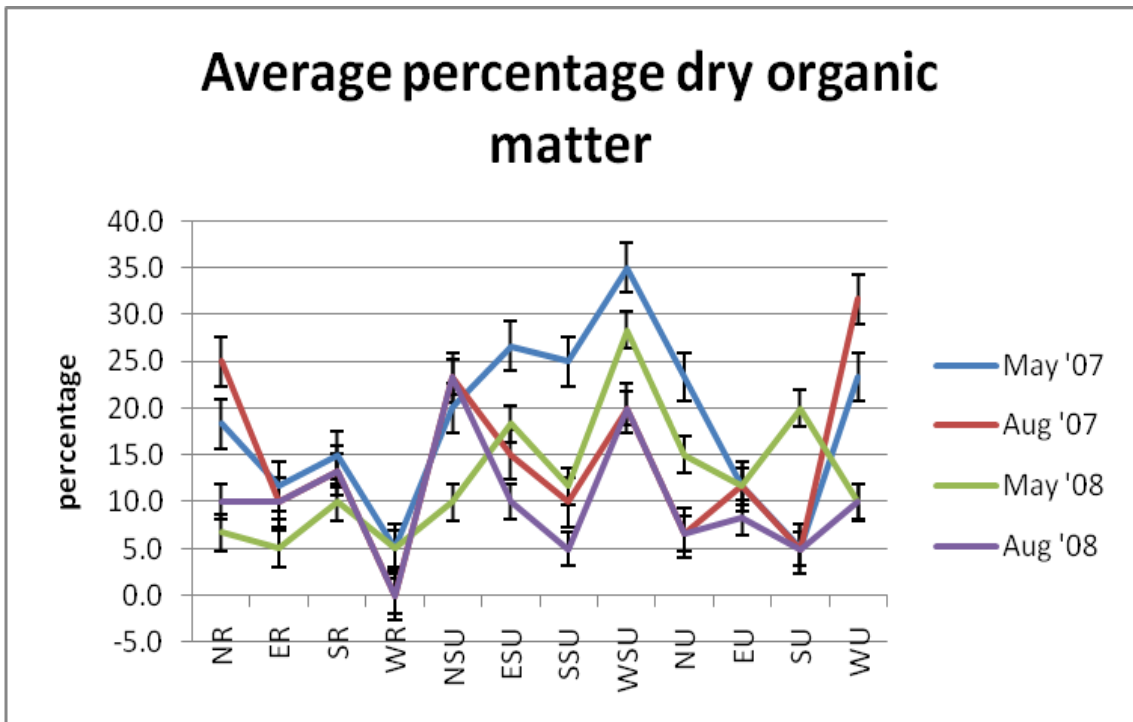


Figure 4.8. The average percentage organic refuse data collected at the different sampling locations over four sampling seasons in the Potchefstroom municipal area along an urbanization gradient.

The average percentages organic matter found in the sampling sites were generally higher during the May (warmer, wet) sampling seasons. It was noted that there was more organic matter on the urban and sub-urban sites. No clear trend was present.

4.2.5 Average percentage bare ground

The average percentage bare ground was estimated at all the sampling plots across the different seasons during the study period. These results are presented in Figure 4.9.

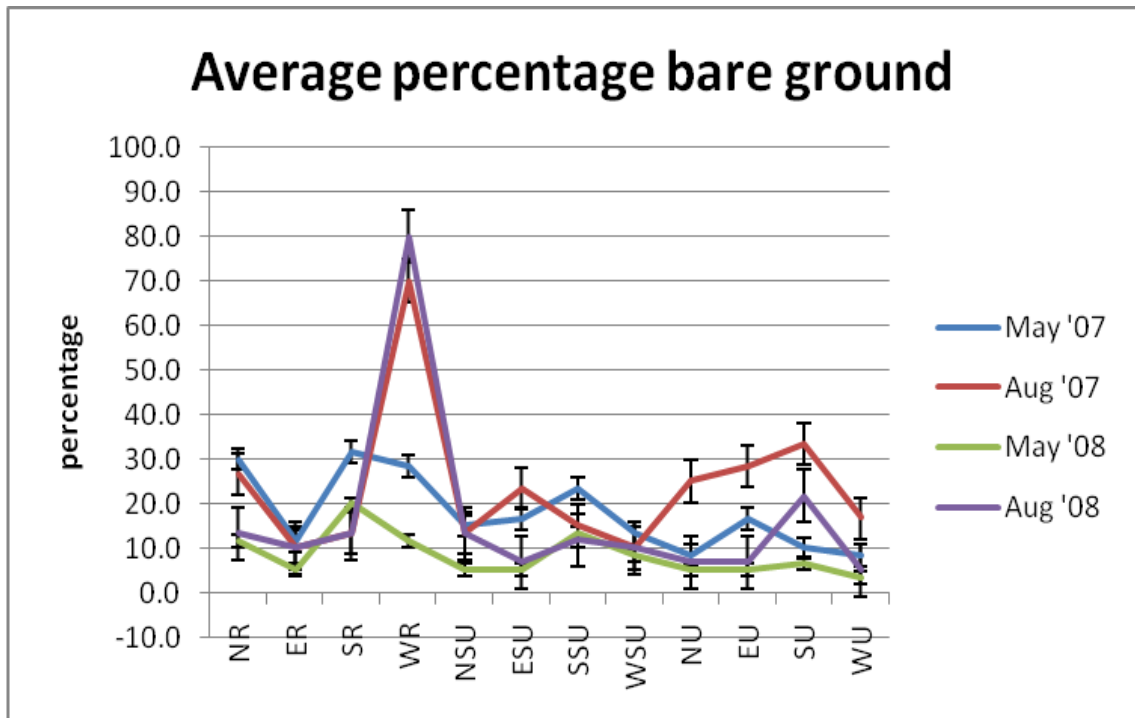


Figure 4.9. The average percentage bare ground data collected at the different sampling locations over four sampling seasons in the Potchefstroom municipal area along an urbanization gradient.

It was noted that there were generally more bare patches on the rural sites than the sub-urban and urban sites. No clear trend was however present.

4.3 Plant species composition results

4.3.1 Plant species richness

The average number of plant species encountered at the urban, sub-urban and rural sampling sites is presented in Figure 4.10.

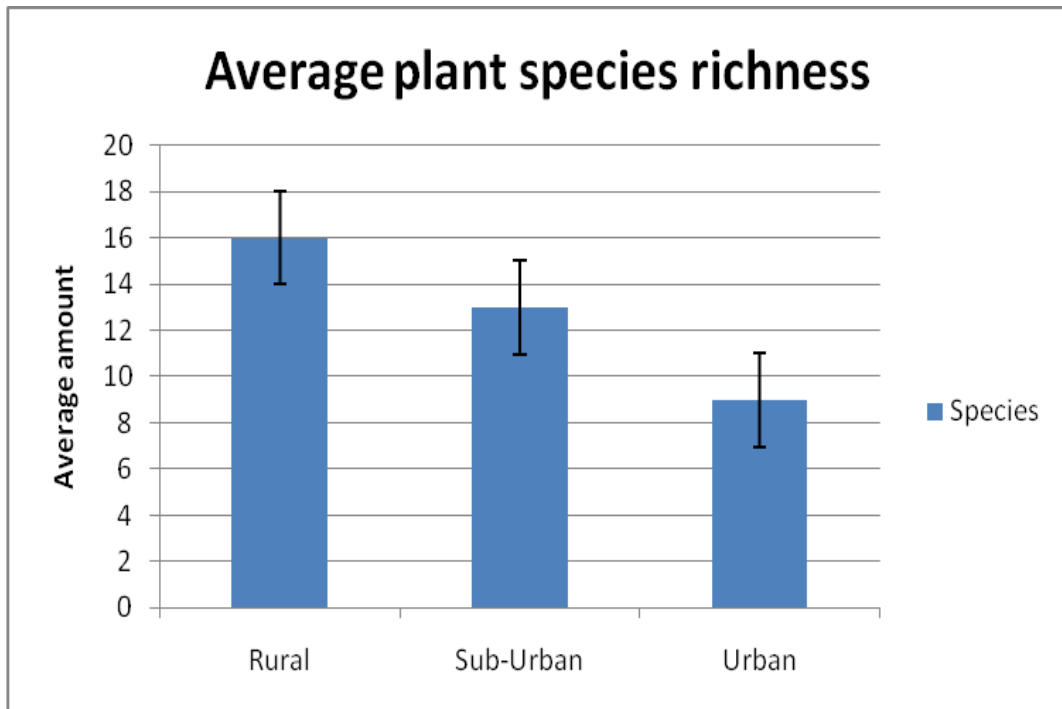


Figure 4.10. Plant species richness of the sampling sites along an urbanization gradient in the Potchefstroom municipal area during May (warmer, wet season) in 2007.

The results indicate that species richness is the highest in rural sampling plots, followed by sub-urban sampling plots, and then urban sampling plots. Statistically significant differences were observed for values from rural, sub-urban and urban sites ($P > 0.05$).

4.3.2 Plant diversity

Using the Shannon-Weaver index, plant data diversity indices were calculated to evaluate the degree of biodiversity. These results are presented in Figure 4.11.

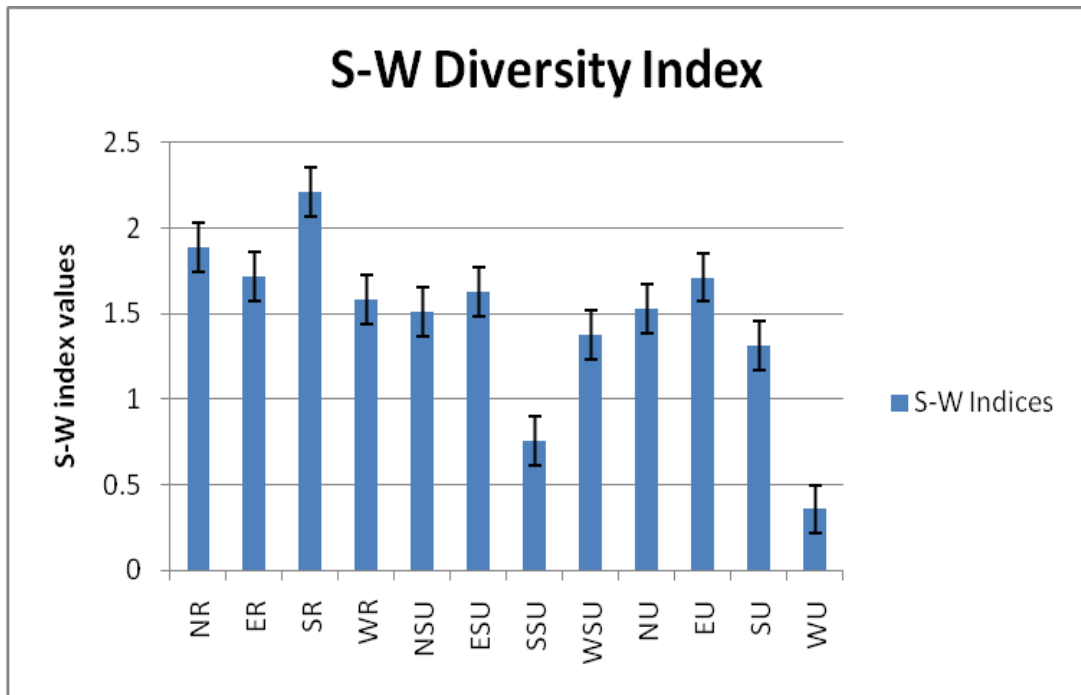


Figure 4.11. Average Shannon-Weaver index values for plant communities encountered at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during May (warmer, wet season) in 2007.

The results showed that for the northern, eastern, southern and western sampling sites, the rural sites have the highest values. The results from the western sampling site showed that the rural value was the highest, followed by the sub-urban site and then the urban site. Results regarding the northern, eastern and southern sampling sites revealed that the urban sites had higher values than the sub-urban sites.

Tukey's HSD revealed that there were no statistically significant differences in terms of the values found at the eastern sampling sites. At northern sites, the urban and sub-urban values differed statistically significantly from the rural site. At the southern sites the urban, sub-urban and rural sites all showed statistically significant differences ($P < 0.05$) from each other. At the western sites the rural and sub-urban site differed statistically significantly from the urban site ($P < 0.05$).

The general trend observed was that the rural sites tended to have a higher biodiversity than the urban and sub-urban sites. The Shannon-Weaver diversity indices were

supported by the data regarding the average plant species richness encountered at each type of sampling site (Figure 4.5).

4.3.3 Plant species characteristics

Plant species encountered at the different sampling locations were subdivided in indigenous species, alien species, annual species, perennial species, and annual/perennial species. Interrogation of this data indicates whether any trends regarding these traits can be observed along the urban-rural gradient. The results are presented in Figure 4.12.

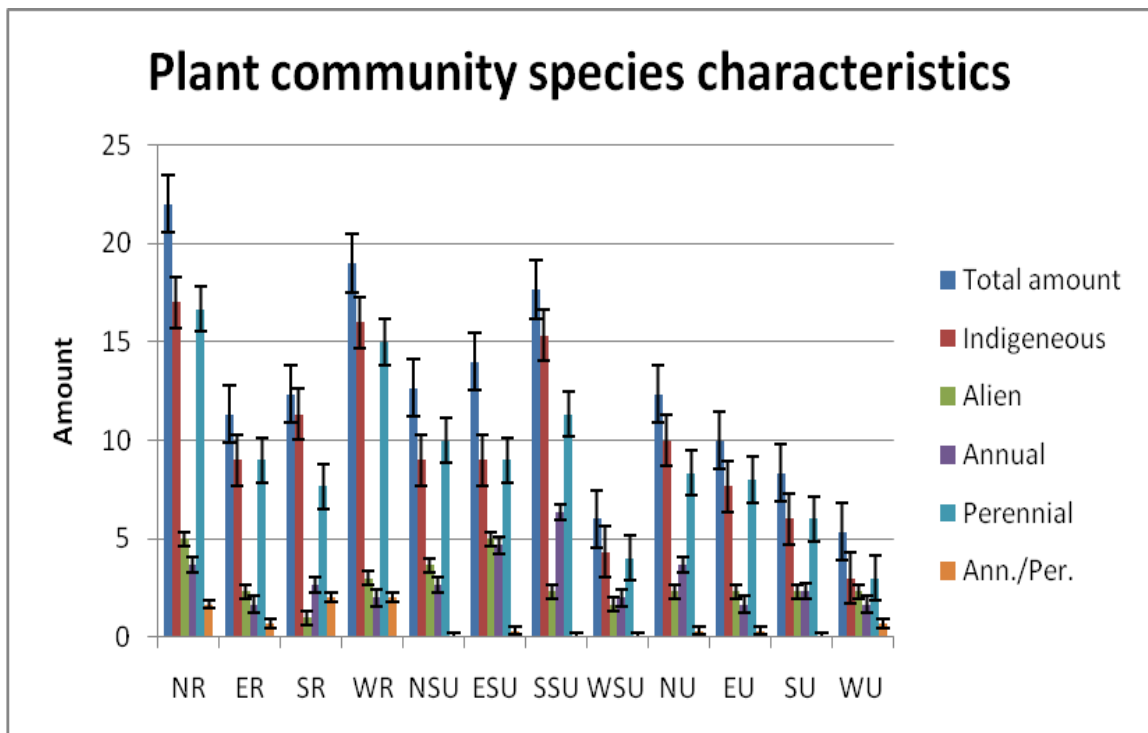


Figure 4.12. Plant species characteristics encountered at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during May (warmer, wet season) in 2007.

The results indicated that the rural sites had the most indigenous and perennial plant species. On the other hand, the sub-urban sites had the most alien species. The sub-

urban and urban sites also had the most annual plant species. All the differences were, however, statistically insignificant ($P > 0.05$).

Plant life form classification results are presented in Figure 4.13. From this figure (Figure 4.13) it is evident that the occurrence of forbs and geophytes are highest in the rural sites, followed by the sub-urban sites.

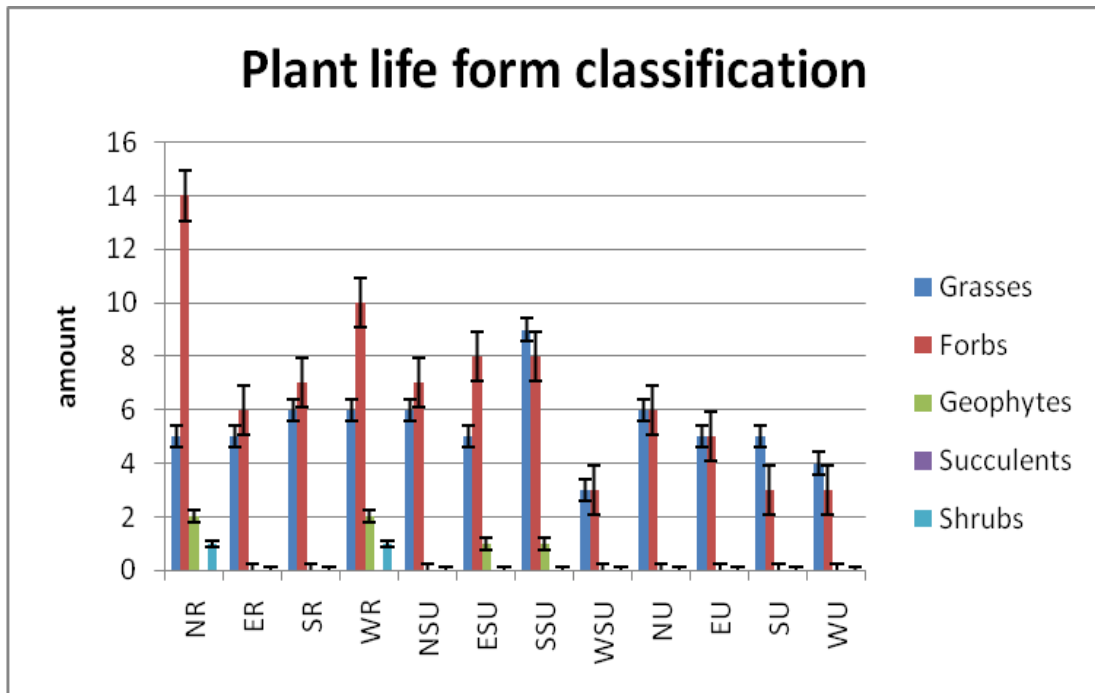


Figure 4.13. Plant community data regarding the plant life forms encountered at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during May (warmer, wet season) in 2007.

The occurrence of forbs follows a trend along the urbanization gradient, with the highest occurrence at rural sites and lowest occurrence at urban sites. It was also noted that geophytes were absent at the urban sites. Differences were, however, statistically insignificant ($P > 0.05$).

4.3.4. Plant species composition and abundance

Plant species data was used in a DCA ordination to establish whether plant species composition and abundance differed along the urbanization gradient. The ordination results are presented in Figure 4.14.

The DCA ordination reveals that the urban, sub-urban and rural sites form distinct clusters based on the plant species composition along ordination axis 1. A strong gradient is visible along ordination axis 1, where the sampling site types tend to be closely related. The ordination illustrates that there is a definite difference in species composition between the urban, sub-urban and rural plant species composition along the urbanization gradient, and that within each category (urban, sub-urban and rural), correlations exist regarding plant species composition. Lists of plant species where they have occurred along the urbanization gradient and their abundances are included in Tables A.3. to A.6. of the Appendix.

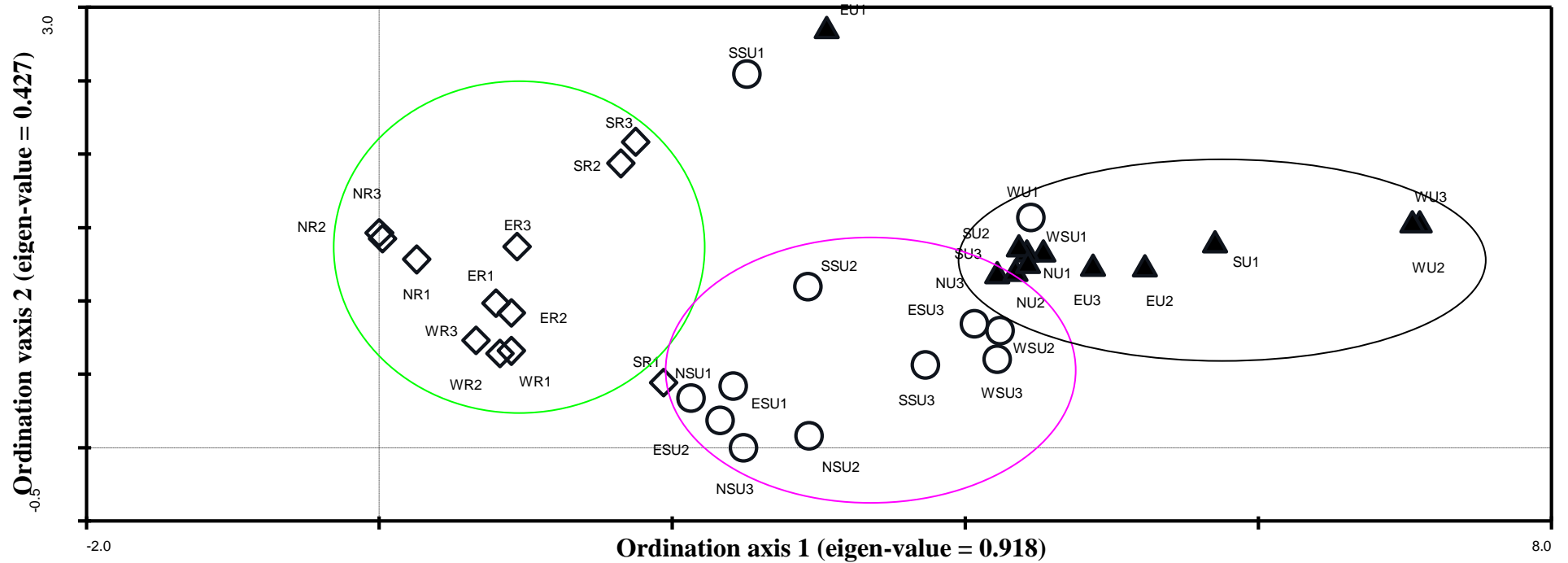


Figure 4.14. DCA ordination illustrating the relationship between the different sampling locations and the plant species diversity encountered at the sampling sites along an urbanization gradient in the Potchefstroom municipal area during May (warmer, wet season) in 2007 (black cluster = mostly urban sites, purple cluster = mostly sub-urban sites, green cluster = mostly rural sites).

4.4 Multivariate ordinations showing relationships between soil data, plant species composition and abundance, and basic sampling site data

4.4.1 Ordinations based on soil chemical and physical data and plant species composition and abundance

A CCA ordination was completed using the plant species data and the chemical and physical data obtained from soil analysis of the different sampling sites (Figure 4.15). Data showing possible trends along the urbanization gradient as described earlier was selected for the ordination.

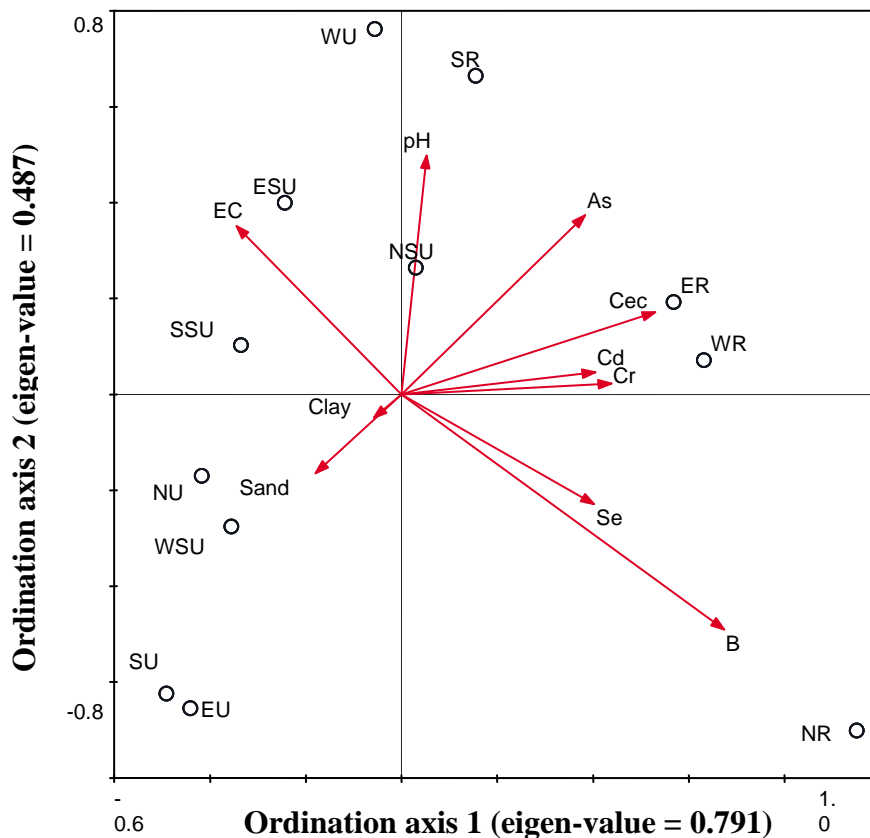


Figure 4.15. CCA ordination illustrating the relationship between the plant species composition and soil environmental factors (sand, clay, pH, electrical conductivity (EC), Boron (B), Chrome (Cr), Cadmium (Cd) Arsenic (As) Selenium (Se) and cation exchange capacity(Cec) data collected at the sampling sites along an urbanization gradient in the Potchefstroom municipal area.

Boron (B) is the variable with the highest positive correlation (0.66) with ordination axis 1 (Table 4.2). From Figure 4.15 it can be observed that a gradient of B (Boron) concentrations exists from bottom right (high) to top left (low) along ordination axis 1. Other positive correlations along ordination axis 1 include cation exchange (Cec) and, to a lesser extent, Chromium (Cr). The pH forms a weak gradient along ordination axis 2 (0.4466).

Table 4.2. Correlation coefficients and cumulative species-environment relation variance of selected environmental factors studied in the Potchefstroom municipal area along an urbanization gradient.

Environmental Factors	Ordination Axis 1	Ordination Axis 2
Sand	-0.1759	-0.1473
Clay	-0.0569	-0.0439
pH	0.0573	0.4466
EC (Electrical conductivity)	-0.3383	0.3146
B (Boron)	0.6601	-0.4393
Cr (Chromium)	0.4296	0.02
Cd (Cadmium)	0.3968	0.0411
As (Arsenic)	0.3755	0.3347
Se (Selenium)	0.3943	-0.2049
Cec (Cation exchange)	0.5197	0.1532
Cumulative species-environment variance	28.5 %	46.1 %

According to the CCA ordination (Figure 4.15), the boron gradient is not closely associated with the urbanization gradient. One of the rural sites had a high boron (B) concentration, two of the rural and two of the urban sites had intermediate boron concentrations, while the remaining sites had low boron concentrations. Only two of the rural sites (WR, ER) are associated with high cation exchange (Cec) and Chromium (Cr) concentrations, while urban and sub-urban sites (NU, SU, EU, WSU) tend to be

least associated with high Chromium (Cr) and cation exchange (Cec) values. Only one sub-urban site (NSU) and one rural site (SR) are closely associated with the pH gradient along ordination axis 2.

No clear patterns in terms of the described urbanization gradient could be observed regarding the soil chemical and physical data used in the ordination. This suggests that the physical and chemical properties of the soil did not differ significantly along the urbanization gradient in the study area. No clear connection could also be established between the physical and chemical properties of the sampling sites and the plant species composition present at these sites.

4.4.2 Ordination based on plant species composition and abundance as well as basic sampling site data

A CCA ordination was created to illustrate the relationship between the plant species composition data and the basic sampling site data collected during the study (Figure 4.16).

Bare ground (BG) is the variable with the highest positive correlation (0.76) with ordination axis 1 (Table 4.3). From Figure 4.16 it can be observed that a clear gradient of bare ground (BG) exists from bottom right (high) to top left (low) along ordination axis 1. None of the other environmental variables exhibit strong correlations with ordination axis 1. Many of the rural and sub-urban sites tend to be more closely associated with high bare ground (BG) values, while most of the urban sites had a low percentage of bare ground.

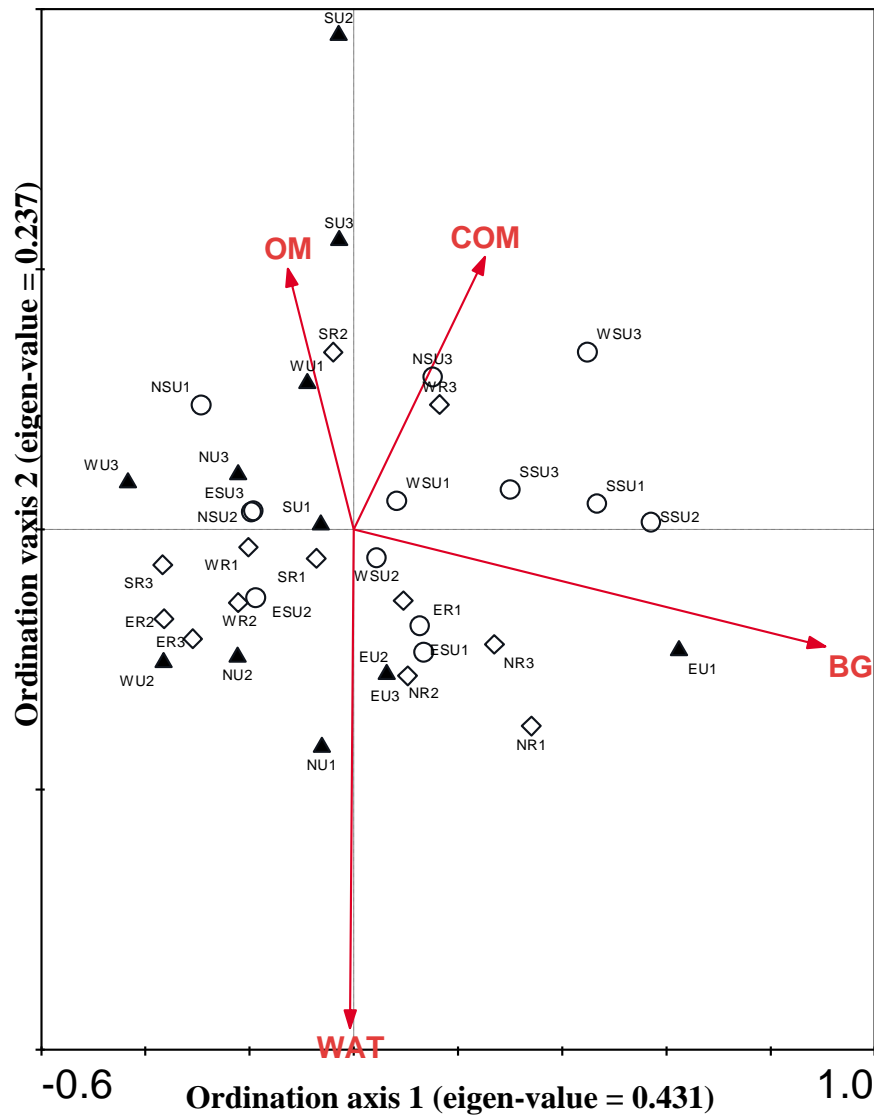


Figure 4.16. CCA ordination illustrating the relationship between the sampling sites regarding plant species composition, gravimetric water content, percentage bare ground, percentage dry organic matter, and soil compaction data collected at the sampling sites along an urbanization gradient in the Potchefstroom municipal area.

Table 4.3. Correlation coefficients and cumulative species-environment relation variance of selected environmental factors studied in the Potchefstroom municipal area along an urbanization gradient.

Environmental Factors	Ordination Axis 1	Ordination Axis 2
OM (organic matter)	-0.1067	0.3570
BG (bare ground)	0.7615	-0.1604
WAT (gravimetric water)	-0.0059	-0.68820
COM (compaction)	0.2122	0.3731
Cumulative species-environment relation variance	47.2 %	73.2 %

A gradient of gravimetric water (WAT) exists from the bottom (high) to the top (low) along ordination axis 2. No clear trend could, however, be established along the urbanization gradient regarding the gravimetric water values. Furthermore, no clear trends could be established regarding the organic matter (OM) and the compaction (COM) values. It would appear as if these results do not characterize the urbanization gradient in this study.

4.5 Microbial culture-dependent techniques

4.5.1 Bacterial levels in soil samples (cfu/g soil).

Bacterial levels (cfu/g soil) were determined for each soil sample collected at the 36 sampling plots during the study period. Average values for the 2007 sampling period are presented in Figure 4.17, and average values for the 2008 sampling period are presented in Figure 4.18.

Rural sites had on average the lowest bacterial cfu/g soil for the 2007 sampling period. The northern rural values were the highest of the rural sites, with the western rural values the lowest. Sub-urban sites tended to have higher bacterial levels than the rural sampling sites in 2007. The northern sub-urban values were the highest of the sub – urban sites, with the eastern-, southern- and western sub-urban values very similar to each other. Urban values had higher bacterial levels than the rural sites. Urban values from the May (warmer, wet) sampling season 2007 tended to be lower than the May (warmer, wet) sampling season 2007 values from the sub-urban sites. The urban values from August (colder, dry) sampling season 2007 tended to be higher than the August (colder, dry) sampling season values from the sub-urban sites. The southern urban August (colder, dry) value was the highest value registered in 2007. The western urban site had the lowest values for the urban sites. Overall, August (colder, dry) sampling had mostly higher values than the May (warmer, wet) sampling values. Urban and sub-urban sites had generally the highest bacterial levels during the 2007 sampling period.

Results regarding the 2008 sampling period revealed that the rural sampling sites had the lowest bacterial levels. The western rural values were the highest for the rural sites, with the eastern- and southern rural values the lowest. Sub-urban sites tended to have higher bacterial levels than the rural sampling sites in 2008. The northern sub-urban values were the highest of the sub-urban sites. The value collected at the northern suburban site in August (colder, dry) 2008 was the highest collected in 2008. The southern sub-urban values were the lowest of the sub-urban sites. Urban values tended to be the highest in the 2008 sampling period. The western urban value collected during May (warmer, wet) was the highest value collected in May of the urban sites, and the eastern urban value collected during August (colder, dry) was the highest value collected in August of the urban sites. Values from both sub-urban and urban samples indicated that the August (colder, dry) values were generally higher than the May (warmer, wet) sampling values.

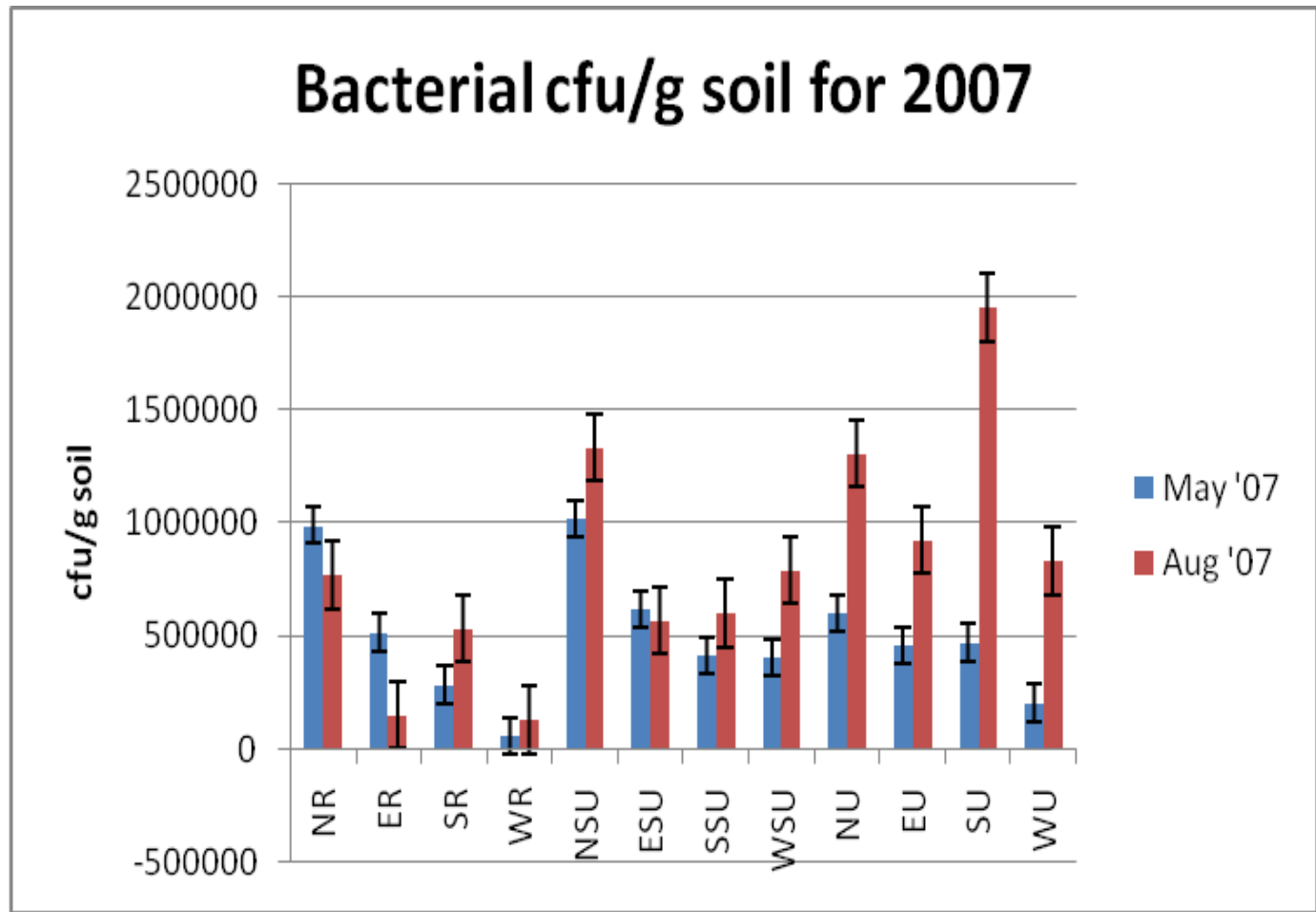


Figure 4.17. Average colony forming units/gram soil for heterotrophic bacteria recorded at the sampling sites during 2007 in the Potchefstroom municipal area along an urbanization gradient.

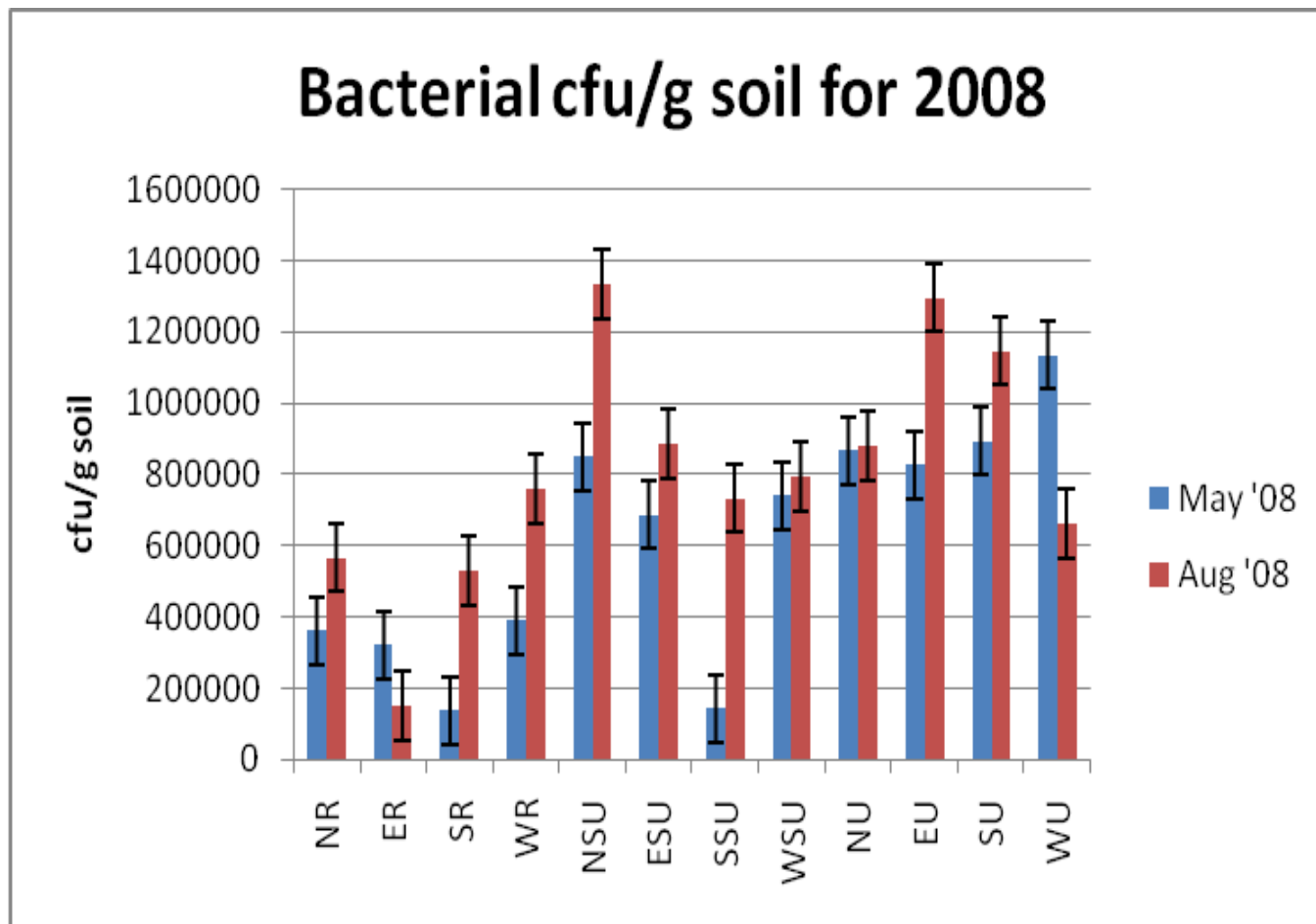


Figure 4.18. Average colony forming units/gram soil for heterotrophic bacteria recorded at the sampling sites during 2008 in the Potchefstroom municipal area along an urbanization gradient.

Summary of the bacterial cfu/g results

The results indicated generally higher cfu/g from August (colder, dry) samples than May (warmer, wet) samples. This was the case in 2007 and 2008. Tukey's HSD revealed that there were generally no statistically significant differences between the seasonal results.

Results indicated that there were generally more viable heterotrophic bacterial cells present in samples from urban soils than in rural samples. The use of Tukey's HSD revealed that differences between results were mostly statistically insignificant ($P > 0.05$). These results suggest that bacterial biomass can be higher in soil environments affected by anthropogenic influences.

4.5.2 Results of the identification of dominant bacterial species

Different morphotypes were isolated from the nutrient agar plates used for the culture-dependent analysis. Morphotypes were characterized by means of phenotypic attributes.

After the morphological characterization, molecular techniques were used to sequence 16S rDNA from the isolates. Sequences were compared to the GenBank database, and the isolates were identified.

The results regarding the visibly dominant bacterial species from samples are presented in Table 4.4.

Table 4.4. Identification of dominant heterotrophic bacterial species collected from agar plates used to determine the colony forming units/gram soil from the Potchefstroom municipal area over May (warmer, wet season) and August (colder, dry season) in 2007 along an urbanization gradient.

Locality where collected	Description of colony morphology and pigmentation	GenBank Id
Rural	Round, white, matted colony.	<i>Bacillus thuringiensis</i>
Rural	Small, white, round colony.	<i>Bacillus thuringiensis</i>
Rural	White, erose edged, flat, dull colony.	<i>Bacillus pumilis</i>
Rural	Beige, umbonate, circular, undulate edged colony.	<i>Bacillus simplex</i>
Rural	White, matted, undulate edged, circular, umbonate, smooth colony.	<i>Bacillus cereus</i>
Rural	Beige, erose edged, raised, shiny colony.	<i>Bacillus pumilis</i>
Sub-urban	Orange, circular, small, shiny, entire edged, flat colony.	<i>Sphingobacterium multivorum</i>
Sub-urban	White, smooth, shiny, flat, entire edged, circular colony.	<i>Arthrobacter</i> sp.
Sub-urban	Beige, circular, shiny, convex, erose edged colony.	<i>Bacillus</i> sp.
Sub-urban	White, erose edged, raised, shiny colony.	<i>Bacillus pumilis</i>
Urban	Beige, erose edged, curled, umbonate, dull, rough colony.	<i>Bacillus pumilis</i>
Urban	White, circular, undulate edged, dull colony.	<i>Bacillus megaterium</i>
Urban	White, circular, umbonate, concave colony.	<i>Bacillus subtilis</i>
Urban	Orange, circular, small, shiny, entire edged, concave colony.	Uncultured bacterium

Identification of the visibly dominant heterotrophic bacteria on culture plates revealed mostly rod-shaped, gram-positive bacteria. Species were mainly from the genus *Bacillus*.

Identification results showed that although the visibly dominant bacteria differed morphologically and with regard to pigmentation, this does not necessarily imply that the organisms belonged to different species. This issue illustrates a problem with regard to using morphological and pigmentation differences to assess bacterial diversity. However, the combination of morphological and sequence data was used to determine Shannon-Weaver indexes for the various sites (section 4.5.3).

4.5.3 Shannon-Weaver diversity indices for bacterial data

The bacterial diversity data that was generated using the culture-dependent techniques was used to calculate Shannon-Weaver diversity indices for all the samples analyzed. The results are presented in Figure 4.19.

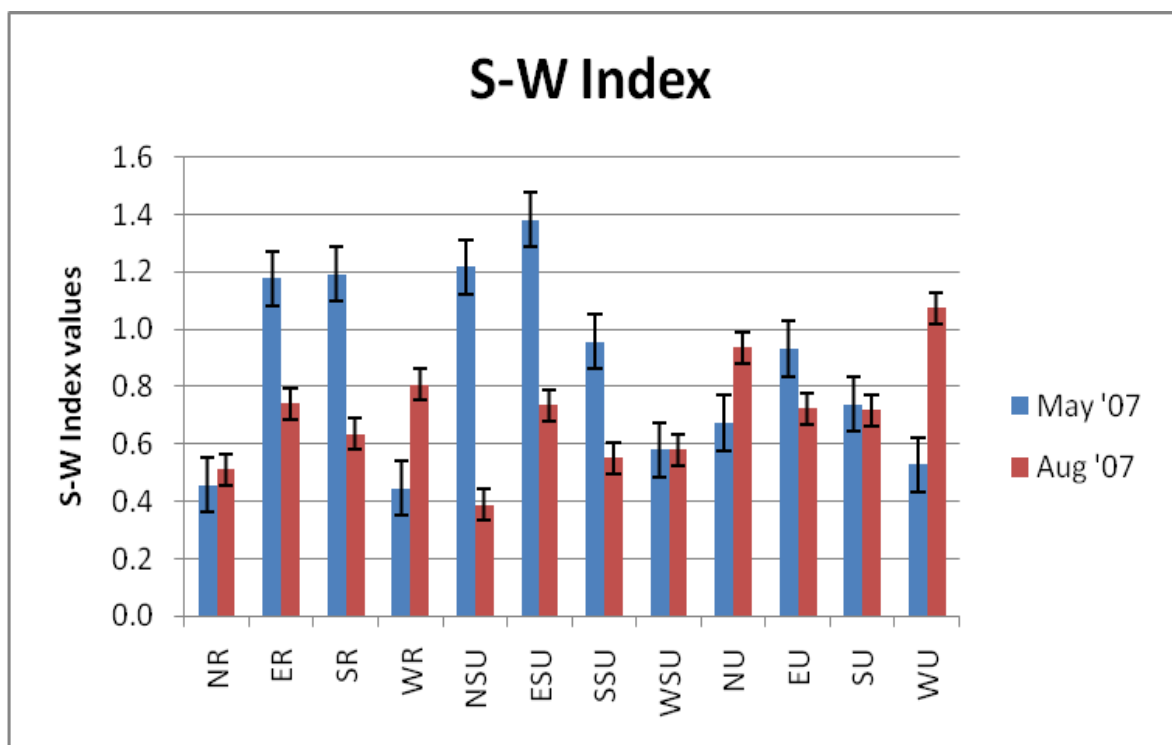


Figure 4.19. Shannon-Weaver Index estimating bacterial diversity. Values were calculated using morphological and sequence data from culture-dependent methods.

The results indicated that the sub-urban and urban sites generally had a higher Shannon-Weaver value than the rural sites, although these differences were mostly statistically insignificant. No clear trends emerged regarding the urbanization gradient or the seasonal differences.

The sequencing of the isolated dominant bacteria revealed that some of the isolates were of the same species of bacteria. It seems as if environmental factors influenced the bacteria, causing colonies of the same species to exhibit different morphological traits. This finding further hampered the significance of the diversity indices calculated from morphological data because of inflation of the results. To obtain a clearer perspective of the bacterial community structure in soil, culture-independent methods must be used in order to eliminate this problem.

4.5.4 Fungal levels in soil samples (cfu/g soil)

Fungal levels (cfu/g) soil was determined for each soil sample collected at the 36 sampling plots during the study period. Average values for the 2007 sampling period are presented in Figure 4.20, and average values for the 2008 sampling period are presented in Figure 4.21.

All the samples collected during the May (warmer, wet) sampling season 2007 yielded higher fungal levels than the samples collected during the August (colder, dry) sampling season 2007. The northern rural value collected during May (warmer, wet) was the highest value collected in 2007. The August (colder, dry) results collected at the rural sites were similar, and much lower than the values collected during the May (warmer, wet) sampling period. The sub-urban values were generally lower than the rural values. The western sub-urban value collected during the May (warmer, wet) was the highest value of the sub-urban sites. The values collected in the August (colder, dry) sampling season were again similar to each other, and lower than the values collected during the May (warmer, wet) sampling season. Urban values tended to be higher than the sub-urban values, but lower than the rural values. The eastern urban value collected during the May (warmer, wet) sampling season was the highest value of the urban sites. Like the rural and the sub-urban values collected in August (colder, dry), the urban

August (colder, dry) values were similar to each other, and lower than the May (warmer, wet) values.

In 2008, the rural values were generally the lowest of all the sites along the urbanization gradient. The eastern rural value collected in the August (colder, dry) sampling season was the highest value of all the rural sites. The western rural values were the lowest of the rural sites. The sub-urban values tended to be higher than the rural values. The western sub-urban value collected in August (colder, dry) was the highest value of the sub-urban sites. The southern sub-urban values were the lowest of the sub-urban sites. The values collected at the urban sites tended to be the highest in 2008. The eastern urban value collected in the August (colder, dry) was the highest value of the 2008 sampling period. The northern urban values were the lowest of the urban sites. The values collected in August (colder, dry) were generally higher than the values collected in May (warmer, wet) in 2008.

Summary of the fungal cfu/g results

May (warmer, wet) results from 2007 were significantly higher than all the other sampling results. The results indicated that fungi cfu/g soil tended to be higher in the rural samples than the sub-urban and urban samples for the 2007 sampling period. Generally, the May (warmer, wet) values collected in 2007 were higher than the values collected in August (colder, dry). The trends present in 2007 were not repeated in 2008. Fungal levels from the urban sites in 2008 tended to be the highest, followed by the sub-urban sites, and then the rural sites. The August (colder, dry) sampling season values collected in 2008 were mostly higher than the values collected in the May (warmer, wet) sampling season 2008. Differences in results from 2007 and 2008 were mostly statistically insignificant ($p > 0.05$).

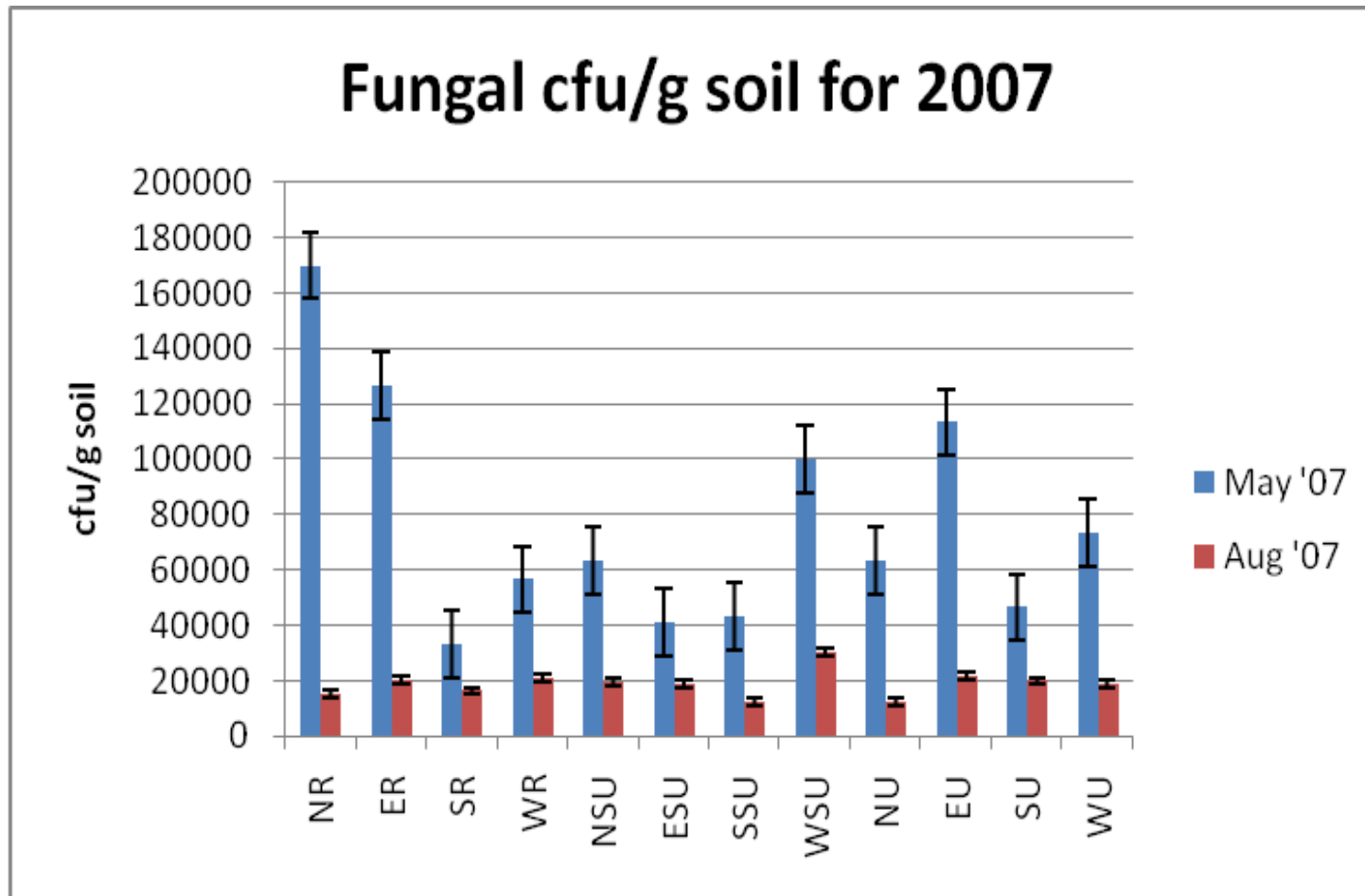


Figure 4.20. Average colony forming units/gram soil for fungi recorded at the sampling sites during 2007 in the Potchefstroom municipal area along an urbanization gradient.

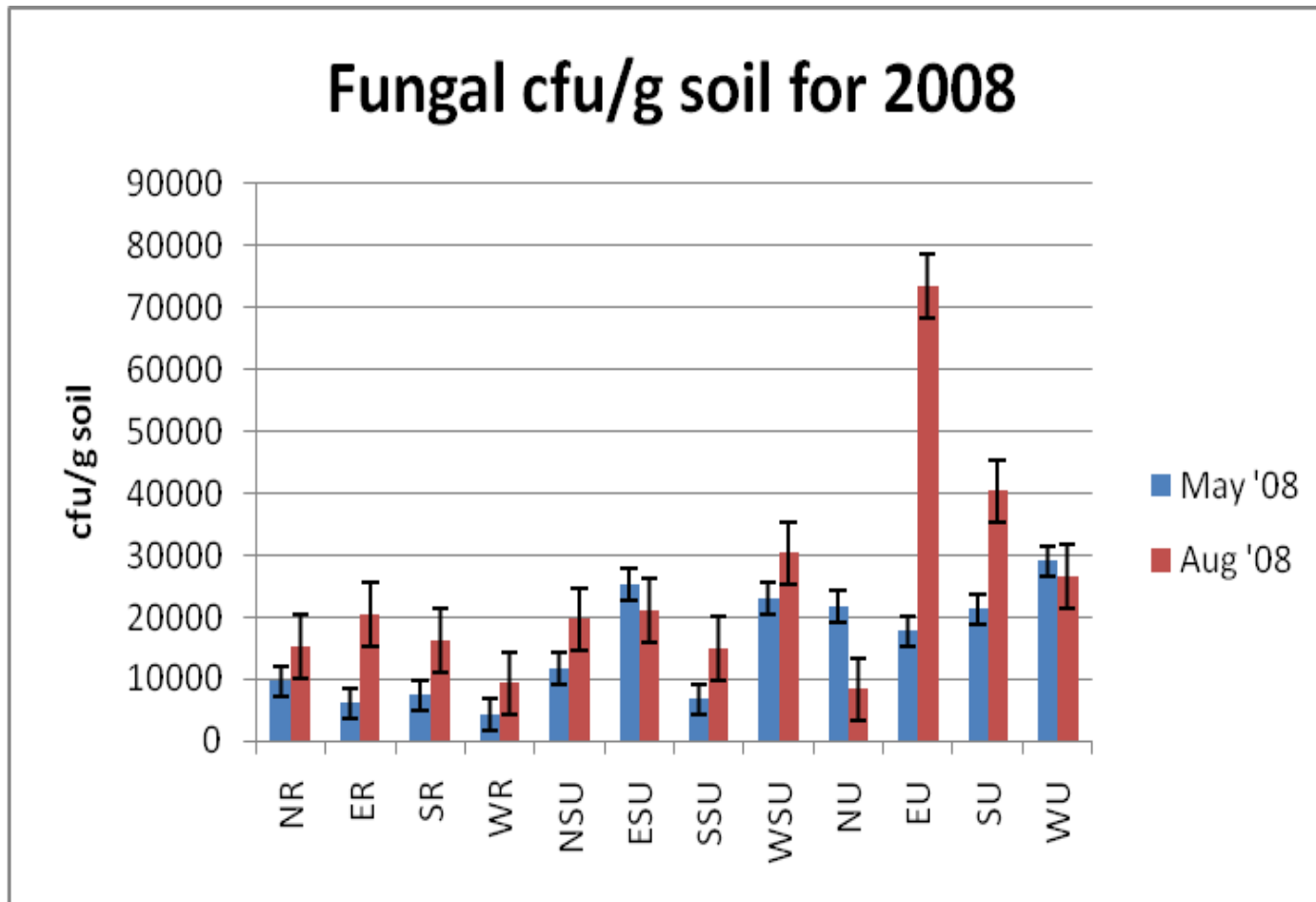


Figure 4.21. Average colony forming units/gram soil for fungi recorded at the sampling sites during 2008 in the Potchefstroom municipal area along an urbanization gradient.

4.6 Microbial culture-independent techniques

4.6.1 Soil bacterial community results

Extracted community DNA were amplified using the PCR reaction. The PCR reactions were conducted using different concentrations of template DNA, BSA, MgCl₂, and primer concentrations. The PCR product amplification was confirmed prior to DGGE analysis. This was done using agarose gel electrophoresis. Figure 4.22 is an example of a gel that demonstrates successful PCR's.

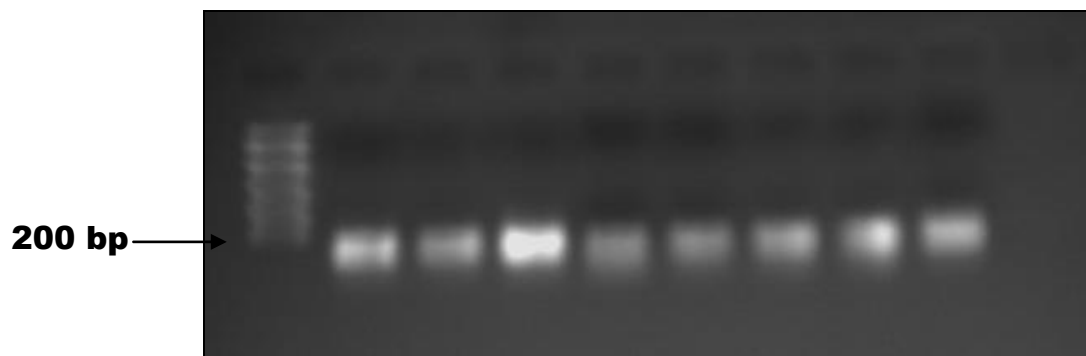


Figure 4.22. Image of the PCR product (200 bp) used for DGGE to analyze the bacterial soil community structure from the Potchefstroom municipal area along an urbanization gradient.

The analysis of the bacterial community structure was affected by poor quality DGGE results. Over the course of the study period, different parameters were used in an attempt to optimize the process. DGGE analysis was performed numerous times, also with little success. The DGGE analysis presented here (Figure 4.23) represent the best recorded results that were obtained.

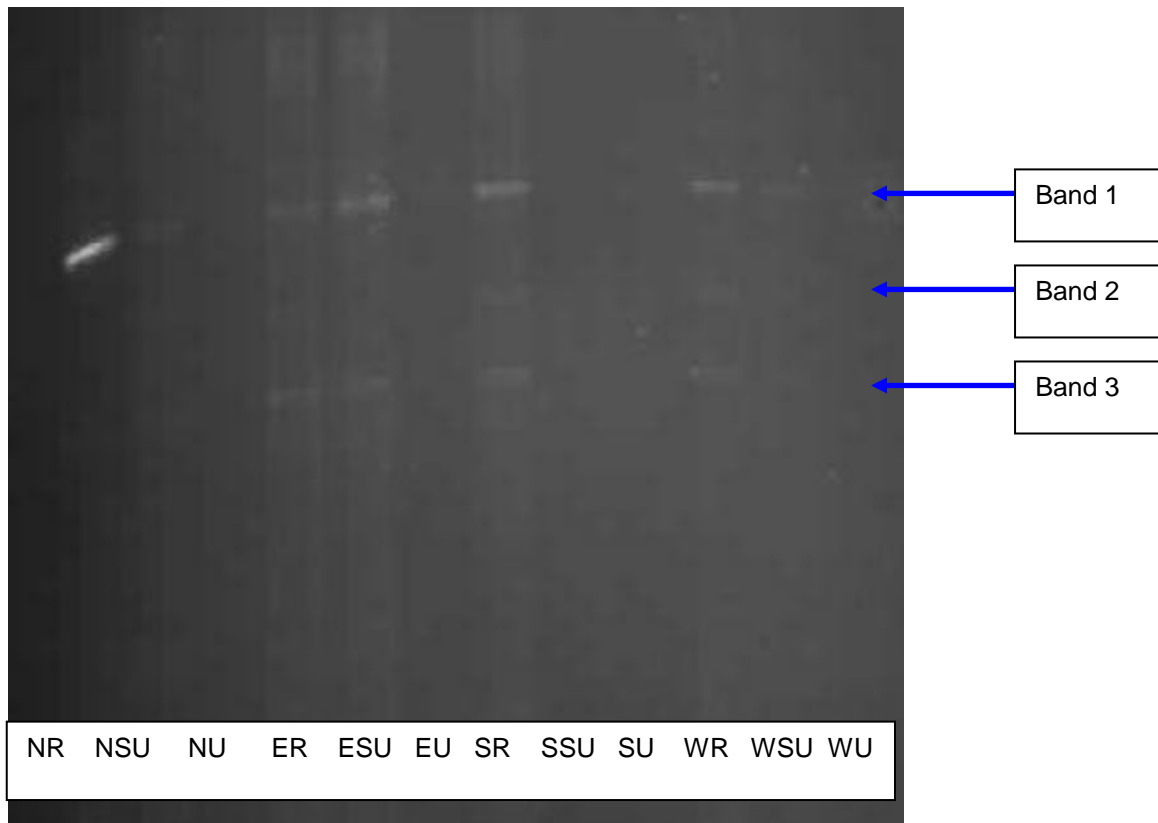


Figure 4.23. DGGE analysis of the bacterial communities from samples collected at the rural, sub-urban and urban sampling locations in the Potchefstroom municipal area along an urbanization gradient. The blue arrows indicate bands that represent dominant species.

Results (Figure 4.23) showed that a few dominant species were prevalent in all the samples analyzed. No further analysis could be conducted. The process therefore needs to be optimized to produce better and more meaningful results. Proposals for further optimization will be presented in Chapter 5.

From the results gathered it can be hypothesized that urbanization does not have a significant effect on the community structure of bacterial component of soil. Further studies are needed to confirm the hypothesis

4.7 Enzyme activity results

Enzyme assay results were processed using the relevant formulas as discussed in the Materials and Methods section. Potential activity units are described in the Materials and Methods section. These results were further processed using Statistica version 7 (Statsoft). Tukey's HSD was used to determine statistical significance between the different sample values.

4.7.1 Dehydrogenase

Potential dehydrogenase activity values collected in the 2007 sampling period is presented in Figure 4.24. Potential dehydrogenase activity values were generally higher in all samples collected during the August (colder, dry) sampling period than those collected during the May (warmer, wet) sampling period. The northern samples typically registered the highest values, with the southern samples mostly registering the lowest values. The urban samples generally registered the highest values, followed by the sub-urban samples, and then the rural samples.

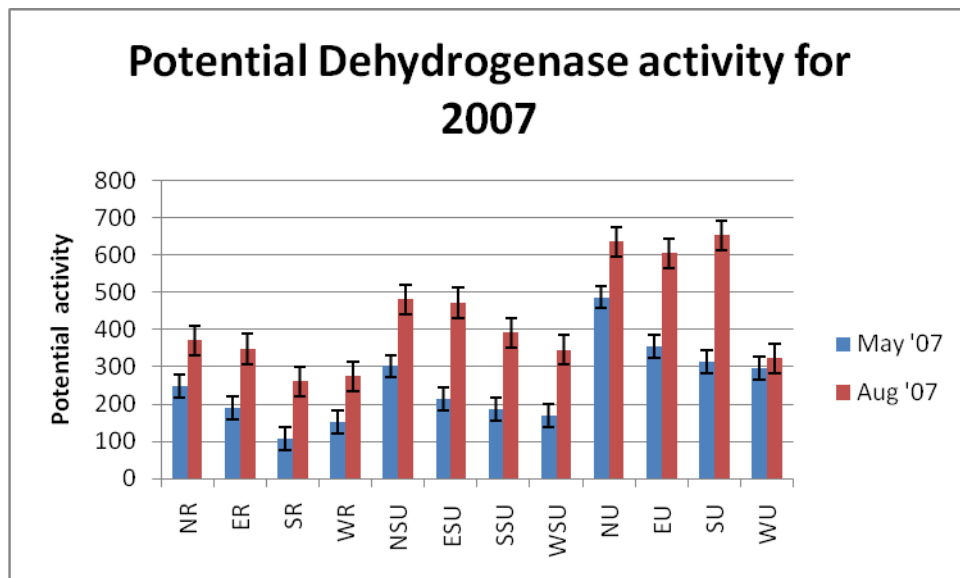


Figure 4.24. Potential dehydrogenase activity for soil samples collected in 2007 at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

Potential dehydrogenase activity values collected in the 2008 sampling period is presented in Figure 4.25. There was no clear trend regarding August (colder, dry) sampling values and May (warmer, wet) sampling values. A similar trend existed as in 2007 regarding higher potential dehydrogenase activity in samples from the urban sampling sites, followed by samples from the sub-urban sampling sites, and then samples from the rural sampling sites. The northern samples typically registered the highest values, with the southern samples mostly registering the lowest values.

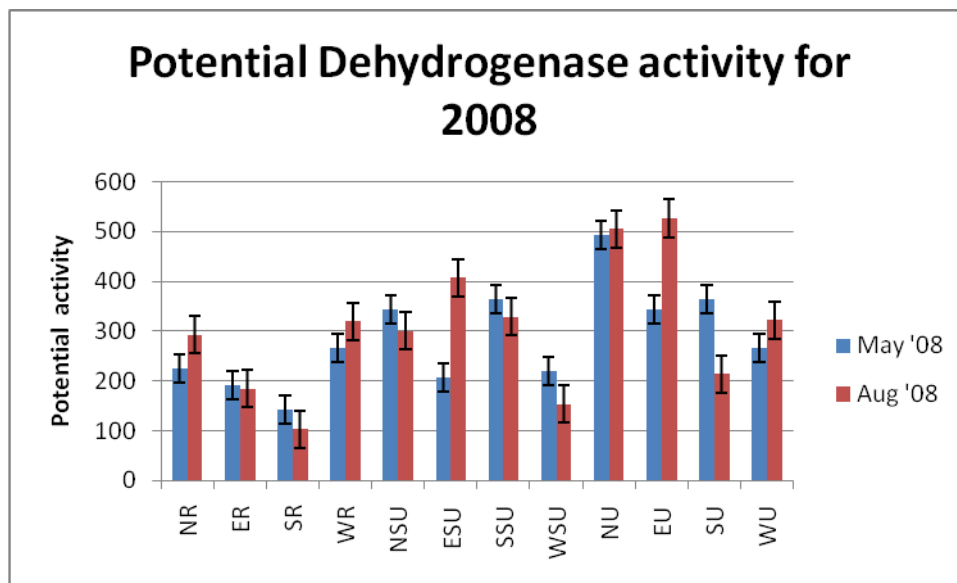


Figure 4.25. Potential dehydrogenase activity for soil samples collected in 2008 at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

Summary of dehydrogenase results

Generally higher levels of potential dehydrogenase activity were observed in samples collected during the August (colder, dry) sampling period. The results generally showed a clear gradient between the urban and rural sites. Urban sites had higher potential dehydrogenase activity, followed by the sub-urban sites, and then the rural sites. Tukey's HSD showed mostly statistically significant differences ($P < 0.05$) between the urban and rural, urban and sub-urban, and sub-urban and rural sampling localities.

4.7.2 β -glucosidase

Potential β -glucosidase activity values collected in the 2007 sampling period is presented in Figure 4.26. The eastern samples generally registered the highest values, with the western samples generally typically the lowest values. Samples collected in the May (warmer, wet) sampling period generally registered higher values than samples collected in the August (colder, dry) sampling period. The urban samples registered the highest values, followed by the sub-urban samples, and then the rural samples.

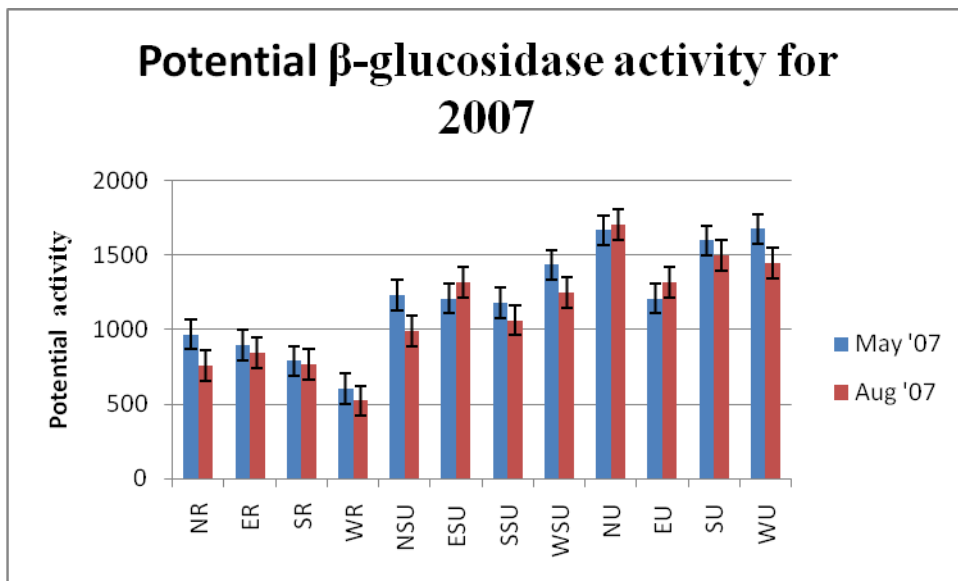


Figure 4.26. Potential β -glucosidase activity for soil samples collected in 2007 at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

Potential β -glucosidase activity values collected in the 2008 sampling period is presented in Figure 4.27. The northern samples generally registered the highest values, with the western samples generally typically the lowest values. Samples collected in the May (warmer, wet) sampling period generally registered lower values than samples collected in the August (colder, dry) sampling period. A similar trend existed as in 2007 regarding the urban samples registering the highest values, followed by the sub-urban samples, and then the rural samples.

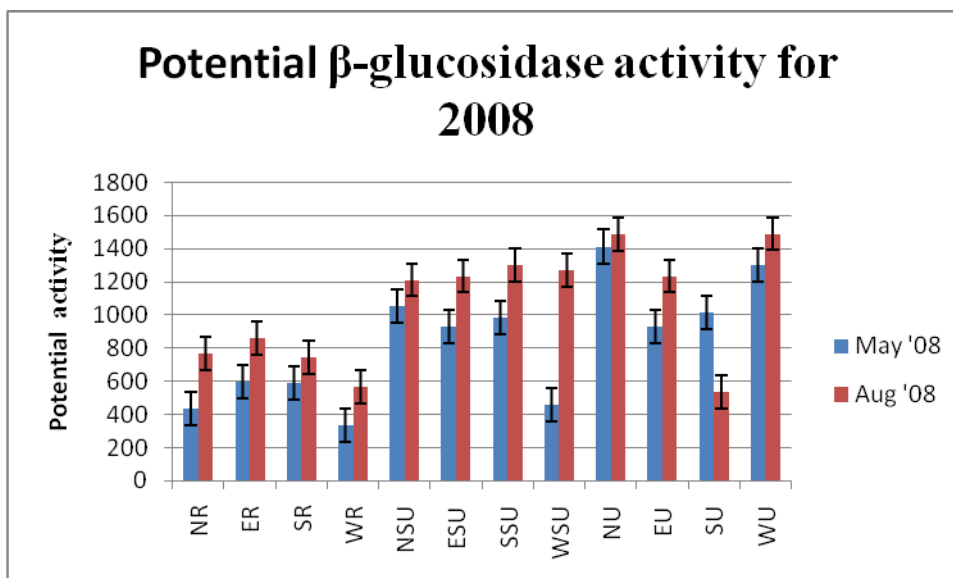


Figure 4.27. Potential β -glucosidase activity for soil samples collected in 2008 at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

Summary of the β -glucosidase results

Overall higher potential β -glucosidase activity was observed with samples collected in the August (colder, dry) sampling period. Results overall showed a clear gradient between the urban and rural sites. Urban sites had higher potential β -glucosidase activity, followed by the sub-urban sites, and then the rural sites. Tukey's HSD showed mostly statistically significant differences ($P < 0.05$) between the urban and rural, urban and sub-urban, and sub-urban and rural sampling localities.

4.7.3 Urease

Potential urease activity values collected in the 2007 sampling period is presented in Figure 4.28. Potential urease activity values were generally higher in all samples collected during the August (colder, dry) sampling periods than those collected during the May (warmer, wet) sampling periods. Potential urease activity was generally the highest in the urban samples, except for the eastern rural and western rural samples

collected in August 2007. The eastern and western samples mostly registered the highest values, with the southern samples generally registering the lowest values.

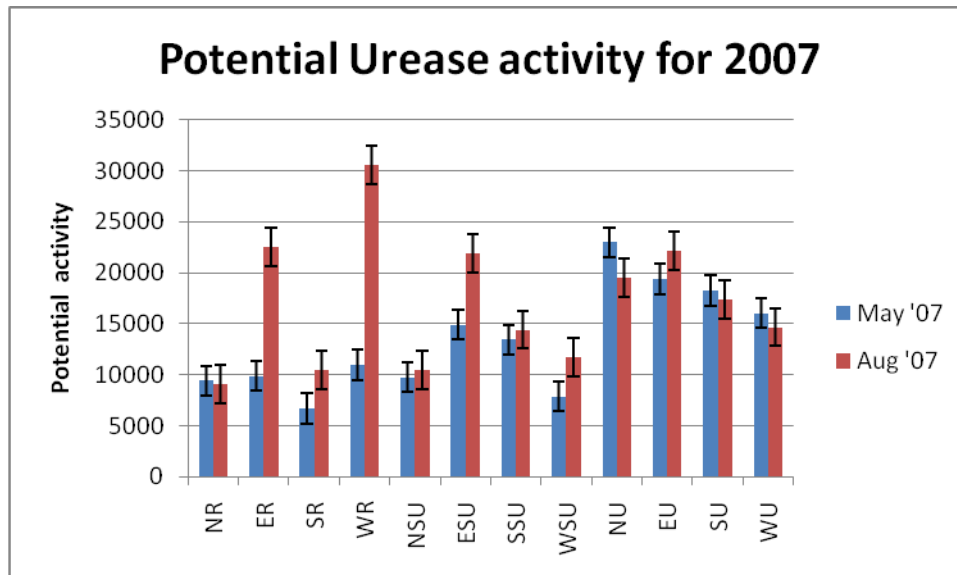


Figure 4.28. Potential urease activity for soil samples collected in 2007 at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

Potential urease activity values collected in the 2008 sampling period is presented in Figure 4.29. No clear trend could be observed regarding the August (colder, dry) sampling values and the May (warmer, wet) sampling values collected in 2008. Urban sample values was generally the highest, with the value from the northern urban sample collected in August and the Eastern sample collected in May being the highest values.

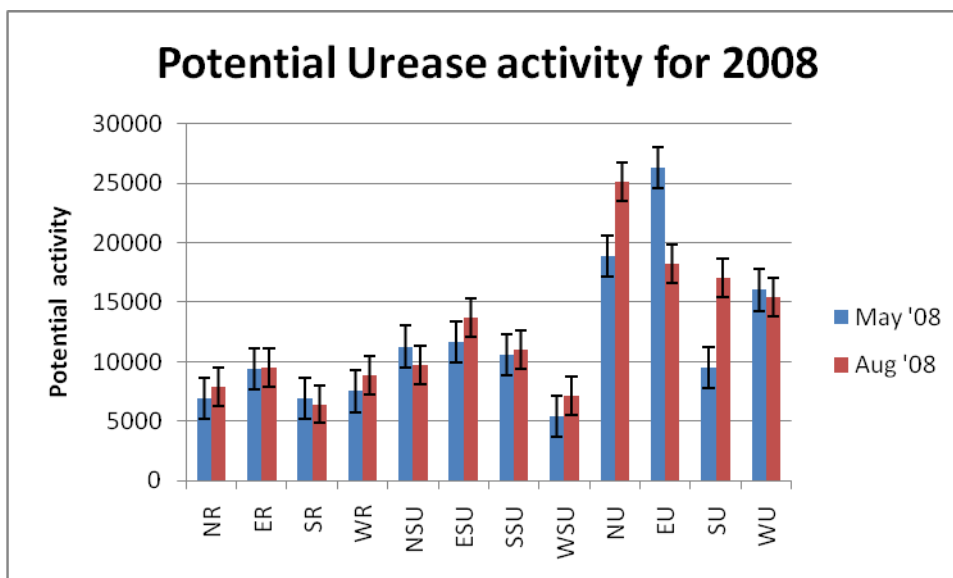


Figure 4.29. Potential urease activity for soil samples collected in 2008 at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

Summary of the urease results

Generally, higher potential urease activity was observed in samples collected in the August (colder, dry) sampling period. For the most part, results showed a clear gradient between the urban and rural sites. Urban sites had higher potential urease activity, followed by the sub-urban sites, and then the rural sites. Tukey’s HSD showed that the differences between the values were mostly insignificant ($P > 0.5$).

4.7.4 Acid phosphatase

Potential acid phosphatase activity values collected in the 2007 sampling period is presented in Figure 4.30. Values from the rural sampling sites were similar to the values from the urban sampling sites.

Potential acid phosphatase activity was, for the most part, the lowest in the samples collected at the sub-urban sampling sites. The southern samples generally registered the highest values. The western urban samples registered the lowest value in the August (colder, dry) sampling period.

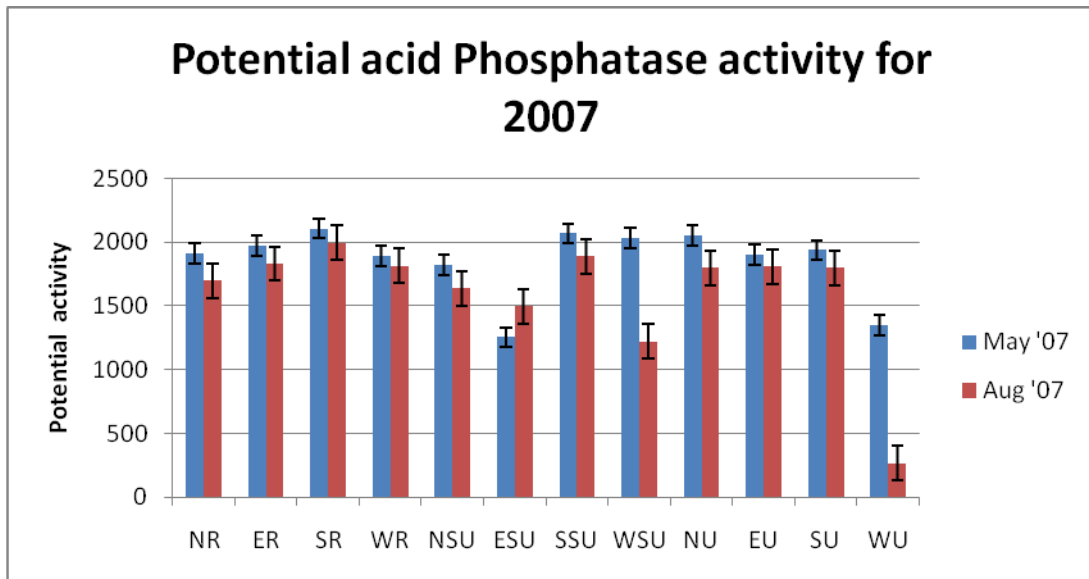


Figure 4.30. Potential acid phosphatase activity for soil samples collected at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

4.9.4.5 Summary of the acid phosphatase results

Potential acid phosphatase activity results revealed no clear trend regarding urbanization. Tukey's HSD indicated mostly statistically significant differences between the urban and rural, urban and sub-urban, and sub-urban and rural sampling localities. May (warmer, wet) values were generally higher than the August (colder, dry) values.

Due to time constraints and the fact that no clear trend was observed in 2007 it was decided not to repeat the acid phosphatase assay in 2008.

4.7.5 Alkaline Phosphatase

Potential alkaline phosphatase activity values collected in the 2007 sampling period is presented in Figure 4.31. Potential alkaline phosphatase activity was typically the lowest in the samples collected at the rural sampling sites. The eastern samples

generally registered the highest values, with the western samples mostly registering the lowest values.

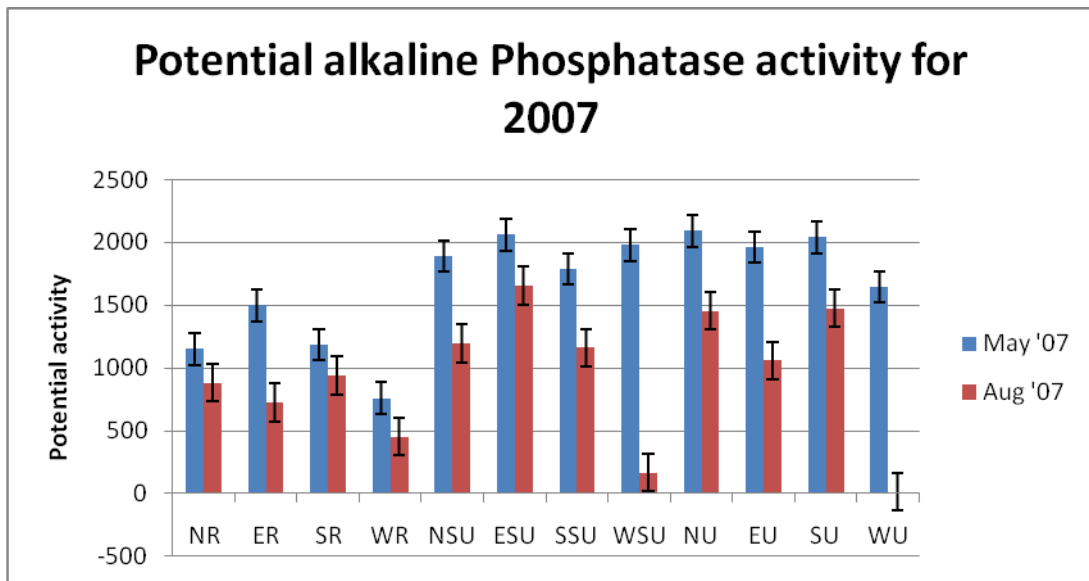


Figure 4.31. Potential alkaline phosphatase activity for soil samples collected at the different sampling locations in the Potchefstroom municipal area along an urbanization gradient.

Potential alkaline phosphatase activity was generally higher in the samples collected at the sub-urban sampling sites than the samples collected in the rural sampling sites. The eastern samples registered the highest values overall, with the western samples generally registering the lowest values.

Potential alkaline phosphatase activity from the urban sampling sites was similar to the results recorded from the sub-urban sampling sites. The northern samples generally registered the highest values, with the western samples for the most part registering the lowest values.

Summary of the alkaline phosphatase results

Potential alkaline phosphatase activity revealed more of a gradient than the acid phosphatase. Tukey's HSD indicated mostly statistically significant differences between the urban and rural, urban and sub-urban, and sub-urban and rural sampling

localities. The trend observed, although weak, suggested that potential alkaline phosphatase activity seemed higher in the urban areas than in the rural areas. Potential alkaline phosphatase activity was significantly higher in the May (warmer, wet) sampling period than in the August (colder, dry) sampling period.

Due to time constraints it was decided not to repeat the alkaline phosphatase assay in 2008.

4.7.6 Ordination results regarding potential dehydrogenase, β -glucosidase and Urease activity

Using CANOCO, a PCA ordination was done using all the data obtained during dehydrogenase, β -glucosidase and urease assays (Figure 4.32). The ordination clearly shows that most of the rural, sub-urban and urban sites tend to be clustered in three groups based on enzyme activity data. The ordination further shows that most of the urban sites had high activities of dehydrogenase, β -glucosidase and urease, while the rural sites showed the lowest activities, with the sub-urban sites in between. The ordination therefore confirmed the results described with the aid of Figures 4.24 to Figure 4.29. It also showed a soil enzyme activity gradient along an urbanization gradient.

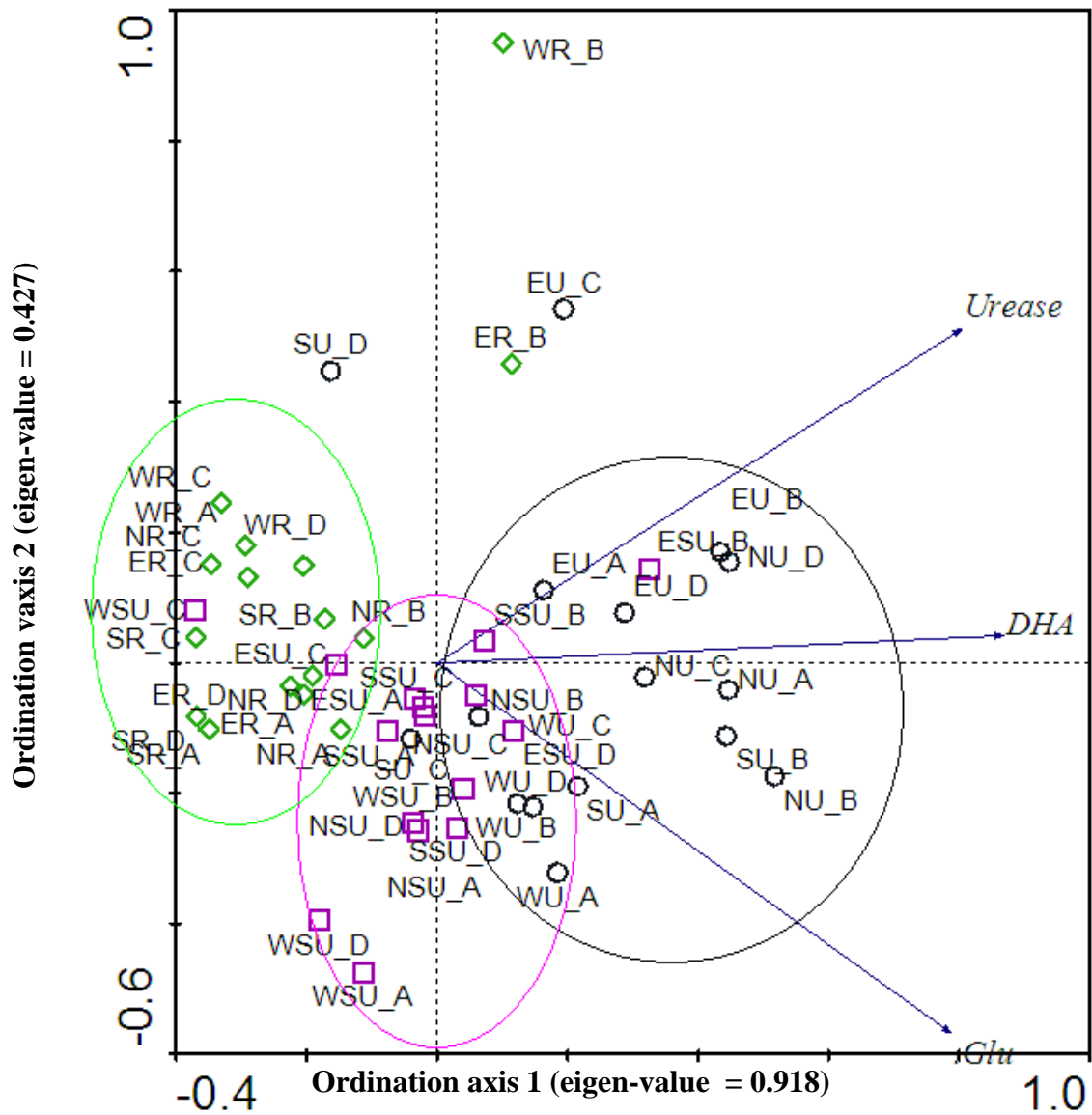


Figure 4.32. PCA ordination showing the relations between site enzymatic assay results for soil samples collected at the different sampling locations over four sampling seasons in the Potchefstroom municipal area along an urbanization gradient. (black cluster = mostly urban sites, purple cluster = mostly sub-urban sites, green cluster = mostly rural sites).

4.8 Ordinations showing relationships between different data sets

To further illustrate the relationship between the different sets of data better, ordinations were created in the CANOCO program (Ter Braak, 1992). Various ordinations using different sets of data are presented in the following section.

4.8.1 Ordination regarding the plant community data and the potential enzymatic activities results

Using CANOCO, a CCA ordination was done using the plant community data and the potential enzymatic activity data (Figure 4.33).

The gradient along ordination axis 1 (Figure 4.33) can be described the best by potential alkaline phosphatase activity (AlkP) and potential β -glucosidase activity (Glu), as they have the highest correlation coefficients (Table 4.5). Sub-urban sites seem to be more closely related to higher potential activity of alkaline phosphatase (AlkP), with the rural sites being the least associated with high potential alkaline phosphatase activity. This could indicate a link between the urban and sub-urban plant communities and higher potential alkaline phosphatase activity. Anthropogenic influences in the urban and sub-urban environment could be responsible for the higher values encountered during this study. β -glucosidase (-Glu), dehydrogenase (DHA) and urease potential activity followed a similar trend, with a gradient stretching from left (high potential activity) to right (low potential activity) along ordination axis 1. It is demonstrated in the graph that urban sites tend to be closely associated to higher potential activity of β -glucosidase, dehydrogenase and urease. This could indicate a possible relationship between the higher potential enzyme activities of these enzymes and the plant community structures encountered at the urban site.

Table 4.5. Correlation coefficients and cumulative species and species-environment relation variance of selected environmental factors studied in the Potchefstroom municipal area along an urbanization gradient.

Environmental Factors	Ordination Axis 1	Ordination Axis 2
Urease	-0.5406	0.4290
DHA (dehydrogenase)	-0.4285	0.4420
-GLu (β-glucosidase)	-0.7720	0.3398
AlkP (alkaline phosphatase)	-0.8376	-0.1996
AcidP (acid phosphatase)	0.1361	-0.1107
Cumulative species- relation variance	8.2 %	12.2%
Cumulative species- environment relation variance	41.8 %	61.7 %

The gradient along the ordination axis 2 is the best described by DHA (potential dehydrogenase activity), although the correlation was relatively weak (Table 4.5). The urban and sub-urban sites form two separate clusters along ordination axis 2, probably indicating that urban plant communities favour dehydrogenase activity.

Sampling sites did not correlate well with potential acid phosphatase activity, with only sampling site SU associated with high potential acid phosphatase activity. The ordination indicates that the potential acid phosphatase activity (AcidP) was not different along the urbanization gradient used in this study. No clear evidence was therefore gathered that anthropogenic influences have altered the enzyme activity.

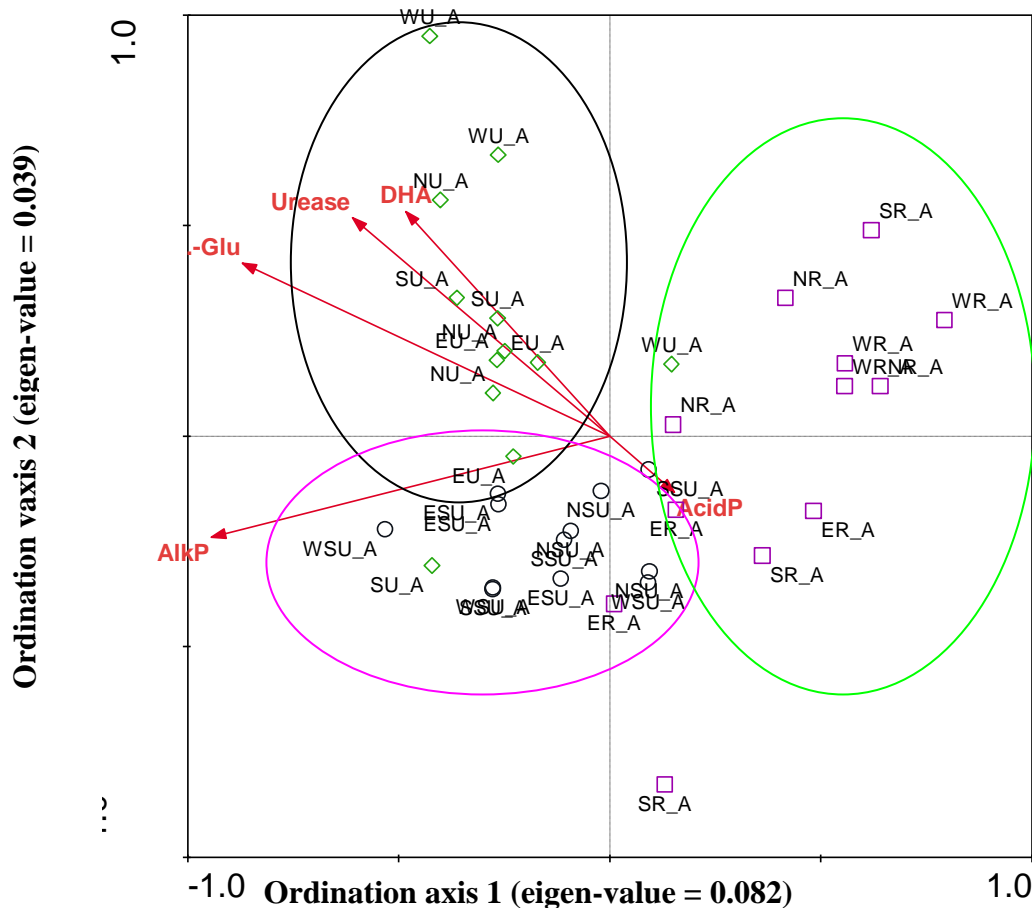


Figure 4.33. CCA ordination illustrating the relationship between the sampling sites regarding the plant community data and the potential enzyme activity of dehydrogenase, β -glucosidase, urease, acid- and alkaline phosphatase data from soil samples collected at the different sampling locations over four sampling seasons in the Potchefstroom municipal area along an urbanization gradient. (black cluster = mostly urban sites, purple cluster = mostly sub-urban sites, green cluster = mostly rural sites).

The ordination clearly illustrates the clusters of the urban, sub-urban and rural sites (Figure 4.33). High potential activity of enzymes involved in the C cycle, namely dehydrogenase and β -glucosidase, are strongly associated with the urban sites. High potential activity of urease (involved in the N cycle) is also strongly associated with the urban sites. The results indicate the highest potential enzymatic activities at the urban sampling sites, followed by the sub-urban sampling sites and the rural sampling sites.

4.8.2 Ordination regarding the plant species composition, plant species characteristics and the potential enzymatic activity of dehydrogenase, β -glucosidase and urease

Using CANOCO, an RDA ordination was performed which included the plant species composition, plant species characteristics, the potential enzymatic activity data from all assays completed, and the levels of bacterial and fungi components obtained from soil samples collected (Figure 4.34). In Figure 4.34 the effect of microbial populations (bacteria and fungi) and potential enzymatic activity of dehydrogenase, β -glucosidase and urease as possible environmental effects of plant species compositional changes is shown by an RDA tri-plot ordination graph.

The first ordination axis of the RDA plot can be best explained by the potential β -glucosidase activity (-Glu) (Table 4.6). Potential alkaline phosphatase activity also correlated positively with ordination axis 1, but to a lesser extent than β -glucosidase (Table 4.6). The second ordination axis was the best described by the bacterial population (bacteria) and potential acid phosphatase activity (Table 4.6).

The ordination reveals that sites experiencing the most anthropogenic influences (sub-urban and urban sites) generally have low numbers of perennial and indigenous plant species. High potential enzymatic activities of urease, dehydrogenase (DHA), alkaline phosphatase (AlkP) and β -glucosidase (-Glu) are, however, closely correlated with these sites, indicating higher enzymatic activity in the anthropogenically affected sites. High bacterial populations (Bacteria) also tended to be more closely associated with the urban and sub-urban sites.

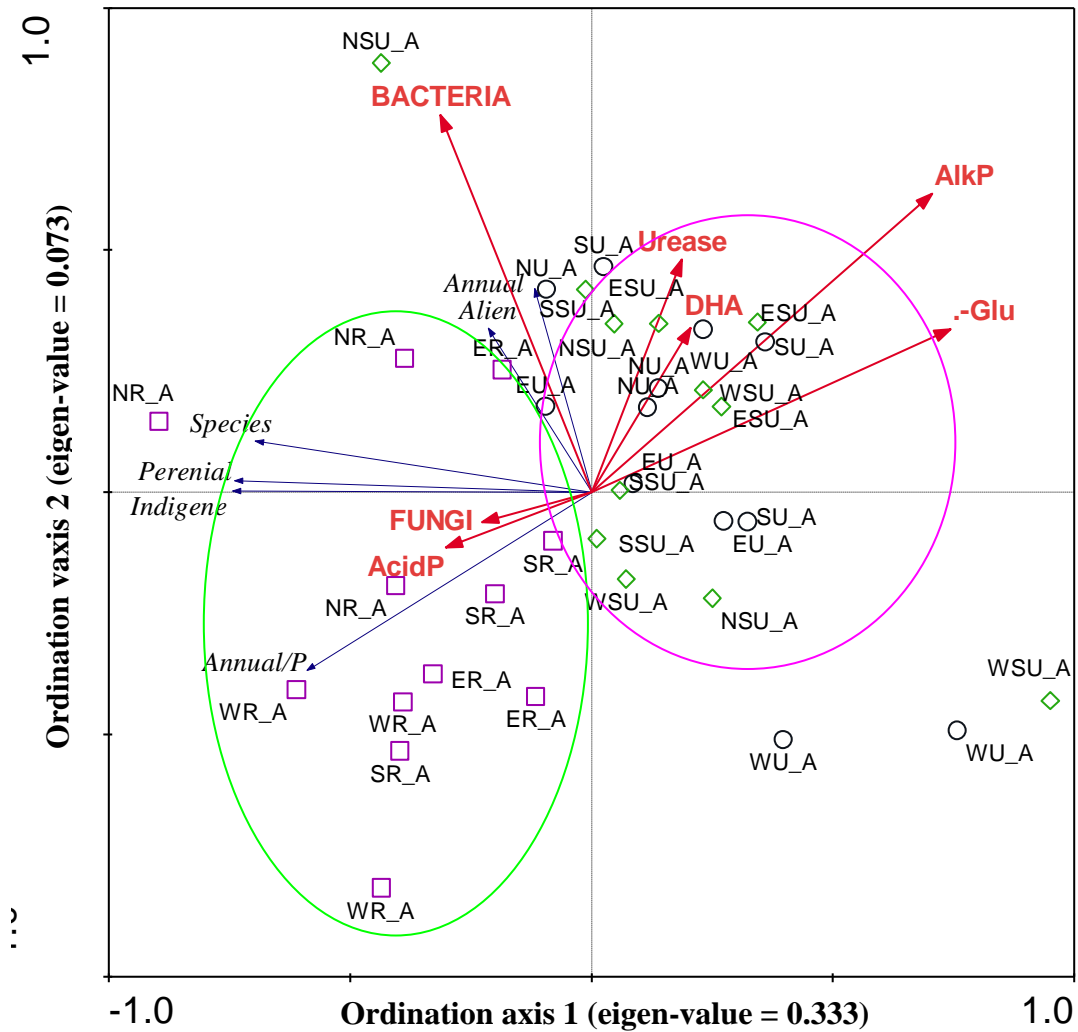


Figure 4.34. RDA ordination illustrating the relationship between the sampling sites regarding the plant species composition, plant species characteristics, the potential enzyme activity of dehydrogenase, β -glucosidase and urease data and the cfu/g soil data of the bacterial and fungal components from soil samples collected at the different sampling locations over four sampling seasons in the Potchefstroom municipal area along an urbanization gradient. (purple cluster = mostly urban and sub-urban sites, green cluster = mostly rural sites).

Rural sites generally have high numbers of annual/perennial plant species. High numbers of fungal populations (fungi) are, however, weakly associated with the rural sites, while potential acid phosphatase activity is more closely associated with the rural sites (Figure 4.34).

Table 4.6. Correlation coefficients and cumulative species and species-environment relation variance of selected environmental factors studied in the Potchefstroom municipal area along an urbanization gradient.

Environmental Factors	Ordination Axis 1	Ordination Axis 2
Urease	0.1401	0.2640
DHA (dehydrogenase)	0.1534	0.1881
-GLu (β-glucosidase)	0.5559	0.1867
AlkP (alkaline phosphatase)	0.5274	0.3411
AcidP (acid phosphatase)	-0.2338	-0.4318
Bacteria (cfu/g soil)	-0.2328	0.4318
Fungi (cfu/g soil)	-0.1701	-0.0342
Cumulative species-relation variance	33.3 %	40.6 %
Cumulative species-environment relation variance	73.5 %	89.7 %

The number of plant species (Species), perennial plant species (Perennial) and indigenous plant species (Indigenous) did not correlate closely with any of the sampling site categories.

Chapter 5 – Discussion

5.1 Soil chemical and physical data along an urbanization gradient

Analysis of clay, sand and silt content of the soil samples revealed that sub-urban and urban soils in the present study had higher clay content than the rural soils (Figure 4.1). Clay particles naturally have a negative charge. Higher clay content therefore increases the cation exchange capacity. This may also affect the electrical conductivity, as ions in clay-rich soils do not leach out as easily as in sandy soils (Camberato, 2001). These factors should be taken into consideration when interpreting cation exchange capacity and electrical conductivity in soil in Potchefstroom.

Soil pH is an important factor in soil ecology, mainly because different groups of microorganisms have different pH preferences (Shah *et al.*, 1990). Most bacteria are neutrophiles, which mean that the optimum pH is between 5.5 and 8. Most fungi prefer slightly acidic pH between pH 4 and 6 (Rousk *et al.*, 2009). The urban, sub-urban and rural sites in the present study all registered pH values in the neutrophile range (Figure 4.2). Rural sites had the lowest pH values, with all sites registering values lower than pH 6 (Figure 4.2), indicating that the rural sites had more favourable conditions for promoting the growth of fungi. The sub-urban sites generally had the highest pH values, while the urban sites had slightly lower values, indicating that sub-urban and urban sites in the present study tended to favour bacterial growth.

pH also has an effect on enzyme activity. Each enzyme works optimum at a specific pH and temperature optimum. If these optima are exceeded, enzyme denaturation can occur. Enzymes analyzed in this study function best at a pH range of 6-8, as demonstrated by a previous study done by Pandey *et al.* 2006. The pH range (5.5 to 7.6) of the sub-urban and urban sites (Figure 4.2) would suggest slightly more favourable conditions for enzyme activity.

In the present study the pH analysis revealed no clear differentiation trend along the urbanization gradient. pH levels, however, tend to be more acidic in the rural sampling sites than the sub-urban and urban sites. Jim (1998) found that urban roadside soil in

Hong Kong was much more alkaline than natural soil not affected by urbanization. He concluded that the release of carbonate from the calcareous construction waste induced alkaline pH conditions. The more alkaline conditions in sub-urban and urban soils of Potchefstroom can possibly be attributed to various causes. These causes include the irrigation of urban and sub-urban soils with calcium-enriched water, atmospheric pollution, and liming of soil to correct suspected deficiencies (Marcotullio *et al.*, 2008). Contrary to the findings of the present study and that of Jim (1998), Pouyat *et al.* (1994) found slightly more acidic pH values at the urban sites in their study. The study was conducted along an urban-rural transect through oak stands in New York, U.S.A. The authors attributed the lower pH values to acidic deposition (Pouyat *et al.*, 1994).

Natural and human activities introduce salts and trace metals into the environment. The presence of heavy metals, salts, and acid deposition products is primarily due to the environmental conditions created by urban areas (Marcotullio *et al.*, 2008). Electrical conductivity results in the current study revealed that sub-urban and urban sites generally showed higher electrical conductivity (EC) values than the rural sites (Figure 4.3). These differences were however insignificant ($P > 0.05$). This higher EC could be an indication of higher salt concentrations present in the sub-urban and urban sampling site soils. The results suggest that the urbanization process might contribute to the salinity of topsoil. Possible causes could be irrigation with saline water and application of fertilizer. These aspects need further investigation. Pouyat *et al.* (2007) found elevated levels of potassium and phosphorous in urban soils in Baltimore, U.S.A. They concluded that the elevated levels of these ions could be due to the addition of fertilizers in the urban areas. Elevated potassium increases the electrical conductivity of soil, and could explain the elevated electrical conductivity levels in the urban soils (and to some degree, sub-urban soils). Although not specifically analysed for, dissolved salts could have affected the electrical conductivity in the present study.

The highest values for cation exchange capacity in the present study were registered for samples from the sub-urban sites (Figure 4.4). There was, however, no clear trend observed along the urbanization gradient. No statistically significant differences were observed, indicating that the urbanization process did not significantly influence cation exchange capacity in this study. A study conducted by Jim (1998) on urban soils in Hong Kong however revealed that highly disturbed urban soils can have significantly

lower cation exchange capacity than natural soils. This was due to the loss of soil structure because of the morphology of soil in the urban environment. However, in the present study the sub-urban sites had the highest clay content (Figure 4.1). This might have had an impact on the cation exchange capacity of the soil at these sites.

Heavy metals are deposited on the soil surface through atmospheric deposition as products of fossil fuel deposition from vehicles, power plants and industrial processes. Anthropogenic sources include production-related activities such as the use, wear and disposal of consumer and commercial products (Marcotullio *et al.*, 2008). The analysis of metals and selected elements in the current study revealed no clear trends along the urbanization gradient. It was noted that the results for all elements were higher in samples from the northern rural sampling site than the other sites (Table 4.1). This could be ascribed to unrepresentative sampling practices on site. Other rural sites did not have such exaggerated values regarding the elemental analysis. Although few soil samples were taken, our study suggests that heavy metal deposition and acidification processes are not as prevalent in this study area (the Potchefstroom municipal area) as they might be in larger urban areas. A study by Pouyat *et al.* (2007) in Baltimore U.S.A also revealed no significant trend along an urbanization gradient regarding heavy metal decomposition. Another study conducted by Pouyat & McDonnell (1991) in New York City indicated evidence of acidification along the urbanization gradient. Levels of Cu, Ni and Pb were elevated in the urban areas. The lack of heavy metal deposition and acidification process in the Potchefstroom municipal area could therefore be attributed to the fact that it is a much smaller city than New York, with much fewer inhabitants and less commercial and industrial activities.

5.2 Basic sampling site information along an urban-rural gradient

In the present study soil compaction was higher during the August (colder, dry) sampling period (Figure 4.5). This could be attributed to lower moisture content of the soil during the sampling period. Urban and sub-urban sites recorded higher soil compaction values than the rural sites (Figure 4.5). Scharenbroch *et al.* (2005) investigated urban soils in various cities across the world. They found that urban soils had generally higher bulk densities than soils not directly affected by anthropogenic

activities. These authors (Scharenbroch *et al.*, 2005) attributed their findings to reduced biological activity in the soil, lower organic content in the soil, as well as compaction due to anthropogenic activities. Higher anthropogenic activities in the urban and sub-urban sites in the present study could be responsible for the higher soil compaction values recorded. Jim (1998) investigated urban soil characteristics in Hong Kong, and found that urban soils were extensively compacted. This characteristic is associated with structural degradation and loss of porosity, and is unfavourable to aeration, drainage, storage of plant-available moisture and root growth.

The gravimetric water content of the soil in the present study was relatively constant during the different sampling periods in the different sampling sites (Figure 4.6). It was hypothesized that urban sites would have higher moisture content than the other sites due to regular watering practices in urban areas. Water practices did, however, not influence the gravimetric water content of the samples in the urban environment significantly. Inadvertent soil compaction in urban environments is a process that reduces water infiltration rates, which can lead to increased water runoff from soil (Gregory *et al.*, 2006). In this study, soil compaction was highest in the sub-urban and urban sampling sites (Figure 4.5). Soil compaction may be a reason for the lower than expected gravimetric water content in the urban areas. Jim (1998) found that urban soils in Hong Kong had diminished transmission of air and water. The soil also exhibited poor retention of plant-available moisture.

Impervious surfaces are more prevalent in urban environments than in rural environments when remote sensing is used to classify land cover (Weeks *et al.*, 2005). The V-I-S model of Ridd (1995) was used to classify the study area in the present study. The resulting classification showed that areas classified as urban have the highest percentage impervious surfaces. Areas classified as rural had the highest percentage vegetation cover (Table 3.1). In a study in New York, McDonnell *et al.* (1997) also found that the percentage impervious spaces were much higher in urban areas than the rural areas. Subjective estimation of the percentage vegetation cover of the plots used in this study revealed that during the May (warmer, wet) sampling period, cover was higher than in the August (colder, dry) sampling period (Figure 4.7). This was expected, due to more favourable conditions for plant growth during the warmer, wet season. No clear trend emerged along the urbanization gradient regarding

subjective estimation of percentage vegetation cover in the study area. Subjective estimation of the percentage bare patches present in the sampling plots revealed that the percentage bare patches were highest in the rural areas (Figure 4.9). This could be mainly attributed to the growth forms of the grass in the rural areas. Grass species commonly found in the grassland biome of South Africa includes grasses from the genera *Themeda*, *Eragrostis*, *Heteropogon* and *Elionurus* (Mucina & Rutherford, 2006). During this study, these common grassland species were mainly found in the rural sampling sites (for grass species names, refer to the Appendix Table A3). Grass species encountered in the rural areas, for example species from the genera *Themeda* and *Eragrostis*, are species that forms mainly tufts. Because of the grass' growth form, more bare ground was exposed in these sampling areas. Urban sites are dominated by rhizomatous and stoloniferous grass species (for example grasses from the genera *Cynodon* and *Pennisetum*; Table A3) that covered the ground more completely, forming mats across the ground.

Subjective estimation of the percentage dry organic matter present on the surface of the sampling sites revealed that during the May (warmer, wet) sampling period, there were more dry organic matter present at the sites than during the August (colder, dry) sampling period (Figure 4.8). This can be attributed to the higher growth rates of plants during the warmer, wet season. Urban and sub-urban sites generally yielded higher values with respect to percentage organic matter on the surface in the sampling sites. This can be explained by anthropogenic maintenance practices of natural and semi-natural grasslands in sub-urban and urban locations in the same way as lawns (Cilliers, 1999). Organic matter resulting from practices such as mowing is not always removed from urban and sub-urban areas, creating more dry organic matter in these areas. The CCA ordination of the plant species composition and the basic sampling site information (Figure 4.16) confirmed that the rural sites had the highest percentage of bare ground.

5.3 Plant species composition along an urbanization gradient.

Urban environments differ from rural environments, which have effects on the plant species composition found in these environments (Pauchard *et al.*, 1997). In the present

study it was demonstrated that the rural areas had higher species richness (Figure 4.10) and higher plant diversity than urban areas (Figure 4.11). It was further indicated that the rural sites had the most indigenous and perennial plant species (Figure 4.12). The differences between urban, sub-urban and rural areas were, however, statistically insignificant. Maestas *et al.* (2003) investigated plant communities along a rural land-use gradient in the American West, and also found a higher native species richness in the rural sites. These results indicate that the ecological succession in the rural areas tended to culminated in a climax community. Plant species composition in a climax community consists mainly of indigenous and perennial species (Ash, 1991). The urban areas, however, are kept at an early successional stage by regular disturbances. These disturbances include anthropogenic activities such as mowing and weeding (Niemelä, 1999). The historical distinctiveness and external control of succession is a very important feature distinguishing urban habitats from more rural, natural habitats (Trepel, 1995).

Sub-urban and urban sampling sites showed lower plant diversity than the rural sites (Figure 4.11). In a study on the effect of urbanization on biota in Chile, Pauchard *et al.* (1997) found that urbanization had a homogenizing effect on the plant communities. These authors (Pauchard *et al.* 1997) recorded higher plant species diversity in the more rural areas. Common plant species (native or alien) usually dominate urban areas (Kuhn & Klotz, 2006).

The urbanization process impacts upon natural areas in and around cities (McConnachie *et al.*, 2008). Factors such as a reduction in extent of natural habitat, fragmentation, transformation and dumping contribute towards the extinction of native plant species (McConnachie *et al.*, 2008). Anthropogenic activities influence in urban and to a lesser extent sub-urban areas through management practices and planting monoculture lawns also influence plant species composition. The over-management of grasslands in and around urban areas by frequent cutting and weeding keeps them in a sub-climax condition and decreases the species diversity tremendously (Nel, 1991; Cilliers, 1999). An opposite trend has also been documented in other urbanization studies. Godefroid & Koedam (2007) stated that urban environments can have high plant species diversity due to adventive garden escapes. Niemelä (1999) concluded that there is a low degree of integration among habitat patches in urban environments. This

leads to variation in colonization and extinction events. Together with their early to mid-successional stage, caused by frequent disturbances, it could explain urban environmental suitability for many plant species. All these factors could explain the high species richness found in some urban areas. Kent *et al.* (1999) documented higher urban plant species richness in housing/urban developments in Plymouth, Devon, than in the more rural areas. These findings were in contrast to the findings in the present study, where the rural areas had higher plant species diversity than the sub-urban and urban areas.

Sub-urban sites showed the most annual and alien plant species (Figure 4.12). During the clearing of rural land to convert it to sub-urban and urban land, natural plant communities are cleared, thus starting secondary succession. As mentioned before, anthropogenic management practices keep urban and sub-urban areas in an early succession stage (Nel, 1991; Cilliers, 1999). Pioneer plant species (mainly annuals) thrive in this environment, thus explaining the higher number of annual plant species. Regular disturbances, such as fluctuating water levels and anthropogenic practices, also favour annual plant species (Ash, 1991).

Results for plant life form classification revealed that rural sites had the highest number of forbs and geophytes (Figure 4.13). The occurrence of forbs followed a trend along the urbanization gradient. Rural sites had the highest number of forbs followed by the sub-urban sites and the urban sites. This could be ascribed to the lack of maintenance in the rural areas, where forbs and geophytes are not removed. Urban areas are regularly maintained, and forbs present on lawns are often removed (Cilliers, 1999). No other clear trends could be observed for the life form classification of the plant encountered at the sampling sites in the present study.

The DCA ordination of plant species composition illustrated a clear gradient from rural to urban areas across the sampling sites (Figure 4.14). Plant species composition and abundance at the different sampling sites along the urbanization gradient in Potchefstroom revealed that the different urban sites had a similar species composition. Similar results were obtained for the sub-urban and rural sampling sites. The ordination illustrated that the plant communities differed significantly across the sampling sites. Godefroid & Koedam (2007) analyzed plant species data in Brussels, Belgium. When

analyzing the plant species data using a CCA ordination, they found that ordination showed a clear differentiation of plant species that prefer urban environments, those tolerant to urban and natural environments, and species that prefer rural environments. Ali & Malik (2010) used a DCA ordination to analyze plant species data from urban open spaces in Islamabad, Pakistan, and also found that plant species found in urban areas could easily be differentiated from plant species found at the rural areas.

In the current study the CCA ordination (Figure 4.15) for the plant species composition and abundance data as well as the chemical and physical results indicated no clear trends. According to the ordination it is probably not the physical and chemical characters in the soil alone that determine the existence of different plant communities along the urbanization gradient. The CCA ordination supported the discussion of the chemical and physical results earlier in this chapter.

5.4 Culture-dependent and culture-independent analysis of the microbial component of soil samples collected at sampling sites.

Culture-dependent results indicated that the bacterial cfu/g soil levels were higher during the August (colder, dry) sampling period than the May (warmer, wet) sampling period (Figures 4.17 and 4.18). The difference between the values were, however, not statistically significant. A study conducted by Tangjang *et al.* (2009) indicated that bacterial cfu/g soil in North-Indian agriforestry systems was highest during spring months. In the study of Tangjang *et al.* (2009) the highest bacterial numbers were recorded in the topsoil, except during the rainy season, when the highest numbers were recorded in the subsoil. In this study the samples consisted of the topsoil, and the findings in the study by Tangjang *et al.* (2009) could explain the higher counts during the August (colder, dry) sampling period.

Furthermore, results for bacterial cfu/g soil counts generally showed higher levels in the urban and sub-urban samples than in the rural samples of Potchefstroom (Figures 4.17 and 4.18). These results suggest that bacterial biomass may be more prevalent in soil environments affected by anthropogenic influences. Although statistically insignificant, reasons for this finding could include soil management inputs such as

fertilization and irrigation that create urban soil environments that are more substrate- and nutrient-rich than their rural counterparts (Pouyat *et al.*, 2002). The results of the latter study suggested that the conditions prevalent in the urban environment influence the heterotrophic bacterial population levels in soil. Kaye *et al.* (2005) investigated soil microbial communities in adjacent urban, native and agricultural ecosystems in Colorado, U.S.A., and found that differences in carbon cycling between the land-use types had a significant effect on nitrogen cycling and microbial biomass. The bacterial biomass was significantly higher in the urban soils than the native and agricultural soils (Kaye *et al.*, 2005).

In the present study fungal counts were slightly higher in the rural samples (Figures 4.20 and 4.21) and could probably be explained by the slightly lower pH recorded at the rural sites (Section 5.1). Generally fungi prefer slightly lower pH levels (pH<6) (Rousk *et al.*, 2009). The differences in fungal levels between rural, sub-urban and urban soils were, however, insignificant. Similar results were reported by Pouyat *et al.* (1994), who studied forest patches in New York along an urban-rural gradient. They found that the abundance of fungi decreased as the sampling sites moved towards more urbanized sampling sites.

The isolation and identification of the visibly dominant species revealed that these are mostly from *Bacillus* spp. (Table 4.4), a bacterium that is widely distributed in soil (Chatterjee *et al.*, 2005). Bacteria from the genus *Bacillus* are gram-positive aerobic or facultative endospore-forming bacteria. Collective features include the degradation of almost all substrates derived from plant and animal sources. Pandey & Palni (1997) investigated the bacterial community of the rhizosphere in soils dominated by tea bushes. They found that *Bacillus* spp. were the dominant type of bacteria in these soils. *Bacillus* spp. has been isolated in a very wide variety of soils, including soils from desert, beach and tundra habitats (Chatterjee *et al.*, 2005).

Community structure of the heterotrophic bacteria was determined. Furthermore Shannon-Weaver diversity indices were also calculated using the culture dependant approach. Results indicated that the urban sampling sites had slightly higher S-W values than the rural and sub-urban sampling sites (Figure 4.19). Differences between the values were, however, statistically insignificant.

Although morphologically dissimilar, some of the isolated dominant colonies were of the same species when identified by 16S rDNA sequences. This suggested that certain environmental factors influenced the morphology and pigmentation of the colonies and thus impacted on the accuracy of the diversity indices that were calculated. Culture-dependent results for the heterotrophic bacterial community structure were thus deemed to be unreliable. Problems with culture-dependent techniques and the estimation of bacterial community diversity have been documented in microbial ecological research (Felske *et al.*, 1998). These problems included that only viable cells can be accounted for. Culture-based approaches can, however, be very useful for understanding the physiological potential of isolated organisms. Culture-dependent methods do not necessarily provide comprehensive information on the composition of microbial communities (Amman *et al.*, 1995; Orphan *et al.*, 2000). It is widely recognized that microbial diversity is underestimated when using culture-dependent approaches (Hori *et al.*, 2006). Due to documented imbalances between cultivatable and *in situ* diversity, it is often difficult to assess the significance of cultured members in resident microbial communities (Orphan *et al.*, 2000). That is why many of the recent studies regarding microbial diversity include culture-dependent and culture-independent methods to complement each other (Aminin *et al.*, 2008; Zhang *et al.*, 2009).

Culture-independent techniques have many advantages. It allows the identification of single bacterial species without cultivation of the organisms. Methods such as PCR-DGGE can be used to investigate bacterial community structure. Furthermore, single bands from the DGGE gel can be excised and the DNA reamplified. The reamplified DNA can be sequenced to get information about single community members in the analyzed community (Handschr *et al.*, 2005). Fingerprinting techniques may be less selective than culture-dependent techniques, and provide a more representative view of a microbial community structure (Reysenbach *et al.*, 1992). Large numbers of samples can be analysed and compared, making them the ideal tool for ecological studies (Smalla *et al.*, 2007). In the present study, the DGGE process (DNA isolation, PCR, DGGE electrophoresis) could not be optimized due to time constraints. Several DNA isolation processes were attempted. They were combined with several PCR reaction mixes. DNA isolated were constantly of poor quality (A260/A280 less than 1.0, Table A9). The DGGE gel presented in the results section showed few bands. However, no conclusive results could be drawn from this gel. In this study, the PCR-DGGE method

needs optimization. The DNA isolation process from the soil samples needs to be reassessed. Thakuria *et al.* (2008) demonstrated that multiple steps of DNA purification should be included in the extraction process to optimize DNA quality. Results from their study indicated that it is essential to extract high quality DNA as it underpins DNA fingerprinting methods. PCR reaction mixtures need to be optimized to retrieve higher quality PCR product for the DGGE process. The approach has been successfully used in other studies involving microbial communities in soil. Nakatsu *et al.* (2000) used PCR-DGGE to investigate the bacterial community structures in soils collected from different agro ecosystems in Norway and the U.S.A. McCaig *et al.* (2001) investigated bacterial communities with PCR-DGGE in grasslands under different land management regimes. Griffiths *et al.* (2003) investigated the bacterial community response to water stress in grasslands using PCR-DGGE. In all these cases PCR-DGGE was successfully applied.

Results from the DGGE technique in the present study indicated that dominant species were distributed in all the samples (rural, sub-urban and urban samples) (Figure 4.23). Although not well resolved, results indicated that there were no differences between the rural, sub-urban and urban samples. This concurred with findings in a study of soil microbial communities in adjacent urban, native and agricultural ecosystems by Kaye *et al.* (2005). These authors concluded that the land use type had a large impact on the biomass of the microbial population, but a small effect on the relative abundance of broad taxonomic groups of microorganisms. These findings are consistent with findings of previous studies (Imhoff *et al.*, 2000; Koerner & Klopatek, 2002).

Considering all the results gathered regarding the bacterial community diversity, it can be hypothesized that urbanization does not significantly influence the composition of the soil bacterial community. Urbanization may influence the bacterial population levels. Further studies are needed to test this hypothesis regarding the soil along an urbanization gradient in South-African grasslands.

5.5 Microbial function along on urban-rural gradient in a South African grassland

The function of microbes along an urban-rural gradient was assessed using five major enzymes that are critical to microbial survival, and are also integral parts of the carbon-, the nitrogen-, and the phosphorous cycles. Enzyme assays were conducted on soil samples collected during the entire sampling period. However, phosphatase activity was only determined in 2007 and not during the 2008 sampling period.

Results of enzymes assays (excluding the alkaline and acid phosphatases) indicated that the August (colder, dry) sampling period typically registered higher potential activity than the May (warmer, wet) sampling period (Figures 4.24-4.31). This can possibly be explained by the shifting of activity from the topsoil (samples analyzed during study were topsoil samples) to the subsurface soil during the rainy season due to persistent water-saturated conditions (Tangjang *et al.*, 2009).

An enzyme activity gradient between urban and rural samples could be observed for the enzymes involved in the carbon cycle. Dehydrogenase and β -glucosidase results indicated statistically significant differences between the urban, sub-urban and rural samples. Higher potential activity was registered in the urban samples, followed by the sub-urban samples, while the lowest potential activity was registered in the rural samples (Figure 4.24-4.27). Potential enzymatic results thus indicated that urbanization has an impact on the carbon cycle in urban areas, and therefore on the enzymes active within the carbon cycle. Influences may include higher carbon deposition and the availability of more carbon sources. Environmental factors such as temperature and water availability may also contribute towards the enhanced potential activity. Results indicated that urban ecosystems have higher carbon cycling rates than rural environments. Our findings concur with results obtained in a study conducted by Kaye *et al.* (2005). Their investigation revealed that urban ecosystems allocate far more carbon belowground and had far greater surface soil carbon storage than non-urban land-use types. Kaye *et al.* (2005) concluded that the urbanization of ecosystems leads to enhanced carbon cycling rates that are of a large enough scale to alter regional carbon budgets.

Urease results indicated a general gradient, with urban sample values higher than the rural sample values (Figures 4.28 and 4.29). The values did, however, not differ statistically significantly ($P > 0.05$). The increased carbon cycling may influence the nitrogen cycling (Kaye *et al.* 2005). An investigation by Pouyat & Carreiro (2003) into nitrogen dynamics of oak leaf litter along an urban-rural gradient indicated that environmental conditions and litter type had important effects on nitrogen dynamics. The specific environmental conditions found in the urban environment seemed to increase the nitrogen cycling rates, as indicated by the higher potential urease activities measured in the urban samples. In another study conducted by Pouyat & Turacheck (2001) on soil in oak-dominated stands in the USA along an urbanization gradient, the results indicated that the net nitrification and net nitrogen mineralization rates were higher in urban and sub-urban stands. Results from the current study suggested that the urban environment in fragmented South African grasslands may have the same effects on nitrogen cycling in soils in the urban environment. Pouyat & Turacheck (2001) concluded that the higher net nitrification rates in urban and sub-urban environments suggest that the urban and sub-urban environments have the potential to lose high amounts of nitrogen through soil leaching. This is an aspect that still needs to be investigated in fragmented South African grasslands.

Enzyme activity results from the alkaline and acid phosphatase analysis did not show any clear trends (Figures 4.30 and 4.31), indicating that urbanization does not have a direct influence on these enzyme activities. However, ordination results indicated that higher potential alkaline phosphatase activities were associated with urban and sub-urban samples, while the higher potential acid phosphatase results were associated with the rural samples (Figure 4.32). The slightly higher alkaline phosphatase activities in the urban and sub-urban areas could be attributed to the higher pH values that were registered for the soils. The same principle could explain the slightly higher acid phosphatase activity in the rural areas, where the pH readings were slightly lower (all pH readings were just below 6). Hall *et al.* (2009) investigated the effect of urbanization on soil microbial function in the Sonoran desert. They found that phosphatase activity were higher in urban soils. Further they concluded that microbial processes in arid soils are responsive to human activities through creation of managed urban landscapes, and through certain factors associated with the urban environment that increase carbon and nitrogen supply. Garcia-Ruiz *et al.* (2008) investigated the

growing interest in the application of acid- and alkaline phosphatases as early indicators of soil quality change under contrasting agricultural management practices. They found that organic management resulted in significantly higher potential enzyme activities, and that tillage intensity slowed down the improvement of soil quality in organic crops.

Ordinations indicated that dehydrogenase, β -glucosidase and urease activities are different between urban, sub-urban and rural areas (Figure 4.32). This clearly illustrated that increased potential activities of the enzymes analyzed were closely related to the urbanization pressures. This ordination supported the discussion presented previously.

5.6 The integration of the microbiological results and plant species composition results along the urbanization gradient.

The CCA ordination of the plant species composition data and the potential activity of dehydrogenase, β -glucosidase and urease (Figure 4.33) revealed clusters of the urban, sub-urban and rural sites. High potential activity of enzymes involved in the carbon cycle (dehydrogenase and β -glucosidase), as well as urease (involved in the nitrogen cycle) were strongly associated with the urban sites.

Ordinations that combined the bacterial and fungal count data, plant species composition data and potential enzymatic activity data (Figure 4.34) revealed that the highest levels of heterotrophic bacteria were associated with the urban sampling sites. This finding concurred with the results of Kaye *et al.* (2005). They found that bacterial levels were higher in the urban soils than the native and agricultural soils. The highest fungal levels were associated with rural sampling sites. Pouyat *et al.* (1994) investigated fungal levels in forest patches along an urban-rural gradient in New York, U.S.A., and found that fungal levels decreased in urban environments, and were the highest in natural, rural areas. This would suggest that the heterotrophic bacterial component thrives in the urban environment, where the fungal component tends to thrive in the more natural, rural environment.

Ordinations of data collected from the study of the microbial component and the plant component along the urbanization gradient supported the results presented. This

illustrated that plant species composition, potential enzyme activities regarding enzymes related to the carbon cycle and nitrogen cycle, and the population levels of heterotrophic bacteria were all influenced by urbanization. It is hypothesized that the plant species composition is not the driving force behind the higher heterotrophic bacterial populations. In a study conducted by Wardle *et al.* (1999), the effect that change of land use had on the microbial community was investigated. The authors removed specific plant groups from sampling sites, and the subsequent analysis revealed that there were no significant differences in the total biomass of bacteria or fungi. Other factors in the urban environment, including anthropogenic influences (irrigation and the application of fertilizers) may well be the driving force behind higher bacterial population values observed in this environment.

The relationship between the aboveground and belowground components was not specifically investigated during the course of this study. General topsoil samples were analyzed, and the results indicated that urbanization does not have a significant effect on the broader soil microbial community structure. It has been suggested by Wardle *et al.* (2004) that plant community composition greatly influences root-associated microbial community structure, but the effect on decomposer communities are context-dependent (Mikola *et al.*, 2002; Wardle *et al.*, 2004).

It has been recognized that soil organisms are responsive to the nature of organic matter that enters the decomposer subsystem (Swift *et al.*, 1979). Plant species differ both in terms of the quantity and quality of resources that they return to the soil. Individual plant species may have important effects on components of the soil biota and the processes that they regulate (Wardle *et al.*, 2004). These differences include microbial community diversity and biomass, altered nutrient cycling and varying soil enzyme activity (Dornbush, 2007). The effects of plant community composition on the decomposer communities are fairly context dependant (Wardle, 2002; Mikola *et al.*, 2002). Setälä *et al.* (2005) suggested that net primary productivity is probably relatively insensitive to decomposer diversity because of the generalist feeding behavior of most consumers in the soil subsystem. The results of the present study indicated that the plant species composition differed along the urbanization gradient, implying different plant material inputs along the urbanization gradient. The microbial community studied (general aerobe, heterotrophic decomposers) indicated no

significant differences in community structure. Enzyme assays indicated that urban areas exhibited higher enzyme activity regarding enzymes active in the carbon- and nitrogen cycles. The relationship between plants and the general microbial community diversity and function could have been influenced by changes induced by the urbanization process. The conceptual framework of Al-Mufti *et al.* (1976) suggests that aboveground consequences of soil biodiversity are strongly context dependent, and that the effects of decomposer organisms may depend on initial site fertility. To test the framework in future studies, more in-depth research is needed focusing on the relationship between aboveground and belowground components. This aspect needs to be investigated in future studies to better understand the regulation and functional significance of biodiversity and of the environmental impacts of human-induced global change phenomena.

Chapter 6 – Final discussion, conclusion and recommendations

In this chapter the achievement of the proposed study objectives are evaluated and the findings of the study summarized.

6.1 Soil chemical and physical data along an urbanization gradient

Analysis of soil samples in this study revealed that the sub-urban and urban sampling sites had slightly higher clay contents than the rural sampling sites. The higher clay content may influence the electrical conductivity and the cation exchange capacity of the soils. Electrical conductivity was slightly higher in the sub-urban and urban sampling sites than the rural sampling sites in this study. Electrical conductivity values may have been influenced by anthropogenic activities, such as irrigation of soil with saline water and the application of fertilizer. No clear trend was observed for the cation exchange capacity in this study, although it was noted that the sub-urban sampling sites generally had the highest values. Examples from other studies were presented that indicated that urbanization can affect the cation exchange capacity, though in this study it was not the case. The results of this study suggested that urbanization may affect soil chemical and physical properties along a gradient.

No clear trend was observed for the pH of the soils along the urbanization gradient in Potchefstroom. Sub-urban and urban sampling sites registered pH levels above pH 6. It was noted that the soil pH recorded in the rural sampling sites were generally lower than the sub-urban and urban sampling sites. Lower soil pH levels in the rural sampling sites suggest that the rural area might be more suitable for growth of fungal communities. The differences in pH levels along the urbanization gradient could also affect potential enzyme activities along the urbanization gradient, as all enzymes have optimal pH ranges.

No clear trends were observed for the heavy metal levels along the urbanization gradient. Previous studies have shown that urbanization can affect heavy metal

precipitation in urban environments. No clear evidence was collected during this study for heavy metal contamination along the urbanization gradient.

6.2 Plant species composition along an urbanization gradient

Investigation of the plant species composition revealed that there was a higher species richness in the rural sampling sites than the sub-urban and urban sampling sites. Shannon-Weaver diversity indices confirmed this, although the differences between the values were statistically insignificant. Lower number of plant species present in the sub-urban and urban sampling sites could be a direct effect of biotic homogenization caused by anthropogenic management practices, and social acceptance of certain landscape in urban areas (such as the planting of monoculture lawns).

In the present study sub-urban sampling sites had the highest species richness of annual and alien plant species. This could be due to anthropogenic management practices that keep the sub-urban areas in an early succession stadium.

No clear trends were observed of the plant life form classification along the urbanization gradient. It was noted that the rural sampling sites had the highest number of forbs and geophytes present, due to the lack of anthropogenic management practices and disturbances.

The DCA ordination showed that the urban, sub-urban and rural sites form three separate clusters, indicating similar plant species composition in these areas. It also illustrated that there were differences in plant species composition along the urbanization gradient.

6.3 Basic sampling site information along an urban-rural gradient

Soil compaction was more prevalent at the sampling sites during the August (colder, dry) sampling period. This could have been due to the lack of rainfall during that sampling period. A lack of moisture could influence the hardness of the soil. When

compaction data of sampling sites were compared, it was found that sub-urban and urban sampling sites had the highest compaction rates. Higher compaction rates in sub-urban and urban areas can be influenced by the higher volumes of human and animal traffic in these areas. Other anthropogenic influences can also contribute to the higher compaction rates in sub-urban and urban areas.

No clear trends were observed for the gravimetric water content of the soil along the urbanization gradient in Potchefstroom. It was hypothesized that urban areas would have the most gravimetric water content due to anthropogenic influences, like regular irrigation. This was found not to be the case. It was demonstrated that higher compaction rates in these areas could have influenced the gravimetric water content of the soil.

The percentage vegetation cover was highest in the May (warmer, wet) sampling period. This can be explained by the fact that the study area is in a summer rainfall area. An optimal temperature range and abundance of water creates optimal growing conditions for the vegetation. No clear trend was however visible along the urbanization gradient regarding the percentage of vegetation cover.

Percentage organic matter was higher in the May (warmer, wet) sampling period, due to more vegetation biomass that is available in this period. It was noted that the percentage organic matter was higher at the urban sampling sites than the sub-urban and rural sites. This could be explained by anthropogenic influences, such as the mowing of lawns, and then not removing the cuttings from the mowed area.

The percentage bare ground values were highest in the rural areas. This could be explained by the tufted growth forms of the grasses encountered in the rural sampling areas, in comparison with the stoloniferous and rhizomatous growth form of grasses in urban areas.

CCA ordination for the plant species composition and the basic sampling sites information showed no clear trends. It was noted that that the highest percentage bare ground was encountered in rural areas.

6.4 Culture-dependent and culture-independent analysis of the microbial component of soil samples collected at sampling sites

Culture-dependent results for bacterial levels in the soil samples indicated generally higher bacterial levels from soil samples collected in the August (colder, dry) sampling period. There were, however, no statistically significant differences between the seasonal bacterial levels results.

Results further indicated higher bacterial levels from the samples collected in the urban sampling sites, although the differences between the values were also mainly statistically insignificant. However, this could suggest that bacterial biomass can be more in soil environments that is influenced by anthropogenic practices.

Dominant bacterial species present in collected soils samples were identified using 16S rDNA sequences. The species were predominantly from the genus *Bacillus*.

Shannon-Weaver indices for bacterial biodiversity in the soil samples indicated sub-urban and urban sites had generally the highest indices values. Differences between the results were, however, also statistically insignificant.

Culture-dependent results for fungi levels in the soil samples indicated generally higher levels in the May (warmer, wet) sampling periods than the August (colder, dry) sampling periods. Results indicated rural sites generally had higher fungal levels than the sub-urban and urban sampling sites, although these differences were mostly statistically insignificant.

The PCR-DGGE process was not optimized. Inconclusive results were thus obtained.

6.5 Microbial function along on urban-rural gradient in a South African grassland

Higher dehydrogenase activity generally was observed for soil samples collected in the August (colder, dry) sampling periods. A clear gradient could be observed, where urban sites had the highest potential dehydrogenase activity, followed by the sub-urban sites, and samples from the rural sites had the lowest values. Differences were mostly statistically significant. Potential β -glucosidase activity followed the same trends as the potential dehydrogenase activity results. This could be expected because both are involved in the carbon cycle.

Potential urease activity was also generally higher in soils samples collected in the August (colder, dry) sampling period. A gradient was observed, although not as profound as the potential dehydrogenase and β -glucosidase activity results. Urban sites had generally the highest values, followed by the sub-urban sites, and then the rural sites. The differences between the values were, however, statistically insignificant.

Activity of potential acid phosphatase from samples collected in the May (warmer, wet) sampling period was higher than samples collected in the August (colder, dry) sampling period. Although the differences between the values were mostly statistically significant, no clear trend was observed along the urbanization gradient.

Furthermore, potential alkaline phosphatase activity of soil samples collected in the May (warmer, wet) sampling period summer were significantly higher than values from samples collected in the August (colder, dry) sampling period. A weak trend was observed along the urbanization gradient. Values from samples collected in the urban sampling sites were the highest, followed by the sub-urban samples, and then the rural samples. The differences between the values were mostly statistically significant.

PCA ordination of the potential dehydrogenase, β -glucosidase and urease activity further illustrated the trends observed along the urbanization gradient. The site values from the different sampling areas tended to be closely associated with each other.

6.6 The integration of the microbiological results and plant species composition results along the urbanization gradient.

The CCA ordination of the plant species composition data and the potential activity of dehydrogenase, β -glucosidase and urease revealed clusters of the urban, sub-urban and rural sites. High potential activity of enzymes involved in the carbon cycle (dehydrogenase and β -glucosidase), as well as urease (involved in the nitrogen cycle) were strongly associated with the urban sites. The results indicated the highest potential enzymatic activities at the urban sampling sites, followed by the sub-urban sampling sites and the rural sampling sites

RDA ordination of data of the microbial component and the botany component along the urbanization gradient supported the results presented. The ordination illustrated that plant species composition was influenced by urbanization. Potential enzymes activities active in the carbon cycle and nitrogen cycle were also clearly influenced by urbanization processes, together with the population levels of heterotrophic bacteria. Higher fungal levels and potential acid phosphatase activity were more closely associated with rural sites. Perennial, annual/perennial and indigenous plant species were also more closely associated with the rural sites. The rural sites registered the highest number of plant species. Higher potential dehydrogenase, β -glucosidase, urease and alkaline phosphatase activity were closely associated with urban and sub-urban sites. These finding were accompanied by higher bacterial levels. Annual and alien plant species were more closely associated with the urban and sub-urban sites. The RDA ordination suggest that plant species composition is influenced by the urbanization process, and that conditions prevalent in the urban topsoil environment is more conducive to higher heterotrophic bacterial populations and enzyme activity of certain enzymes. The RDA ordination illustrated that the investigated enzymes active in the carbon and nitrogen cycle tend to have higher activities in the urban environment than in rural environments.

6.7 Conclusion

Urbanization has definite effects on the plant communities. In this study it was demonstrated that differences exist in plant species composition, species diversity and plant traits along the urbanization gradient. Urbanizations also influence soil microbial communities and function. It would seem that microbial levels, diversity and function are artificially maintained in urban environments. Furthermore, soil enzyme assays of important enzymes in the carbon cycle (dehydrogenase and β -glucosidase) and the nitrogen cycle (urease) suggest enhanced nutrient cycling in the urban environment. The full effect of this is not yet fully understood.

All the objectives of the study were achieved, except the screening of bacterial soil communities using PCR-DGGE. Further optimization of the process (as described in this document) needs to be done. Even so, this study demonstrates the potential of using the tools described to investigate microbial diversity and function in fragmented South African grasslands along an urbanization gradient. Microbial data sets could be positively associated with botanical data sets. Future studies should focus more on specific aboveground and belowground interactions and relations between plant communities and general decomposers in soil along urbanization gradients. Insight into these aspects may broaden the understanding of the ecological processes taking place in urban ecosystems.

6.8 Recommendations for future studies

- It is recommended that future studies optimize the PCR-DGGE technique to study the soil microbial community structure in fragmented South African grasslands along an urbanization gradient.
- It is further recommended that in future urbanization gradient studies the gradient should be quantified using physical demographic and landscape metric variables (Du Toit, 2009)
- The possibility of urban environments losing carbon and nitrogen at higher rates than rural environments should be investigated. Results from this study illustrated higher carbon and nitrogen cycling rates in the urban environment. By investigating these aspects in more detail a clearer understanding of regional C and N budgets could be achieved. Such a study should also have it as goal to better understand the relationship between higher carbon sequestration rates and carbon dioxide emissions in urban environments compared to rural ones.
- An investigation of the effect that different litter qualities have on the microbial community along an urbanization gradient in South African grasslands will also add value to future studies.
- Controlled studies (such as microcosm studies) could be conducted. Impacts of urbanization on grasslands can be better understood if the effects of specific anthropogenic influences (like fertilizing, irrigation etc.) on South African grasslands are investigated.
- To further investigate the above- and belowground interactions in South African grasslands along an urbanization gradient. Such a study can give more insight into energy consumption in urbanized environments.

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Appendix

Table A1. Chemical and Physical data regarding the soil (All elements presented as parts per million (ppm))

Sample name	Sandcn (%)	Siltcn (%)	Claycn (%)	pH	Ec mS/m	Cu	Fe	Mn	Zn	B	Pb	Cr	Cd	As	Se	Cec
NR1	72.8	14	13	6.1	0.1	1.57	78	4.23	2.32	0.5	8.8	740	0.08	5.5	7.4	13.5
NR2	72.8	14	13	6.1	0.1	1.57	78	4.23	2.32	0.5	8.8	740	0.08	5.5	7.4	13.5
NR3	72.8	14	13	6.1	0.1	1.57	78	4.23	2.32	0.5	8.8	740	0.08	5.5	7.4	13.5
NSU1	34.6	39	26	7.6	0.2	0.01	0.6	0.01	0.01	0	0.01	0.04	0.01	3.14	4.35	20.45
NSU2	34.6	39	26	7.6	0.2	0.01	0.6	0.01	0.01	0	0.01	0.04	0.01	3.14	4.35	20.45
NSU3	34.6	39	26	7.6	0.2	0.01	0.6	0.01	0.01	0	0.01	0.04	0.01	3.14	4.35	20.45
NU1	66.5	9.8	24	6.7	0.2	0.03	3.7	0.08	0.02	0	0.01	0.07	0.01	1.905	1.195	17.185
NU2	66.5	9.8	24	6.7	0.2	0.03	3.7	0.08	0.02	0	0.01	0.07	0.01	1.905	1.195	17.185
NU3	66.5	9.8	24	6.7	0.2	0.03	3.7	0.08	0.02	0	0.01	0.07	0.01	1.905	1.195	17.185
ER1	47.8	26	27	5.6	0.1	0.51	80	1.52	0.41	10	0.61	34	0.01	0.01	5.9	26.25
ER2	47.8	26	27	5.6	0.1	0.51	80	1.52	0.41	10	0.61	34	0.01	0.01	5.9	26.25
ER3	47.8	26	27	5.6	0.1	0.51	80	1.52	0.41	10	0.61	34	0.01	0.01	5.9	26.25

ESU1	33.8	24	42	7.3	0.2	0.03	6	0.02	0.01	0.1	0.01	0.05	0.01	1.235	3.26	26.215
ESU2	33.8	24	42	7.3	0.2	0.03	6	0.02	0.01	0.1	0.01	0.05	0.01	1.235	3.26	26.215
ESU3	33.8	24	42	7.3	0.2	0.03	6	0.02	0.01	0.1	0.01	0.05	0.01	1.235	3.26	26.215
EU1	21.5	35	39	6.9	0.2	0.03	5.3	0.02	0.02	0.1	0.01	0.02	0.01	0.01	2.61	22.415
EU2	21.5	35	39	6.9	0.2	0.03	5.3	0.02	0.02	0.1	0.01	0.02	0.01	0.01	2.61	22.415
EU3	21.5	35	39	6.9	0.2	0.03	5.3	0.02	0.02	0.1	0.01	0.02	0.01	0.01	2.61	22.415
SR1	45.8	26	28	5.6	0.2	0.01	3.3	0.14	0.02	0.1	0.01	0.01	0.01	0.01	6.655	17.525
SR2	45.8	26	28	5.6	0.2	0.01	3.3	0.14	0.02	0.1	0.01	0.01	0.01	0.01	6.655	17.525
SR3	45.8	26	28	5.6	0.2	0.01	3.3	0.14	0.02	0.1	0.01	0.01	0.01	0.01	6.655	17.525
SSU1	37.7	28	35	6.9	0.3	0.09	6	0.11	0.18	0.1	0.05	0.34	0.01	0.01	1.77	20.845
SSU2	37.7	28	35	6.9	0.3	0.09	6	0.11	0.18	0.1	0.05	0.34	0.01	0.01	1.77	20.845
SSU3	37.7	28	35	6.9	0.3	0.09	6	0.11	0.18	0.1	0.05	0.34	0.01	0.01	1.77	20.845
SU1	86.2	3.6	10	5.5	0.1	0.01	5.2	0.05	0.01	0	0.01	0.01	0.01	0.01	5.44	3.06
SU2	86.2	3.6	10	5.5	0.1	0.01	5.2	0.05	0.01	0	0.01	0.01	0.01	0.01	5.44	3.06
SU3	86.2	3.6	10	5.5	0.1	0.01	5.2	0.05	0.01	0	0.01	0.01	0.01	0.01	5.44	3.06

WR1	67	6.5	27	6	0.1	0.01	5.4	0.08	0.03	0	0.01	0.07	0.01	0.09	3.97	6.6
WR2	67	6.5	27	6	0.1	0.01	5.4	0.08	0.03	0	0.01	0.07	0.01	0.09	3.97	6.6
WR3	67	6.5	27	6	0.1	0.01	5.4	0.08	0.03	0	0.01	0.07	0.01	0.09	3.97	6.6
WSU1	38.1	12	50	6.3	0.2	0.02	2.5	0.1	0.03	0.2	0.01	0.01	0.01	0.01	1.37	20.34
WSU2	38.1	12	50	6.3	0.2	0.02	2.5	0.1	0.03	0.2	0.01	0.01	0.01	0.01	1.37	20.34
WSU3	38.1	12	50	6.3	0.2	0.02	2.5	0.1	0.03	0.2	0.01	0.01	0.01	0.01	1.37	20.34
WU1	53.7	18	28	6.6	0.2	0.02	6.8	0.05	0.01	0	0.01	0.09	0.01	1.66	2.02	21.09
WU2	53.7	18	28	6.6	0.2	0.02	6.8	0.05	0.01	0	0.01	0.09	0.01	1.66	2.02	21.09
WU3	53.7	18	28	6.6	0.2	0.02	6.8	0.05	0.01	0	0.01	0.09	0.01	1.66	2.02	21.09

Table A2. Physical and subjective botanical results

Site	% Vegetation cover	% Organic matter	% Rockiness	% Bare ground	GRAW H ₂ O	COMPACTION
NR_A	65	30	5	35	95.92547	2.1
NR_A	65	15	5	25	95.40201	2.4
NR_A	70	10	5	30	95.84812	2.8
NSU_A	80	10	5	20	94.03594	2.2
NSU_A	80	25	5	10	94.66266	2.3
NSU_A	65	25	5	15	95.87371	2.8
NU_A	90	20	5	10	91.23127	1.2
NU_A	70	30	5	10	86.99443	2.1
NU_A	70	20	5	5	92.88027	1.9
ER_A	90	15	5	10	92.88607	2.2
ER_A	85	10	40	10	94.34931	2.1
ER_A	80	10	5	15	88.9972	2.2
ESU_A	85	30	5	10	90.3815	3.2
ESU_A	85	20	5	15	93.34524	3.2
ESU_A	70	30	20	25	89.86609	3.7
EU_A	70	10	5	25	95.56762	2.8
EU_A	80	10	5	15	95.43234	2.9
EU_A	80	15	5	10	92.26031	2.4
SR_A	60	10	5	35	93.28119	3.6
SR_A	60	15	5	35	92.01037	2.7
SR_A	60	20	10	25	90.87683	2.4
SSU_A	65	30	5	30	93.86176	4.2
SSU_A	70	35	5	25	93.11907	2.9
SSU_A	60	10	5	30	89.79602	3.8
SU_A	80	5	5	15	96.7279	1.3
SU_A	85	5	5	10	95.60403	1.9
SU_A	90	5	5	5	91.47159	2.2
WR_A	50	5	5	40	95.72607	2.8

WR_A	60	5	5	25	97.38059	3
WR_A	70	5	5	20	95.49636	1.8
WSU_A	85	30	10	15	91.21692	2
WSU_A	80	40	10	10	81.39112	3.9
WSU_A	85	35	10	15	86.30137	3.6
WU_A	80	35	5	10	87.48556	2
WU_A	75	25	5	10	95.29979	2
WU_A	85	10	5	5	93.31758	3.2
NR_B	65	30	5	30	96.54253	1.8
NR_B	65	30	5	25	96.35774	3.1
NR_B	75	15	5	25	94.81701	2.4
NSU_B	75	10	5	15	97.02662	1.7
NSU_B	80	25	5	10	93.00684	2.4
NSU_B	64	35	5	15	95.30047	2.1
NU_B	50	5	5	40	89.26091	1.3
NU_B	70	5	5	20	79.77163	3.9
NU_B	75	10	5	15	90.68174	2.4
ER_B	85	10	5	5	93.51774	2
ER_B	85	10	40	15	95.18706	1.9
ER_B	85	10	5	10	96.41311	1.9
ESU_B	70	25	5	20	95.40928	2.7
ESU_B	70	10	5	15	95.13339	5
ESU_B	60	10	5	35	94.66831	3.6
EU_B	40	10	5	65	96.37451	2.1
EU_B	75	15	5	10	94.53922	3.1
EU_B	75	10	5	10	96.68397	2.4
SR_B	70	15	5	15	95.17134	4.7
SR_B	70	15	5	15	95.62135	3
SR_B	70	10	10	10	96.49725	2.5
SSU_B	65	10	5	20	95.46384	4
SSU_B	70	10	5	15	105.5335	3.5
SSU_B	60	10	5	20	87.60837	5.5

SU_B	40	5	5	60	97.93539	1.2
SU_B	60	5	5	30	97.21737	1.9
SU_B	80	5	5	10	92.75432	2.4
WR_B	30	0	5	70	96.26423	1.9
WR_B	30	0	5	70	97.58746	1.9
WR_B	30	0	5	70	97.60955	2.3
WSU_B	80	20	5	10	94.60462	1.7
WSU_B	80	20	5	10	96.18218	3.2
WSU_B	70	20	5	10	92.86476	2.9
WU_B	70	30	5	20	97.02783	1.8
WU_B	80	30	5	10	95.29031	2.3
WU_B	60	35	5	20	93.63452	3
NR_C	65	10	5	15	94.04732	1.9
NR_C	65	5	5	10	95.01924	2.5
NR_C	70	5	5	10	93.71697	2.3
NSU_C	80	10	5	5	86.94946	2.3
NSU_C	80	10	5	5	88.56971	2.7
NSU_C	65	10	5	5	90.58995	2.4
NU_C	90	5	5	5	86.63426	1.8
NU_C	70	20	5	5	89.6917	2
NU_C	70	20	5	5	90.05676	2.2
ER_C	90	5	5	5	90.79588	2.2
ER_C	85	5	15	5	91.38042	2.2
ER_C	80	5	5	5	91.17911	2.4
ESU_C	85	5	5	5	88.9981	2.9
ESU_C	85	25	5	5	86.3099	2.9
ESU_C	70	25	5	5	87.46362	1.9
EU_C	70	10	5	5	89.96135	2.2
EU_C	80	15	5	5	94.82408	2.2
EU_C	80	10	5	5	91.08191	2.1
SR_C	60	10	10	20	87.62476	2.2
SR_C	60	10	10	20	89.01623	1.8

SR_C	60	10	10	20	91.4495	1.9
SSU_C	65	10	10	20	88.03346	2.2
SSU_C	70	10	10	15	89.02891	3.1
SSU_C	60	15	10	15	90.15478	2.5
SU_C	80	10	5	10	95.70039	1.5
SU_C	85	25	5	5	92.02091	2
SU_C	90	25	5	5	90.43902	2.4
WR_C	50	5	5	15	93.17869	3
WR_C	60	5	5	10	90.54809	3
WR_C	70	5	5	10	90.16665	2.3
WSU_C	85	30	5	10	89.38321	3.6
WSU_C	80	30	5	5	88.96261	3.6
WSU_C	85	25	5	10	92.92665	3.1
WU_C	80	10	5	10	90.29493	2.6
WU_C	75	10	5	0	91.27303	4
WU_C	85	10	5	0	89.51275	3.7
NR_D	65	10	5	15	93.5992	2.4
NR_D	65	10	5	10	96.82826	2.5
NR_D	60	10	5	15	96.29574	2.4
NSU_D	75	10	5	15	97.02662	1.7
NSU_D	80	25	5	10	93.00684	2.4
NSU_D	64	35	5	15	95.30047	2.1
NU_D	75	5	5	10	75.62624	2.7
NU_D	80	5	5	5	77.99761	3.1
NU_D	75	10	5	5	84.05102	2.1
ER_D	85	10	5	5	93.51774	2
ER_D	85	10	40	15	95.18706	1.9
ER_D	85	10	5	10	96.41311	1.9
ESU_D	50	15	5	5	94.63979	3.9
ESU_D	65	5	5	10	95.63161	2.9
ESU_D	70	10	5	5	92.62761	3.2
EU_D	70	10	5	5	97.84641	2.1

EU_D	65	5	5	10	97.70602	2.1
EU_D	75	10	5	5	93.60294	2.5
SR_D	70	15	5	15	95.17134	4.7
SR_D	70	15	5	15	95.62135	3
SR_D	70	10	10	10	96.49725	2.5
SSU_D	40	5	5	50	95.67265	2.2
SSU_D	65	5	5	15	96.48975	2.6
SSU_D	75	5	5	10	94.98476	2.5
SU_D	50	5	5	40	98.04539	1.4
SU_D	65	5	5	15	96.62619	1.9
SU_D	80	5	5	10	92.3459	2.4
WR_D	10	0	5	80	96.26423	2.5
WR_D	10	0	5	80	97.1894	2
WR_D	10	0	5	80	96.79554	1.9
WSU_D	80	20	5	10	94.60462	1.7
WSU_D	80	20	5	10	96.18218	3.2
WSU_D	70	20	5	10	92.86476	2.9
WU_D	85	10	5	5	95.62084	2.6
WU_D	80	10	5	5	97.18843	2
WU_D	85	10	5	5	89.4521	2.9

Table A3. List of identified plant species and where they have been encountered along the urban-rural gradient.

Plantspecies	Urban	Sub-urban	Rural
<i>Acacia karroo</i> Hayne			X
<i>Albuca setosa</i> Jacq.		X	X
<i>Aloe greatheadii</i> Schönland			X
* <i>Alternanthera pungens</i> Kunth.	X		
* <i>Amaranthus hybridus</i> L.		X	
<i>Aristida bipartita</i> (Nees) Trin. & Rupr.			X
<i>Aristida canescens</i> Henrard.		X	X
<i>Aristida congesta</i> Roem. & Schult.			X
<i>Berkheya radula</i> (Harv.) De Wild		X	
* <i>Bidens bipinnata</i> L.		X	X
<i>Blepharis integrifolia</i> (L.f.) E.Meg. Ex Schinz			X
<i>Blepharis serrulata</i> (Nees) Ficalho & Hiern.			X
<i>Brachiaria eruciformis</i> (SM) Griseb.		X	X
<i>Brachiaria serrata</i> (Thunb.) Stapf			X
<i>Bulbine narcissifolia</i> Salm-Dyck.			X
<i>Chaetacanthus costatus</i> Nees			X
<i>Chascanum hederaceum</i> (Sond.) Moldenke			X
* <i>Chenopodium album</i> L.	X		
<i>Chloris pycnothrix</i> Trin.	X		
<i>Chloris virgata</i> SW.	X	X	
* <i>Ciclospermum leptophyllum</i> (Pers.) Sprague		X	
* <i>Convolvulus arvensis</i> L.			X
<i>Convolvulus sagittatus</i> Thunb.			X
* <i>Conyza bonariensis</i> Cronquist	X		X

<i>Conyza podocephala</i> DC.	X	X	X
<i>Corchorus asplenifolius</i> Barch.		X	X
<i>Cotula australis</i> (Spreng.) Hook.f.	X	X	
<i>Crabbea acaulis</i> N.E. Br.			X
<i>Crabbea angustifolia</i> Nees.			X
<i>Cymbopogon pospischillii</i> (<i>Cymbopogon plurinodes</i>) (K. Schum.) C.E. Hubb.			X
<i>Cynodon dactylon</i> (L.) Pers.	X	X	X
<i>Cynodon hirsitus</i> Stent.	X	X	
* <i>Dactyloctenium aegyptium</i> (L.) Willd.	X		
<i>Deverra burchellii</i> (DC.) Eckl. & Zeyh.			X
* <i>Dichondra micrantha</i> (<i>Dichondra repens</i>) Urb.	X	X	
<i>Digitaria ternata</i> (A. Rich) Stapf	X	X	
<i>Elephantorrhiza elephantina</i> (Burch.) Skeels.			X
<i>Elionurus muticus</i> (Spreng.) Kuntze			X
<i>Eragrostis chloromellas</i> Steud.		X	X
<i>Eragrostis lehmanniana</i> Nees		X	
<i>Eragrostis obtusa</i> Munro ex Ficalho & Hiern		X	
<i>Eragrostis plana</i> Nees		X	X
<i>Eragrostis racemosa</i> (Thunb.) Steud.			X
* <i>Euphorbia hirta</i> (<i>Chamaesyce hirta</i>) (L.) Millsp.	X		
<i>Euphorbia inaequilatera</i> (<i>Chamaesyce inaequilatera</i>) (Sond.) Sojak			X
<i>Falkia oblonga</i> Bernh. Ex C. Krauss		X	
<i>Felicia muricata</i> (Thund.) Nees		X	X
<i>Gazania krebsiana</i> var. <i>Serrulata</i> Less.		X	X
<i>Gomphrena celosiodes</i> Mart.	X	X	
* <i>Guilleminea densa</i> (Willd. Ex Roem. %	X		

Schult.) Moq.			
<i>Hermannia depressa</i> N.E. Br.			X
<i>Helichrysum rotundatum</i> (DC.) Harv.			X
<i>Helichrysum rugulosum</i> Less.			X
<i>Heteropogon contortus</i> (L.) Roem. & Schult.			X
<i>Hibiscus pusillus</i> Thunb.			X
<i>Hibiscus trionum</i> L.		X	X
<i>Hyparrhenia hirta</i> (L.) Stapf		X	X
<i>Indigofera sp. L.</i>			X
<i>Jamesbrittenia aurantiaca</i> (Burch.) Hilliard			X
<i>Lactuca sp. L.</i>	X	X	X
<i>Ledebouria revoluta</i> (L.f.) Jessop			X
* <i>Lepidium bonariensis</i> L.	X		
<i>Leucas capensis</i> (Benth.) Engl.			X
<i>Macleodium zeyheri</i> (<i>Dicoma zeyheri</i>) Sond.			X
* <i>Malva neglecta</i> Wallr.	X		
* <i>Medicago laciniata</i> (L.) Mill.	X	X	
* <i>Medicago sativa</i> L.		X	
<i>Melinis nerviglumis</i> (Franch.) Zizka			X
* <i>Modiola carolinianum</i> (L.) G. Don.	X	X	
<i>Monsonia angustifolia</i> E. Mey. Ex A. Rich.		X	X
<i>Moraea sp. Mill.</i>	X	X	
* <i>Oenothera tetraptera</i> Cav.		X	
* <i>Oxalis corniculata</i> L.	X	X	X
<i>Oxalis obliquifolia</i> Steud. Ex Rich.		X	
<i>Pachystigma pygmaeum</i> (Schltr.) Robyns			X
<i>Panicum coloratum</i> L.			X
* <i>Paspalum dilatatum</i> Poir.	X	X	
* <i>Pennisetum clandestinum</i> Hochst. Ex Chiov.	X		

<i>*Plantago lanceolata</i> L.	X	X	
<i>Physalis viscosa</i> L.	X	X	
<i>Pollichia campestris</i> Aiton			X
<i>*Pseudognaphalium undulatum</i> Hillard & B.L. Burt			X
<i>Raphionacme velutina</i> (<i>Raphionacme burkei</i>) N.E. Br.			X
<i>Rhynchosia totta</i> (Thund.) DC.			X
<i>Salvia runcinata</i> L.f.		X	
<i>Scabiosa columbaria</i> L.			X
<i>*Schkuhria pinnata</i> (Lam.) Cabrera		X	X
<i>Setaria pumila</i> (<i>Setaria pallide-fusca</i>) (Schumach.) Stapf & C.E. Hubb		X	
<i>Setaria sphacelata</i> var. <i>torta</i> (Schumach.) Moss			X
<i>Sida rhombifolia</i> L.			X
<i>Sida spinosa</i> L.		X	X
<i>Solanum panduriforme</i> E. Mey.		X	X
<i>*Solanum sisymbriifolium</i> Lam.	X		
<i>Sporobolus africanus</i> (Poir.) Robyns & Tournay.	X		
<i>Sporobolus fimbriatus</i> (Trin) Nees		X	
<i>Striga asiatica</i> (L.) Kuntze			X
<i>*Tagetes minuta</i> L.		X	X
<i>*Taraxacum officinale</i> Weber	X		
<i>Tephrosia</i> sp. Pers.		X	X
<i>Themeda triandra</i> Forssk		X	X
<i>Thesium</i> sp. L.			X
<i>Tripteris aghillana</i> (<i>Osteospermum scariosum</i>) DC.			X
<i>Urochloa mosambicensis</i> (Hack.) Stapf			X
<i>Urochloa panicoides</i> P. Beauv.	X	X	

* <i>Verbena bonariensis</i> L.		X	X
* <i>Verbena aristigera</i> Briq.		X	
<i>Vernonia oligocephala</i> (DC.) Sch. Bip. Ex Walp.			X
<i>Ziziphus zeyheriana</i> Sond.			X

Table A4. Plant community data collected at the RURAL sampling sites.

Plantspecies	NR1	NR2	NR3	ER1	ER2	ER3	SR1	SR2	SR3	WR1	WR2	WR3
<i>Acacia karroo</i>	0	0	0.5	0	0	0	0	0	0	0	0	0
<i>Albuca setosa</i>	0.5	0	0	0	0	0	0	0	0	1	0.5	0.5
<i>Aloe greatheadii</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>Alternanthera pungens</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amaranthus hybridus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aristida bipartita</i>	0	0	0	0	0	2	0	0	0	2	0	0
<i>Aristida canescens</i>	0	0	0	3	1	6	0	0	0	0	0	0
<i>Aristida congesta</i>	0	0	0	7	2	0	0	2	2	0.5	0	0
<i>Aristida diffusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Berkheya radula</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bidens bipinnata</i>	0.5	0	0	0	0	0	0	0	0	0	0	0
<i>Blepharis integrifolia</i>	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Blepharis serrulata</i>	0	0	0	0	0	0	0	0	0	0.5	0.5	0

<i>Brachiaria eruciformis</i>	0	0	0	0	0	0	4	4	5	0	0	0
<i>Brachiaria serrata</i>	0	8	5	0	0	0	0	0	0	1	0	8
<i>Bulbine narcissifolia</i>	0.5	0	0.5	0	0	0	0	0	0	0	0	0
<i>Chaetacanthus costatus</i>	0	0.5	1	0	0	0	0	0	0	0	0	0.5
<i>Chascanum hederaceum</i>	0	0	0	0	0	0	0	0	0	0	0	0.5
<i>Chenopodium album</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chloris pycnothrix</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chloris virgata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ciclospermum leptophyllum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Convolvulus arvensis</i>	0	0.5	1	0	0	0	0	0	0	0	0	0
<i>Convolvulus sagittatus</i>	0	0	0	0	0	0	0.5	0	0.5	2	1	1
<i>Conyza bonariensis</i>	2	0.5	0	0	0	0	0	1	0	0	0	0
<i>Conyza podocephala</i>	6	2	2	0	0	0	0	0	0	1	0	1
<i>Corchorus asplenifolius</i>	0	0	0	0.5	0	0	0	0.5	0	0	0	0
<i>Cotula australis</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Crabbea acaulis</i>	0	0	0	0	0	0	0	0	0	0	0.5	0
<i>Crabbea angustifolia</i>	1	0	1	0	0	0	0	0	0	1	0	0
<i>Cymbopogon pospischillii</i> (<i>Cymbopogon plurinodes</i>)	8	0	2	0	0	0	0	0	0	0	0	0
<i>Cynodon dactylon</i>	0	0	0	0	0	0	15	0	2	0	0	0
<i>Cynodon hirsitus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dactyloctenium aegyptium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Deverra burchellii</i>	0.5	0	0	0	0	0	0	0	0	0	0	0
<i>Dichondra micrantha</i> (<i>Dichondra repens</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Digitaria ternata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Elephantorrhiza elephantina</i>	0	0	0	0	0	0	0	0	0	0	0	3
<i>Elionurus muticus</i>	40	50	57	24	25	15	2	0	0	12	25	20
<i>Eragrostis chloromelas</i>	0	0	0	2	6	12	0	35	25	2	0	0
<i>Eragrostis lehmanniana</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Eragrostis obtusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eragrostis plana</i>	0	0	0	0	0	0	0	3	2	0	0	0
<i>Eragrostis racemosa</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Euphorbia hirta</i> (<i>Chamaesyce hirta</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euphorbia inaequilatera</i> (<i>Chamaesyce inaequilatera</i>)	0	0	0	0.5	0.5	0.5	0	0.5	0	0	0	0
<i>Falkia oblonga</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Felicia muricata</i>	0	0	0	0	0	0	0	0	0	1	1	0
<i>Gazania krebsiana</i>	0	0.5	0	0	0	0	0	0	0	0	0	0
<i>Gomphrena celosiodes</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Guilleminea densa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hermannia depressa</i>	6	1	2	2	0	1	0	1	1	1	1	1
<i>Helichrysum rotundatum</i>	0	1	10	0	0	0	0	0	0	0	0	3
<i>Helichrysum rugulosum</i>	0	8	0	0	0	0	0	0	0	0	1	0

<i>Heteropogon contortus</i>	0	0	2	0	0	15	0	0	0	0	0	2
<i>Hibiscus pusillus</i>	0	0.5	0.5	1	2	0	0	1	0	0.5	0	0
<i>Hibiscus trionum</i>	0.5	0	0	0	0	0	0	0	0	0	0	0
<i>Hyparrhenia hirta</i>	0	0	2	0	0	0	0	15	13	0	0	0
<i>Indigofera sp.</i>	0	0	0	0	0	0	0	0.5	0	0	0	0
<i>Jamesbrittenia aurantiaca</i>	0.5	1	1	0	0	0	3	2	0	2	0	1
<i>Lactuca sp.</i>	0	0.5	0	0	0	0.5	0	0	0	0	0	0
<i>Ledebouria revoluta</i>	0	0.5	0	0	0	0	0	0	0	2	0	1
<i>Lepidium bonariense</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucas capensis</i>	0	0	0	0	0	0	0	0	0	3	0	0.5
<i>Macledium zeyheri (Dicoma zeyheri)</i>	0	0	0	0	0.5	0	0	0	0	0	0	0
<i>Malva neglecta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Medicago laciniata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Medicago sativa</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Melinis nerviglumis</i>	0	0	0	0	0	0	0	0	0	0	0	4
<i>Modiola carolinianum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Monsonia angustifolia</i>	1	0.5	0.5	0	0.5	0	0.5	0	0.5	0	0	0
<i>Moraea sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oenothera tetraptera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oxalis corniculata</i>	0	0.5	0	0	0	0	0.5	0	0	0	0	0
<i>Oxalis obliquifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pachystigma pygmaeum</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>Panicum coloratum</i>	0	0	0	0	0	0	0	0	0	2	2	0
<i>Paspalum dilatatum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pennisetum clandestinum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Plantago lanceolata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Physalis viscosa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pollichia campestris</i>	0.5	1	1	1	0.5	0.5	0	0	0	0.5	0	1
<i>Pseudognaphalium undulatum</i>	0.5	0.5	0	0	0	0	0	0	0	0	0	0

<i>Raphionacme velutina</i> (<i>Raphionacme burkei</i>)	1	0	0	0	0	0	0	0	0	0	0	0	1
<i>Rhynchosia totta</i>	0	0	0	0.5	0.5	0	0.5	0	0	0	0	0	0
<i>Salvia runcinata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scabiosa columbaria</i>	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Schkuhria pinnata</i>	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Setaria pumila</i> (<i>Setaria pallide- fusca</i>)	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Setaria sphacelata</i> var. <i>torta</i>	25	20	8	0	0	0	0	0	0	4	4	0	0
<i>Sida rhombifolia</i>	0.5	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sida spinosa</i>	0	0	0	0	0	0	0.5	0	0	0	0	0	0
<i>Solanum panduriforme</i>	0.5	0	0	0	0	0	0	0	0	0	0	0	0
<i>Solanum sisymbriifolium</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sporobolus africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sporobolus fimbriatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Striga asiatica</i>	0	0	0	0	0	0	0	0	0	0	0	0.5
<i>Tagetes minuta</i>	0.5	0	0	0	0	0	0	0.5	0	0.5	0	0
<i>Taraxacum officinale</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tephrosia sp.</i>	0	0.5	0	0	0	0.5	0	0	0.5	0	0	0
<i>Themeda triandra</i>	7	4	6	35	40	30	60	20	10	45	50	45
<i>Thesium sp.</i>	0	0	0	0	0.5	0	0	0	0	0	0	0
<i>Tripteris aghillana (Osteospermum scariosium)</i>	0	0.5	0	0	0	0	0	0	0	1	0.5	0
<i>Urochloa mosambicensis</i>	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Urochloa panicoides</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Verbena bonariensis</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Verbena aristigera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Vernonia oligocephala</i>	0	0	3	0	0	0	0	0	0	0	0	0
<i>Ziziphus zeyheriana</i>	0	0	0	0	0	0	0	0	0	0	0	5

Table A5. Plant community data collected at the SUB-URBAN sampling sites.

Plantspecies	NSU1	NSU2	NSU3	ESU1	ESU2	ESU3	SSU1	SSU2	SSU3	WSU1	WSU2	WSU3
<i>Acacia karroo</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Albuca setosa</i>	0	0	0	1	0	0	0	0	1	0	0	0
<i>Aloe greatheadii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alternanthera pungens</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amaranthus hybridus</i>	0	0	0	0	0	0	0	0	0	0	0.5	0
<i>Aristida bipartita</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aristida canescens</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Aristida congesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aristida diffusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Berkheya radula</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Bidens bipinnata</i>	0	0	0.5	0	0	0	0	0	0	0	0	0
<i>Blepharis integrifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Blepharis serrulata</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Brachiaria eruciformis</i>	0	0	0	4	2	0	14	12	0	0	0	0
<i>Brachiaria serrata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bulbine narcissifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chaetacanthus costatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chascanum hederaceum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chenopodium album</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chlorus pycnothrix</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chlorus virgata</i>	0	0	0	0	0	0	14	7	1	0	0	0
<i>Ciclospermum leptophyllum</i>	0	0	0	0	0.5	0	0	0	0	0	0	0
<i>Convolvulus arvensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Convolvulus sagittatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conyza bonariensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conyza podocephala</i>	6	0	3	6	6	0	0	0	0.5	0	0	0
<i>Corchorus asplenifolius</i>	0	0	0	1	0	0	0	0	0.5	0	0	0
<i>Cotula australis</i>	0	0	0	3	0	0	15	3	0	0	0	0

<i>Crabbea acaulis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Crabbea angustifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbopogon pospischillii</i> (<i>Cymbopogon plurinodes</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cynodon dactylon</i>	15	18	5	14	9	63	6	8	28	82	50	2
<i>Cynodon hirsitus</i>	0	0	0	0	0	0	0	0	0	0	0	13
<i>Dactyloctenium aegyptium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Deverra burchellii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dichondra micrantha</i> (<i>Dichondra repens</i>)	0	1	0	0	5	0	1	3	15	0	0	0
<i>Digitaria ternata</i>	0	0	0	0	1	0	12	0	1	0	0	0
<i>Elephantorrhiza elephantina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Elionurus muticus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eragrostis chloromelas</i>	0	0	0	0	0	0	26	9	0	1	0	6
<i>Eragrostis lehmanniana</i>	0	0	0	2	0	0	0	0	0	0	0	0

<i>Eragrostis obtusa</i>	0	0	0	0	0	0	0.5	0.5	1	0	0	0
<i>Eragrostis plana</i>	0	0	0	0	0	0	2	0	0	0	0	0
<i>Eragrostis racemosa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euphorbia hirta</i> (<i>Chamaesyce hirta</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euphorbia inaequilatera</i> (<i>Chamaesyce inaequilatera</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Falkia oblonga</i>	0	0	0	0	0	0	0	0	0.5	0	0	0
<i>Felicia muricata</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Gazania krebsiana</i>	0	0	0	0	0	0	0.5	0	0	0	0	0
<i>Gomphrena celosiodes</i>	0	0	0	0	0	0	0.5	0.5	0	0	0	0
<i>Guilleminea densa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hermannia depressa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helichrysum rotundatum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helichrysum rugulosum</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Heteropogon contortus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hibiscus pusillus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hibiscus trionum</i>	0	0	0	0	0	0	1	0	1	0	0	0
<i>Hyparrhenia hirta</i>	4	1	0	0	0	0	0	0	0	0	0	0
<i>Indigofera sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Jamesbrittenia aurantiaca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lactuca sp.</i>	0	2	2	1	0.5	2	0	0	0	0	0	0
<i>Ledebouria revoluta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepidium bonariense</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucas capensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Macledium zeyheri (Dicoma zeyheri)</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Malva neglecta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Medicago laciniata</i>	1	0	0	1	0.5	1	0	0.5	1	0	0	0
<i>Medicago sativa</i>	0	3	0	1	1	6	0	0	0	0	0	0

<i>Melinis nerviglumis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Modiola carolinianum</i>	0	0	1	0	0	0	0	0	0	0.5	0	0
<i>Monsonia angustifolia</i>	0	0	0	2	0	0	1	0	5	0	0	0
<i>Moraea sp.</i>	0	0	0	0	0.5	0	0	0	0	0	0	0
<i>Oenothera tetraptera</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>Oxalis corniculata</i>	0	0	0	0	0	0	0	0	2	0	0	0
<i>Oxalis obliquifolia</i>	0	0.5	0	0	0	0	0	0	0	0	0	0
<i>Pachystigma pygmaeum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Panicum coloratum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paspalum dilatatum</i>	1	1	0	0	2	10	0	0	0	0	0	0
<i>Pennisetum clandestinum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Plantago lanceolata</i>	5	25	13	12	14	6	5	15	6	0	0	1
<i>Physalis viscosa</i>	2	2	0	1	0	0	0	0	0	3	1	2
<i>Pollichia campestris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pseudognaphalium undulatum</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Raphionacme velutina</i> (<i>Raphionacme burkei</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhynchosia totta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Salvia runcinata</i>	0	0	0	0.5	0	0	0.5	2	2	0	0	0
<i>Scabiosa columbaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Schkuhria pinnata</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>Setaria pumila</i> (<i>Setaria pallide- fusca</i>)	1	2	0	0	0	0	1	0	0	0	0	0
<i>Setaria sphacelata</i> var. <i>torta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sida rhombifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sida spinosa</i>	0	0.5	0	0	0	0	0	0	0	0	0	0
<i>Solanum panduriforme</i>	0	2	1	0	0	0	0	0	1	2	0	1
<i>Solanum sisymbriifolium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sporobolus africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sporobolus fimbriatus</i>	0	0	1	0	0	0	0	0	0	0	0	0

<i>Striga asiatica</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tagetes minuta</i>	0	0.5	0.5	0	0	0	0	0	0	0	0	0
<i>Taraxacum officinale</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tephrosia sp.</i>	0	0	0	0.5	0	0	0	15	20	0	0	0
<i>Themeda triandra</i>	55	26	44	30	43	0	0	0	0	0	0	0
<i>Thesium sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tripteris aghillana (Osteospermum scariosium)</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Urochloa mosambicensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Urochloa panicoides</i>	1	15	20	1	1	12	2	8	22	2	45	60
<i>Verbena bonariensis</i>	0	0.5	0	0	1	0	0	0.5	0.5	0	0	1
<i>Verbena aristigera</i>	0	0	0	2	0.5	0	0	0	0	0	0	0
<i>Vernonia oligocephala</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ziziphus zeyheriana</i>	0	0	0	0	0	0	0	0	0	0	0	0

Table A6. Plant community data collected at the URBAN sampling sites.

Plantspecies	NU1	NU2	NU3	EU1	EU2	EU3	SU1	SU2	SU3	WU1	WU2	WU3
<i>Acacia karroo</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Albuca setosa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aloe greatheadii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alternanthera pungens</i>	0	0	0	0	0.5	0.5	0.5	0	0	0.5	0	0
<i>Amaranthus hybridus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aristida bipartita</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aristida canescens</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aristida congesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aristida diffusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Berkheya radula</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bidens bipinnata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Blepharis integrifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Blepharis serrulata</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Brachiaria eraciformis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachiaria serrata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bulbine narcissifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chaetacanthus costatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chascanum hederaceum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chenopodium album</i>	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Chloris pycnothrix</i>	1	1	0	7	1	0	3	21	17	0	0	0
<i>Chloris virgata</i>	0	0	0	27	1	0	0	0	0	0	0	0
<i>Ciclospermum leptophyllum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Convolvulus arvensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Convolvulus sagittatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conyza bonariensis</i>	0	0	0	0	0	0.5	0	0	0	4	0	1
<i>Conyza podocephala</i>	0	0	0	0	0	1	0	0	0	0	0	0
<i>Corchorus asplenifolius</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cotula australis</i>	0	0	0	35	0	0	0	0	0	0	0	0

<i>Crabbea acaulis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Crabbea angustifolia (E)</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbopogon pospischillii</i> (<i>Cymbopogon plurinodes</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cynodon dactylon</i>	81	47	85	7	11	12	0	33	43	68	0	1
<i>Cynodon hirsitus</i>	0	0	0	0	43	2	57	0	0	0	0	0
<i>Dactyloctenium aegyptium</i>	2	0	0	0	0	0	1	0	2	6	0	0.5
<i>Deverra burchellii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dichondra micrantha (Dichondra repens)</i>	1	18	18	0	0	13	2	0	6	0	0	0
<i>Digitaria ternata</i>	0	1	0	1	0	0	0	0	0	0	0	0
<i>Elephantorrhiza elephantina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Elionurus muticus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eragrostis chloromelas</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eragrostis lehmanniana</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Eragrostis obtusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eragrostis plana</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eragrostis racemosa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euphorbia hirta</i> (<i>Chamaesyce hirta</i>)	0	0	2	1	0	3	0	0	0	0	0	0
<i>Euphorbia inaequilatera</i> (<i>Chamaesyce inaequilatera</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Falkia oblonga</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Felicia muricata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gazania krebsiana</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphrena celosiodes</i>	0	0	0.5	0	0	1	0	0	0	0	0	0
<i>Guilleminea densa</i>	0	0	0	0	1	0	1	3	6	0	0	0
<i>Hermannia depressa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helichrysum rotundatum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helichrysum rugulosum</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Heteropogon contortus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hibiscus pusillus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hibiscus trionum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hyparrhenia hirta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Indigofera sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Jamesbrittenia aurantiaca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lactuca sp.</i>	1	20	6	0	0	0	0	0	0.5	0.5	0	0
<i>Ledebouria revoluta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepidium bonariense</i>	0	0.5	0	0	1	0	0	0	0	1	0	0
<i>Leucas capensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Macledium zeyheri (Dicoma zeyheri)</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Malva neglecta</i>	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Medicago laciniata</i>	0	2	1	1	0	0	0	0	0	0	0	0
<i>Medicago sativa</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Melinis nerviglumis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Modiola carolinianum</i>	0	0.5	0	0	0	0	0	0	4	0	0	0
<i>Monsonia angustifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Moraea sp.</i>	0	0.5	0.5	0	0	0	0	0	1	0	0	0
<i>Oenothera tetraptera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oxalis corniculata</i>	1	1	0.5	0	0	0	0	0	4	0	0	0
<i>Oxalis obliquifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pachystigma pygmaeum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Panicum coloratum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paspalum dilatatum</i>	3	1	2	0	0	0	0	0	0	0	0	0
<i>Pennisetum clandestinum</i>	6	0	0	0	0	6	5	0	0	0	100	100
<i>Plantago lanceolata</i>	0	0.5	6	3	0	1	0	0	0	0	0	0
<i>Physalis viscosa</i>	0	0	0	0	0	0	0	0	0	5	0	0
<i>Pollichia campestris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pseudognaphalium undulatum</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Raphionacme velutina</i> (<i>Raphionacme burkei</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhynchosia totta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Salvia runcinata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scabiosa columbaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Schkuhria pinnata</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Setaria pumila</i> (<i>Setaria pallide- fusca</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Setaria sphacelata</i> var. <i>torta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sida rhombifolia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sida spinosa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Solanum panduriforme</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Solanum sisymbriifolium</i>	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Sporobolus africanus</i>	7	26	14	1	0	10	2	2	6	0	0	0
<i>Sporobolus fimbriatus</i>	0	0	0	0	0	0	0	0	0	0	0	0

<i>Striga asiatica</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tagetes minuta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taraxacum officinale</i>	0	0	0.5	0	0	0	0	0	0	0	0	0
<i>Tephrosia sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Themeda triandra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thesium sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tripteris aghillana</i> (<i>Osteospermum scariosium</i>)	0	0	0	0	0	0	0	0	0	0	0	0
<i>Urochloa mosambicensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Urochloa panicoides</i>	0.5	0.5	3	1	15	8	7	25	4	1	0	0
<i>Verbena bonariensis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Verbena aristigera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Vernonia oligocephala</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ziziphus zeyheriana</i>	0	0	0	0	0	0	0	0	0	0	0	0

Table A7. The amount of plant species observed in each plot, subdivided into groups indigenous, alien, perennial, annual or annual/perennial.

Site	Species	Indigenous	Alien	Annual	Perennial	Annual/Perennial
ER1	11	9	2	1	9	1
ER2	12	10	2	1	10	1
ER3	11	8	3	3	8	0
ESU1	18	11	7	6	11	1
ESU2	17	11	6	7	10	0
ESU3	7	5	2	1	6	0
EU1	10	9	1	2	8	0
EU2	8	6	2	1	6	1
EU3	12	8	4	2	10	0
NR1	21	16	5	6	14	1
NR2	24	18	6	4	18	2
NR3	21	17	4	1	18	2
NSU1	10	8	2	0	10	0
NSU2	16	12	4	4	12	0
NSU3	12	7	5	4	8	0
NU1	10	9	1	3	7	0
NU2	14	11	3	4	9	1
NU3	13	10	3	4	9	0
SR1	12	11	1	2	8	2
SR2	14	13	1	3	9	2
SR3	11	10	1	3	6	2
SSU1	17	15	2	6	11	0
SSU2	16	13	3	7	9	0
SSU3	20	18	2	6	14	0
SU1	9	7	2	2	7	0
SU2	5	4	1	1	4	0
SU3	11	7	4	4	7	0
WR1	25	21	4	3	19	3

WR2	12	10	2	2	9	1
WR3	20	17	3	1	17	2
WSU1	6	4	2	2	4	0
WSU2	4	2	2	1	3	0
WSU3	8	7	1	3	5	0
WU1	11	5	6	4	5	2
WU2	1	1	0	0	1	0
WU3	4	3	1	1	3	0

Table A8. Bacterial and Fungal CFU/g soil. Included in the table is the Shannon-Weaver Indices regarding the culture dependent bacterial results and the plant community data. Data includes standard deviations and Tukey's HSD. (Seasons : A= May '07; B=Aug '07; C=May '08; D=Aug'08).

	Bacterial CFU/g	Fungi CFU/g	Bacterial Shannon-Weaver indices	Plant Shannon-Weaver Indices
ER_A	516667±132455.4 ^{a,b}	126666.7±37118.43 ^{e,f}	1.177697±0.124006 ^{b,c,d}	1.714504±0.244335 ^{a,b}
ER_B	150000±3464.1 ^{a,b}	20333.3±2666.67 ^{a,b}	0.667293±0.047960 ^{a,b,c,d}	
ER_C	320000±5773.5 ^{a,b}	6000.0±1000.00 ^a		
ER_D	150000±3464.1 ^{a,b}	20333.3±2666.67 ^{a,b}		
ESU_A	620000±60277.1 ^{a,b}	41000.0±14294.52 ^{a,b,c}	1.381613±0.016109 ^d	1.626498±0.173598 ^{a,b}
ESU_B	570000±45825.8 ^{a,b}	19000.0±4932.88 ^{a,b}	0.735584±0.039757 ^{a,b,c,d}	
ESU_C	686667±177043.6 ^{a,b}	25333.3±3282.95 ^{a,b}		
ESU_D	886667±74461.0 ^{a,b,c}	21000.0±10503.97 ^{a,b}		
EU_A	460000±5773.5 ^{a,b}	113333.3±24037.01 ^{d,e,f}	0.931858±0.057838 ^{a,b,c,d}	1.711804±0.254097 ^{a,b}
EU_B	923333±146553.0 ^{a,b,c}	21666.7±666.67 ^{a,b}	0.723921±0.041863 ^{a,b,c,d}	

EU_C	826667±20275.9 ^{a,b,c}	17666.7±3382.96 ^{a,b}		
EU_D	1296667±193419.5 ^{a,b,c}	73333.3±12333.33 ^{b,c,d,e}		
NR_A	990000±150996.7 ^{a,b,c}	170000.0±11547.01 ^f	0.458080±0.180464 ^{a,b,c,}	1.884979±0.024091 ^a
NR_B	770000±295691.3 ^{a,b,c}	15333.3±4666.67 ^{a,b}	0.594601±0.132025 ^{a,b,c,}	
NR_C	363333±41766.5 ^{a,b}	9666.7±1201.85 ^{a,b}		
NR_D	566667±88756.8 ^{a,b}	15333.3±4666.67 ^{a,b}		
NSU_A	1020000±270246.8 ^{a,b,c}	63333.3±13333.33 ^{a,b,c,d,e}	1.217188±0.063611 ^{c,d}	1.509965±0.112573 ^{a,b}
NSU_B	1333333±381677.6 ^{b,c}	19666.7±2333.33 ^{a,b}	0.389261±0.059936 ^a	
NSU_C	850000±140475.4 ^{a,b,c}	11666.7±2848.00 ^{a,b}		
NSU_D	1333333±381677.6 ^{b,c}	19666.7±2333.33 ^{a,b}		
NU_A	600000±81445.3 ^{a,b}	63333.3±3333.33 ^{a,b,c,d,e}	0.673140±0.104102 ^{a,b,c,d}	1.528021±0.187921 ^{a,b}
NU_B	1306667±171302.2 ^{a,b,c}	12333.3±3480.10 ^{a,b}	0.935526±0.091653 ^{a,b,c,d}	
NU_C	866667±44095.9 ^{a,b,c}	21666.7±5238.74 ^{a,b}		
NU_D	880000±97125.3 ^{a,b,c}	8333.3±2333.33 ^a		
SR_A	283333±117945.4 ^{a,b}	33333.3±13333.33 ^{a,b}	1.191849±0.122904 ^{b,c,d}	2.209762±0.052302 ^a

SR_B	531667±369533.2 ^{a,b}	16333.3±3179.80 ^{a,b}	0.592288±0.158402 ^{a,b,c}	
SR_C	135667±5044.2 ^{a,b}	7333.3±1452.97 ^a		
SR_D	531667±369533 ^{a,b}	16333.3±3179.80 ^{a,b}		
SSU_A	413333±89504.8 ^{a,b}	43333.3±8819.17 ^{a,b,c}	0.955909±0.016481 ^{a,b,c,d}	0.757735±0.174360 ^{b,c}
SSU_B	600000±244199.4 ^{a,b}	12333.3±2333.33 ^{a,b}	0.552042±0.258840 ^{a,b,c}	
SSU_C	142667±10268.6 ^{a,b}	6666.7±666.67 ^a		
SSU_D	733333±76883.8 ^{a,b,c}	15000.0±1732.05 ^{a,b}		
SU_A	470000±110151.4 ^{a,b}	46666.7±12018.50 ^{a,b,c}	0.738981±0.120949 ^{a,b,c,d}	1.312887±0.206570 ^{a,b,c}
SU_B	1953333±560991.2 ^c	20000.0±2309.40 ^{a,b}	0.716653±0.198312 ^{a,b,c,d}	
SU_C	893333±273820.7 ^{a,b,c}	21333.3±3844.19 ^{a,b}		
SU_D	1146667±193677.9 ^{a,b,c}	40333.3±24333.33 ^{a,b,c}		
WR_A	58000±16093.5 ^a	56666.7±23333.33 ^{a,b,c,d,e}	0.445519±0.222963 ^{a,b}	1.583335±0.245132 ^{a,b}
WR_B	128000±11532.6 ^{a,b}	21000.0±6245.00 ^{a,b}	0.778678±0.061214 ^{a,b,c,d}	
WR_C	391667±131540.0 ^{a,b}	4333.3±666.67 ^a		
WR_D	760000±138684.3 ^{a,b,c}	9333.3±2403.70 ^{a,b}		

WSU_A	406667±174865.0 ^{a,b}	100000.0±15275.25 ^{c,d,e}	0.580294±0.296154 ^{a,b,c}	1.373294±0.201682 ^{a,b}
WSU_B	793667±331949.0 ^{a,b,c}	30333.3±6960.20 ^{a,b}	0.579519±0.084529 ^{a,b,c}	
WSU_C	740000±215715.9 ^{a,b,c}	23000.0±7000.00 ^{a,b}		
WSU_D	793667±331949.0 ^{a,b,c}	30333.3±6960.20 ^{a,b}		
WU_A	204000±148028.2 ^{a,b}	73333.3±27284.51 ^{b,c,d,e}	0.527736±0.228194 ^{a,b,c}	0.358158±0.290798 ^c
WU_B	831333±503595.5 ^{a,b,c}	18666.7±7125.85 ^{a,b}	0.965338±0.077043 ^{a,b,c,d}	
WU_C	1133333±398594.8 ^{a,b,c}	29000.0±15947.83 ^{a,b}		
WU_D	663333±84129.5 ^{a,b}	26666.7±2848.00 ^{a,b}		

Table A9. Qualitative data regarding the isolated bacterial community DNA generated by nanodrop spectrophotometer analysis. DNA used for analysis was extracted from soil sampled in May 2007.

Sample ID	Sampling period	DNA yield ng/ul	A260	A280	260/280	260/230
NR	May '07	25.95	0.519	0.787	0.66	0.59
NSU	May '07	25.19	0.504	0.748	0.67	0.66
NU	May '07	32.64	0.653	0.97	0.67	0.87
ER	May '07	31.37	0.627	0.923	0.68	0.68
ESU	May '07	18.25	0.365	0.636	0.57	0.74
EU	May '07	18.6	0.372	0.579	0.64	0.7
SR	May '07	34.41	0.688	1.056	0.65	0.7
SSU	May '07	39.24	0.785	1.055	0.74	0.67
SU	May '07	34.33	0.687	0.934	0.74	0.78
WR	May '07	35.92	0.718	1.058	0.68	0.73
WSU	May '07	17.91	0.358	0.425	0.84	0.96
WU	May '07	29.15	0.583	0.778	0.75	0.78

Table A10. Enzyme assay data (Dehydrogenase, B-Glucosidase, Urease, Alkaline and Acid Phosphatase). Data includes standard deviations and Tukey's HSD. (Seasons : A= May '07; B=Aug '07; C=May '08; D=Aug'08).

	DHA	β --Glu	Urease	Alk P	Acid P
ER_A	190.66±32.16 ^{a,b,c,d,e}	896.568±106.6091 ^{a,b,c,d,e,f,g,h,i}	9862.03±1868.40 ^{a,b,c}	1498.267±218.0338 ^{d,e,f,g,h,I,j}	1971.361±39.3666 ^{e,f,g}
ER_B	347.87±31.68 ^{a,b,c,d,e,f,g,h,i}	847.538±158.7099 ^{a,b,c,d,e,f,g,h}	22522.42±13419.86 ^{a,b,c}	724.768±211.0107 ^{a,b,c,d}	1831.377±16.9395 ^{d,e,f,g}
ER_C	191.86±14.25 ^{a,b,c,d,e}	597.761±32.446 ^{a,b,c,d,e,f}	9413.73±1422.38 ^{a,b}		
ER_D	184.75±32.13 ^{a,b,c,d,e}	858.354±95.8594 ^{a,b,c,d,e,f,g,h,i}	9531.25±1838.23 ^{a,b}		
ESU_A	213.66±21.37 ^{a,b,c,d,e,f}	1206.803±89.5376 ^{c,d,e,f,g,h,I,j,k}	14905.43±782.95 ^{a,b,c}	2061.496±28.1871 ^j	1254.742±137.2680 ^b
ESU_B	471.72±65.75 ^{b,c,d,e,f,g,h,i}	1318.089±137.8127 ^{f,g,h,I,j,k}	21888.56±3134.38 ^{a,b,c}	1656.881±9.5247 ^{e,f,g,h,I,j}	1497.802±91.8255 ^{b,c,d}

ESU_C	207.09±44.90 ^{a,b,c,d,e,f}	928.059±177.2568 ^{a,b,c,d,e,f,g,h,I,j}	11653.71±2661.30 ^{a,b,c}		
ESU_D	407.15±115.87 ^{a,b,c,d,e,f,g,h,i}	1234.425±141.5157 ^{d,e,f,g,h,I,j,k}	13686.98±652.90 ^{a,b,c}		
EU_A	354.90±26.23 ^{a,b,c,d,e,f,g,h,i}	1446.643±30.4385 ^{g,h,I,j,k}	19374.48±2912.02 ^{a,b,c}	1964.713±26.7012 ^{h,I,j}	1899.363±26.7091 ^{e,f,g}
EU_B	604.39±53.53 ^{g,h,i}	1592.146±150.1231 ^{h,I,j,k}	22117.10±5755.12 ^{a,b,c}	1061.542±45.4767 ^{c,d,e,f,g}	1809.148±30.2082 ^{d,e,f,g}
EU_C	343.01±43.79 ^{a,b,c,d,e,f,g,h,i}	1313.116±66.0507 ^{f,g,h,I,j,k}	26326.78±7517.51 ^{b,c}		
EU_D	526.24±39.19 ^{f,g,h,i}	1612.703±132.9641 ^{I,j,k}	18261.97±2802.42 ^{a,b,c}		
NR_A	249.02±26.19 ^{a,b,c,d,e,f}	966.438±146.5061 ^{a,b,c,d,e,f,g,h,I,j,k}	9419.91±984.71 ^{a,b}	1151.629±203.1742 ^{c,d,e,f,g,h}	1913.478±13.7519 ^{e,f,g}
NR_B	370.72±19.39 ^{a,b,c,d,e,f,g,h,i}	757.739±75.6744 ^{a,b,c,d,e,f,g}	9072.47±167.68 ^{a,b}	882.118±67.9003 ^{b,c,d,e}	1696.458±14.3474 ^{c,d,e,f}
NR_C	223.83±26.58 ^{a,b,c,d,e,f}	437.406±140.9808 ^{a,b}	6951.38±1066.65 ^{a,b}		

NR_D	292.83±5.99 ^{a,b,c,d,e,f,g}	765.843±110.7925 ^{a,b,c,d,e,f,g}	7908.21±1090.33 ^{a,b}		
NSU_A	302.17±39.58 ^{a,b,c,d,e,f,g}	1231.656±99.2341 ^{d,e,f,g,h,I,j,k}	9737.15±412.62 ^{a,b,c}	1890.224±56.3522 ^{g,h,I,j}	1819.333±27.1723 ^{d,e,f,g}
NSU_B	480.89±28.40 ^{c,d,e,f,g,h,i}	990.249±129.7286 ^{a,b,c,d,e,f,g,h,I,j,k}	10459.28±2889.54 ^{a,b,c}	1195.595±149.3806 ^{c,d,e,f,g,h,i}	1636.812±53.6006 ^{c,d,e}
NSU_C	343.13±51.96 ^{a,b,c,d,e,f,g,h,i}	1055.624±190.7733 ^{a,b,c,d,e,f,g,h,I,j,k}	11282.32±3373.28 ^{a,b,c}		
NSU_D	300.31±34.62 ^{a,b,c,d,e,f,g,h,i}	1210.988±83.7322 ^{c,d,e,f,g,h,I,j,k}	9738.33±409.97 ^{a,b,c}		
NU_A	486.62±9.94 ^{d,e,f,g,h,i}	1664.864±21.4106 ^{j,k}	22963.79±4067.00 ^{a,b,c}	2092.438±44.0197 ^j	2050.504±44.3724 ^g
NU_B	635.71±40.17 ^{h,i}	1704.484±143.6692 ^k	19465.35±2919.81 ^{a,b,c}	1456.078±276.9483 ^{d,e,f,g,h,I,j}	1798.519±100.4901 ^{d,e,f,g}
NU_C	492.10±48.94 ^{d,e,f,g,h,i}	1412.965±63.5337 ^{g,h,I,j,k}	18911.00±2301.27 ^{a,b,c}		
NU_D	504.81±155.67 ^{e,f,g,h,i}	1484.718±306.6932 ^{g,h,I,j,k}	25085.31±4199.70 ^{a,b,c}		

SR_A	107.25±15.27 ^a	789.378±66.3422 ^{a,b,c,d,e,f,g}	6707.60±977.49 ^{a,b}	1185.359±361.3602 ^{c,d,e,f,g,h,i}	2107.482±40.9778 ^g
SR_B	260.49±40.91 ^{a,b,c,d,e,f}	768.396±88.1711 ^{a,b,c,d,e,f,g}	10494.11±1845.80 ^{a,b,c}	941.172±139.4616 ^{b,c,d,e,f}	1995.474±4.5498 ^{f,g}
SR_C	142.79±13.16 ^{a,b}	592.169±100.8819 ^{a,b,c,d,e,f}	6948.40±1550.99 ^{a,b}		
SR_D	102.84±13.59 ^a	743.385±53.6706 ^{a,b,c,d,e,f,g}	6435.01±925.43 ^{a,b}		
SSU_A	186.68±20.05 ^{a,b,c,d,e}	1177.960±32.5725 ^{b,c,d,e,f,g,h,I,j,k}	13439.66±1913.59 ^{a,b,c}	1789.677±157.4869 ^{f,g,h,I,j}	2068.762±31.5306 ^g
SSU_B	391.03±66.94 ^{a,b,c,d,e,f,g,h,i}	1063.142±130.8006 ^{a,b,c,d,e,f,g,h,I,j,k}	14416.64±3821.58 ^{a,b,c}	1160.509±324.1160 ^{c,d,e,f,g,h}	1887.414±115.2507 ^{e,f,g}
SSU_C	363.88±66.86 ^{a,b,c,d,e,f,g,h,i}	980.311±213.2387 ^{a,b,c,d,e,f,g,h,I,j,k}	10544.61±2505.37 ^{a,b,c}		
SSU_D	328.64±53.48 ^{a,b,c,d,e,f,g,h,i}	1299.824±57.9843 ^{e,f,g,h,I,j,k}	11005.06±1738.39 ^{a,b,c}		
SU_A	313.77±156.71 ^{a,b,c,d,e,f,g,h}	1598.098±132.3422 ^{h,I,j,k}	18265.85±3021.01 ^{a,b,c}	2041.805±37.7639 ^{l,j}	1937.811±142.1468 ^{e,f,g}

SU_B	652.97±19.97 ⁱ	1500.078±31.1357 ^{g,h,I,j,k}	17395.98±3033.84 ^{a,b,c}	1473.131±49.5639 ^{d,e,f,g,h,I,j}	1797.399±48.6954 ^{d,e,f,g}
SU_C	364.85±68.32 ^{a,b,c,d,e,f,g,h,i}	1016.486±134.7655 ^{a,b,c,d,e,f,g,h,I,j,k}	9527.40±1719.19 ^{a,b}		
SU_D	214.32±40.69 ^{a,b,c,d,e,f}	536.401±72.6715 ^{a,b,c,d,e}	17000.38±3008.62 ^{a,b,c}		
WR_A	151.66±28.14 ^{a,b,c}	602.389±31.9169 ^{a,b,c,d,e,f}	10984.38±2183.00 ^{a,b,c}	758.780±72.6763 ^{a,b,c,d}	1894.075±20.8124 ^{e,f,g}
WR_B	274.24±6.23 ^{a,b,c,d,e,f}	522.543±36.1421 ^{a,b,c,d}	30549.60±1133.29 ^c	452.142±47.5950 ^{a,b,c}	1816.371±10.1865 ^{d,e,f,g}
WR_C	265.67±20.36 ^{a,b,c,d,e,f}	334.677±57.2180 ^a	7527.29±565.47 ^{a,b}		
WR_D	319.66±5.56 ^{a,b,c,d,e,f,g,h}	566.818±44.4748 ^{a,b,c,d,e,f}	8902.96±2130.24 ^{a,b}		
WSU_A	170.39±36.24 ^{a,b,c,d}	1433.468±244.6500 ^{g,h,I,j,k}	7852.03±1685.00 ^{a,b}	1980.061±203.4209 ^{h,I,j}	2030.024±93.3536 ^{f,g}
WSU_B	345.73±21.13 ^{a,b,c,d,e,f,g,h,i}	1247.220±77.9586 ^{d,e,f,g,h,I,j,k}	11680.96±1685.03 ^{a,b,c}	166.941±75.5282 ^{a,b}	1223.543±14.6136 ^b
WSU_C	218.74±23.54 ^{a,b,c,d,e,f}	459.766±198.8812 ^{a,b,c}	5389.94±773.51 ^a		
WSU_D	153.33±28.27 ^{a,b,c}	1269.635±162.9039 ^{d,e,f,g,h,I,j,k}	7137.05±1551.51 ^{a,b}		

WU_A	295.76±67.73 ^{a,b,c,d,e,f,g}	1673.612±257.5138 ^{j,k}	16028.50±5152.37 ^{a,b,c}	1646.470±113.8476 ^{e,f,g,h,I,j}	1349.426±61.3618 ^{b,c}
WU_B	323.41±140.42 ^{a,b,c,d,e,f,g,h}	1442.899±153.4814 ^{g,h,I,j,k}	14666.74±8887.71 ^{a,b,c}	11.603±29.4304 ^a	266.191±32.7163 ^a
WU_C	267.24±33.54 ^{a,b,c,d,e,f}	1302.371±186.2191 ^{f,g,h,I,j,k}	16028.50±5152.37 ^{a,b,c}		
WU_D	322.19±108.46 ^{a,b,c,d,e,f,g,h}	1489.706±110.6630 ^{g,h,I,j,k}	15393.28±4376.10 ^{a,b,c}		

