Capturing baseline vegetation data, including an assessment of plant sensitivity to increased acidity, in the Waterberg

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Declaration

I declare that this dissertation, submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg, is my own unaided work, unless acknowledged to the contrary in the text. It has not been submitted before for any degree or examination at any other University.

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Belinda Flood

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Abstract

Global environmental change due to anthropogenic activity results in alterations to the Earth's biogeochemical cycles. This study focused on nitrogen and sulphur deposition, which results in the acidification of ecosystems. Alterations to these processes will have an effect on the diversity and ecophysiology of the vegetation; moreover, little is known about the long-term impacts on the vegetation structure and composition.

Increased development, mining and industrialization, within the Waterberg area, particularly with the construction of the Medupi power station, have resulted in this area coming under study. The impacts of additional air pollution to the vegetation in this area are currently unknown. The aim of this research was to determine whether increased ambient levels of atmospheric nitrogen and sulphur, resulting from the power stations, will impact the structure, functional type and the composition of the vegetation, and the resultant impacts on vegetation structure and growth as a result of the added nitrogen and sulphur to the system and increased acidity. A baseline of quantitative data was needed in order for future comparisons to be made to assess whether biodiversity is changing and at what rate. One part of the study involved the collection of baseline vegetation data along a pre-determined transect in the Waterberg area. A transect was identified which could serve as a vehicle for monitoring changes over time, with areas downwind of the Matimba and Medupi power stations assumed to be more impacted than areas upwind. The two downwind sites and the two sites closest to the power stations were found to have the highest beta diversities, with the two downwind sites having a value of 0.60 and the downwind Landelani site and upwind Withoutpan site having a value of 0.53. The high species variation between these sites may already be an indication of pollution impacts within the area. The downwind site closest to the power stations, Landelani, is particularly vulnerable as it has a high Shannon diversity index, with a value of 2.84, and high tree biomass, 46.64 tonnes/ha, with low tree density, 625 trees per hectare.

Functional groups are assumed to react similarly under changing environmental conditions. The second part of this research focussed on photosynthetic pathways, by using two C3 woody species and two C4 grasses. The experiment was conducted in the greenhouse at the University of the Witwatersrand and showed that both tree and both grass species selected were sensitive to sulphur additions to varying degrees. Additionally *Acacia sieberiana* was found to be sensitive to nitrogen addition, however *Combretum erythrophyllum* responded to nitrogen when it was added alone. A positive response to nitrogen was seen in both grass species; however the positive response was negated by the addition of any sulphur in *Eragrostis curvula*. In *Panicum maximum* the positive response in growth to nitrogen addition exceeded any negative effects from the sulphur addition. The results of plant growth to increasing soil acidity were different between functional groups and within functional groups. A relationship between the increase in the average above and below ground mass was found to exist with a decrease in soil acidity in *Acacia sieberiana* (R^2 = 0.45). A relationship between an increase in the average above and below ground mass with a decrease in soil acidity in *Eragrostis curvula* was also seen (R^2 = 0.31).

Changes to the structure and composition of vegetation in this area will impact land use and the management thereof, impacting land users and owners ability to generate an income and therefore their livelihoods. Changes to vegetation structure and composition will also have a greater overall effect by impacting ecosystem functioning and resilience to future disturbances.

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Chapter 1: Introduction

Rapid increase in human population and the resultant increase in anthropogenic activities have resulted in a profound change in the global environment. These changes have altered the structure of the environment as well as its functioning (Vitousek 1994). One such change in the global environment will ultimately be a change in global biodiversity. There are five pressures which are directly impacting global biodiversity; climate change, land use change, over-exploitation, the introduction of alien species and pollution (Secretariat of the Convention on Biological Diversity 2010; Driver *et al.* 2012). These disturbances result in ecosystem degradation by decreasing biodiversity, lowering primary production and decreasing the systems resilience to natural disturbances (Rapport and Whitford 1999). All ecosystems are unique with differing climates, soils, species composition, species diversity, productivity and nutrient cycling; ultimately varying in their responses to anthropogenic disturbances.

Biodiversity has both intrinsic and utilitarian value, and is important in ensuring ecosystem functioning. Ecosystem functioning is composed of ecosystem properties including the size of compartments and pools, and the rates of processes between these pools, which impact ecosystem goods and services (Hooper *et al.* 2005). Ecosystem goods and services can be categorised into four groups; provisioning, supporting, regulating and cultural. Maintaining the biodiversity of natural ecosystems is important in ensuring the provision of ecosystem services, as well as reducing ecosystems vulnerability to these changes (Millenium Ecosystem Assessment 2005; Secretariat of the Convention on Biological Diversity 2010). Determining the biodiversity of an area is therefore important in determining the biotic properties of ecosystems, their responses, and thereby their vulnerabilities to these changes (Bazzaz 1997; Crawley 1997; Chapin III *et al*. 2002).

South Africa is a biologically diverse country, encompassing a variety of biomes, plant and animal life. South Africa is a developing country, which is highly reliant on a coal-based economy. In May 2007 Eskom, South Africa's largest energy producer who generates 93% of its energy from coal-fired power stations, began building the largest dry-cooled power station in the world, in Lephalale, in the Waterberg, in the Limpopo Province. The building of the 4800 MW Medupi power station is intended to meet South Africa's rapidly increasing energy demands (Eskom 2011). In 2012 the Department of Environmental Affairs (DEA) declared the Waterberg, one of five districts in the Limpopo Province, a priority area as a result of its increasing development, particularly with regards to its vast coal resources. This has resulted in growing concern regarding decreased air quality as a result of these increased activities (DEA 2010). The Waterberg priority area includes the Waterberg district in the Limpopo Province and the Bojanala District in the North West Province. In the Waterberg the Matimba power station has already been in operation since 1987. In addition to the power stations, there is extensive development in the Waterberg area, including open-cast coal mines, road building, formal and informal settlements, construction, and increased volumes of traffic. The operation of the Medupi power station will result in an increase in carbon dioxide (CO_2), nitrogen oxide (NO_x) and sulphur dioxide (SO_2) emissions and an increase in sulphur and nitrogen deposition in the Waterberg area, which will ultimately have an effect on air quality, and an unknown effect on the surrounding ecosystems. Increases in the emissions of sulphur dioxide (SO_2) and nitrogen oxides (NO_x) will result in increases in sulphuric acid and nitric acid being deposited back into the system, impacting and acidifying soils and water sources. The impacts of both wet and dry deposition on vegetation are mostly unknown, particularly regarding indigenous vegetation found in savanna systems in South Africa.

Research areas for this project were identified, using a range of stakeholder meetings, and these areas were prioritised and implementation strategies were put in place. This research project was done in two parts: 1) a biodiversity study and 2) a plant sensitivity study to increased nitrogen and sulphur levels, and increased acidity. The vegetation diversity study took place along a predetermined transect in the Waterberg area, taking into account areas downwind of the operational Matimba power station and under construction Medupi power station, and areas upwind of these. The effects of nitrogen and sulphur deposition on four vegetation species were determined in an experiment in the greenhouse at the University of the Witwatersrand.

The aim, objectives and key questions of this study are as follows:

1.1 Aim, Objectives and key questions

The aim of this research was to determine whether increased ambient levels of atmospheric nitrogen and sulphur, resulting from the power stations, will impact the structure (height and biomass), functional type and the composition (alpha and beta diversity) of the vegetation, and the resultant impacts on vegetation structure and growth as a result of the added nitrogen and sulphur to the system and increased acidity.

The first objective was to create a baseline study of vegetation (trees, grasses and forbs) along selected transects in the Waterberg area. The key questions for this objective were:

1. What are the alpha and beta diversities of the vegetation identified in the area?
2. What functional types are dominant within the area?
3. What is the aboveground standing biomass of the vegetation in the area?

The second objective was to assess the changes in growth pattern of plant species from two functional types, to increasing soil acidity resulting from nitrogen and sulphur deposition. The key questions for this objective were:

1. How will the addition of nitrogen and sulphur, both separately and in combination, affect plant growth?
2. How will increasing soil acidity affect plant growth?

Chapter 2: Literature Review

2.1 Introduction

The current literature relevant to the study is reviewed in this chapter. It is divided into two broad sections. The first section, vegetation diversity and baseline data begins by looking at the area under study, the Waterberg in the Limpopo Province in South Africa. This is followed by reviewing global environmental change, with particular attention to climate change. An overview of biodiversity within South Africa and specifically in savanna biomes in the Waterberg follows and attention is given to the definitions and measurements used to assess this biodiversity. The importance of baseline vegetation data is also reviewed.

The second section looks at soil acidification and nitrogen and sulphur deposition and begins by reviewing past research on the impacts of pollution on plant growth, as well as the role that both water and nutrients play in these impacts. This is followed by a review of biogeochemical processes, initially covering the amount of nitrogen and sulphur found within coal and their atmospheric transformations and then taking a closer look at terrestrial cycling and transformations of both nitrogen and sulphur, and the resulting acidic deposition. The form that deposition can take as well as deposition within South Africa follows. This deposition may have varying impacts on vegetation structure and composition, plant growth, and different ecosystems and this is reviewed next. This is followed by a brief review on the co-deposition of base cations. This section concludes with information on phenotypic elasticity and functional types, to assess the possible impacts of deposition in causing differing responses in C3 trees and C4 grasses.

2.2 Vegetation diversity and baseline data

2.2.1 The Waterberg area

The Waterberg is situated in the Limpopo Province and borders the North West Province, Gauteng Province and Capricorn District Municipality in South Africa, and Botswana. The Waterberg priority area, which includes the Waterberg in the Limpopo Province and the Bojanala District in the

North West Province, can be seen in Figure 1. The Waterberg has a semi-arid climate which is characterised by mildly warm, dry seasons and hot, wet seasons with high inter-annual variation. It has a mean annual rainfall of 421mm, predominantly in summer, and a mean annual temperature of approximately 20°C. The Waterberg is found at an altitude between 1500 – 1800m (Mucina and Rutherford 2006).

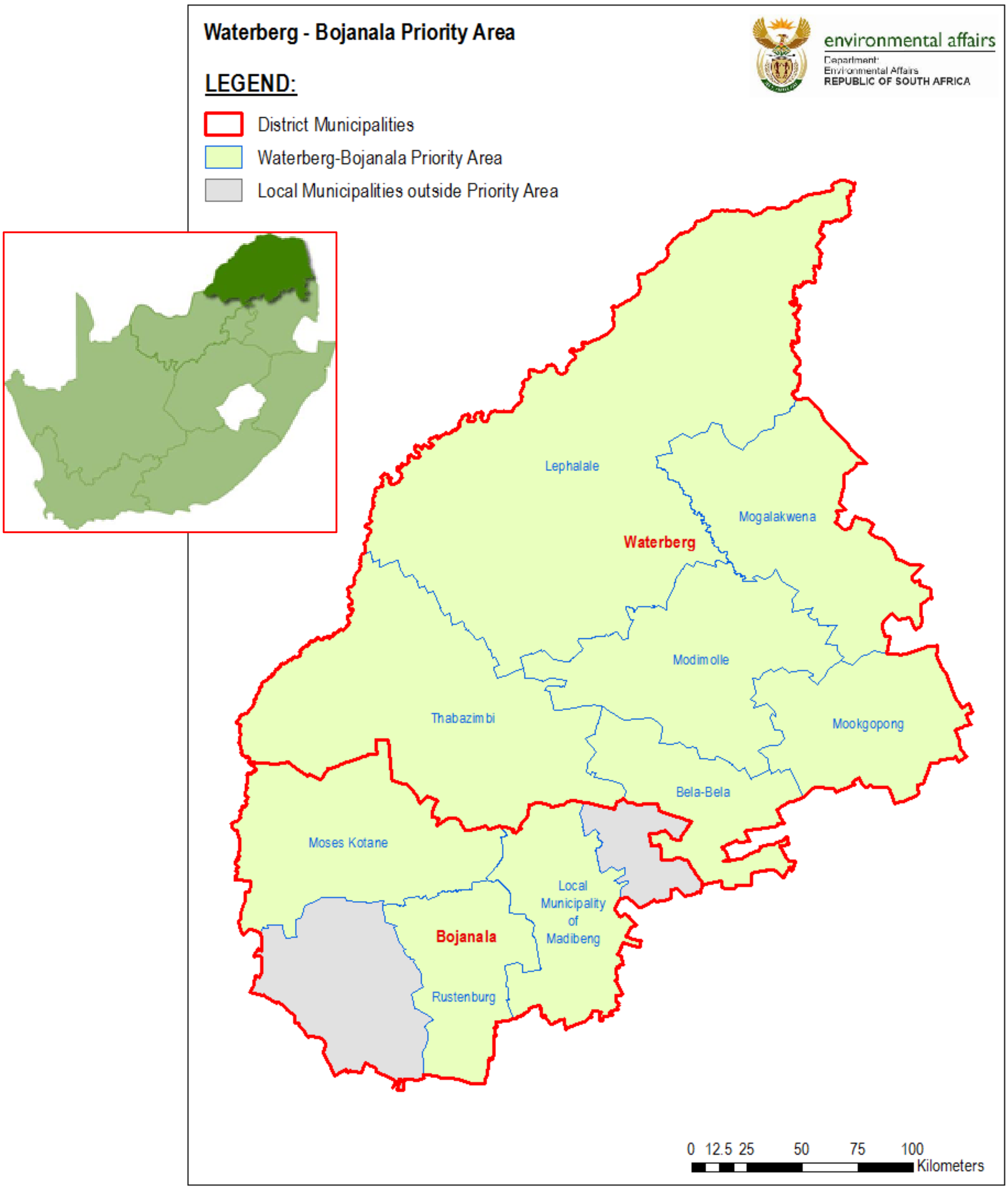


Figure 1. The Waterberg priority area, including the Waterberg district in the Limpopo Province and the Bojanala District in the North West Province (Scott 2012).

Most of the vegetation within the Waterberg area falls within the savanna biome, which is characterised by a discontinuous woody layer and a continuous grassy ground layer. The form and functioning of savanna ecosystems are determined largely by nutrient availability, water availability, and fire and herbivore disturbances (Scholes and Walker 1993). These interactions, and the competitive ability of trees versus grasses in securing these resources, as well as the ecophysiology of trees and grasses, largely determine the grass-tree ratio within this ecosystem (Scholes and Archer 1997). Savanna systems can be divided into nutrient-poor and nutrient-rich savannas. These nutrient-rich and nutrient-poor savannas develop as a result of a number of processes including differences in parent material geology, and cantenal processes which result in landscapes which differ in fertility, with uphill areas being less fertile than areas in downhill positions (Scholes 1990). The nutrient-poor savannas are characterised as having broad-leaved trees, a greater root: shoot ratio, increased woody biomass and a decrease in plant palatability. These savanna systems are dominated by the plant families Combretaceae and Caesalpiniaceae. Nutrient-rich savannas are characterised by decreased leaf size, lower root: shoot ratios, decreased woody biomass and an increase in palatable vegetation. Nutrient-rich savannas are dominated by the family Mimosaceae (Scholes 1990; Mucina and Rutherford 2006).

Protected areas are important in providing a buffering capacity to future disturbances, pollution impacts and climate change (Claasen *et al.* 2010). The amount of land that is statutorily protected within the Waterberg area is shown in Figure 2. In 2001 UNESCO named areas of the Waterberg a biosphere reserve to ensure conservation, sustainable resource use and economic growth within this area. The Waterberg and the Kruger to Canyon Biosphere Reserve are the only savanna biosphere reserves within South Africa. Many of the statutorily protected areas fall within the biosphere reserve with many smaller reserves falling within the core area (Figure 2). There is a small area which is statutorily protected close to Lephalale and the study sites.

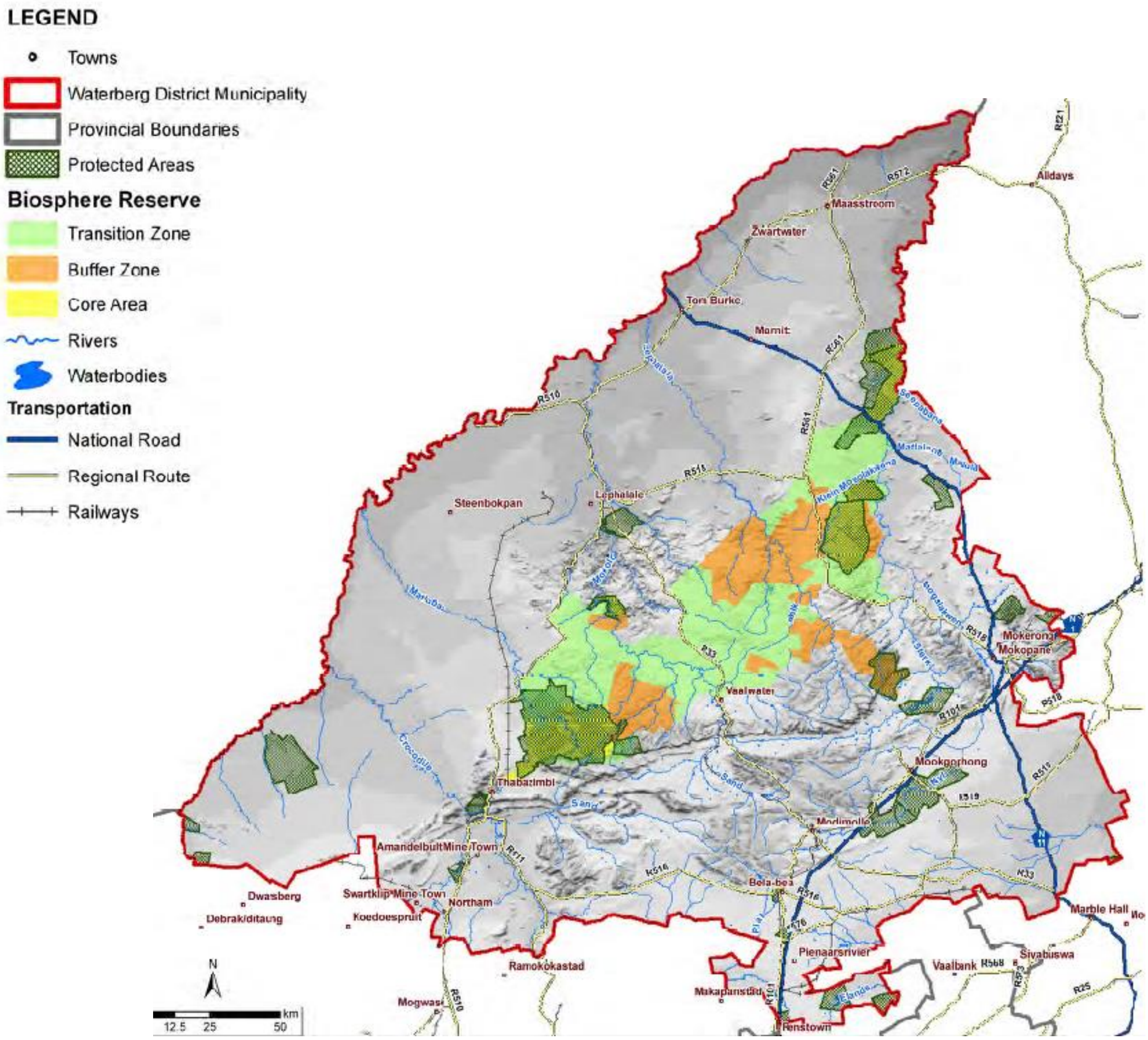


Figure 2. Protected areas found within the Waterberg District Municipality (Claasen *et al.* 2010).

The Waterberg, and in particular Lephalale is rich in mineral resources such as iron, platinum and coal. This area also has an abundance of wildlife. The current land use of the Waterberg includes mining, agriculture, game farming, gaming hunting, eco-tourism, conservation and to a lesser extent cattle farming (Claasen *et al.* 2010).

The current air quality of the Waterberg district is measured in Lephalale, Mogalakwena and Thabazimbi (Mogotlane 2012). Increases in development within the area, as well as adjacent areas in

South Africa, for example between the Waterberg District Municipality and the Bojanala Platinum District Municipality in the North West Province, and neighbouring countries, in particular Botswana, may result in air quality being negatively impacted in the near future. As a result the Waterberg was declared a National Priority Area in 2012, to ensure that air pollution levels remain within national ambient air quality standards (DEA 2012).

Sources of emissions in the Waterberg include the generation of power, industrial emissions such as Hanglip Brickwares and small boiler sources, mining activities including opencast coal mining at Grootgeluk Coal Mine, biomass burning, vehicle emissions, agricultural activities and domestic fuel burning (Mogotlane 2012). The emissions inventory compiled by the Waterberg District Air Quality Management Plan in 2009 shows that Lephalale has the greatest contribution to industrial emissions, particulate matter ($PM_{10\mu m}$), sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) (Table 1). The generation of power contributes to 95% of SO_2 and 93% of NO_2 in the area (WDM 2009).

Table 1. The emissions inventory compiled for the various municipalities in the Waterberg District (WDM 2009), showing relative contributions.

Municipality	**Industrial emissions**	**Domestic fuel burning**	**Vehicle emissions**	**$PM_{10\mu m}$**	**SO_2**	**NO_2**
Lephalale	95.9%	19.1%	24.1%	86.2%	95.4%	94.3%
Bela-Bela	0.0%	48.0%	17.0%	0.4%	0.02%	1.0%
Mookgophong	0.0%	3.5%	6.1%	0.2%	0.01%	0.3%
Thabazimbi	3.6%	10.9%	28.1%	0.8%	4.5%	1.6%
Mogalakwena	0.4%	52.0%	13.2%	11.7%	0.05%	2.2%
Modimolle	0.0%	9.6%	11.4%	0.6%	1.8%	0.6%

Dispersion modelling simulations have shown that SO_2 emissions from Matimba result in ambient standards being exceeded. Unless Medupi is fitted with flue gas desulphurisation (FGD) which removes SO_2 from the power plant, the SO_2 emissions will not be controlled, and this will therefore result in elevated concentrations of SO_2, ensuring that the national standards are further exceeded (WDM 2009). Current studies in the area have predicted that with both Medupi and

Matimba power stations in operation, and excluding any other emission sources, the hourly accepted SO_2 levels will be exceeded (Figure 3) (Feig 2010).

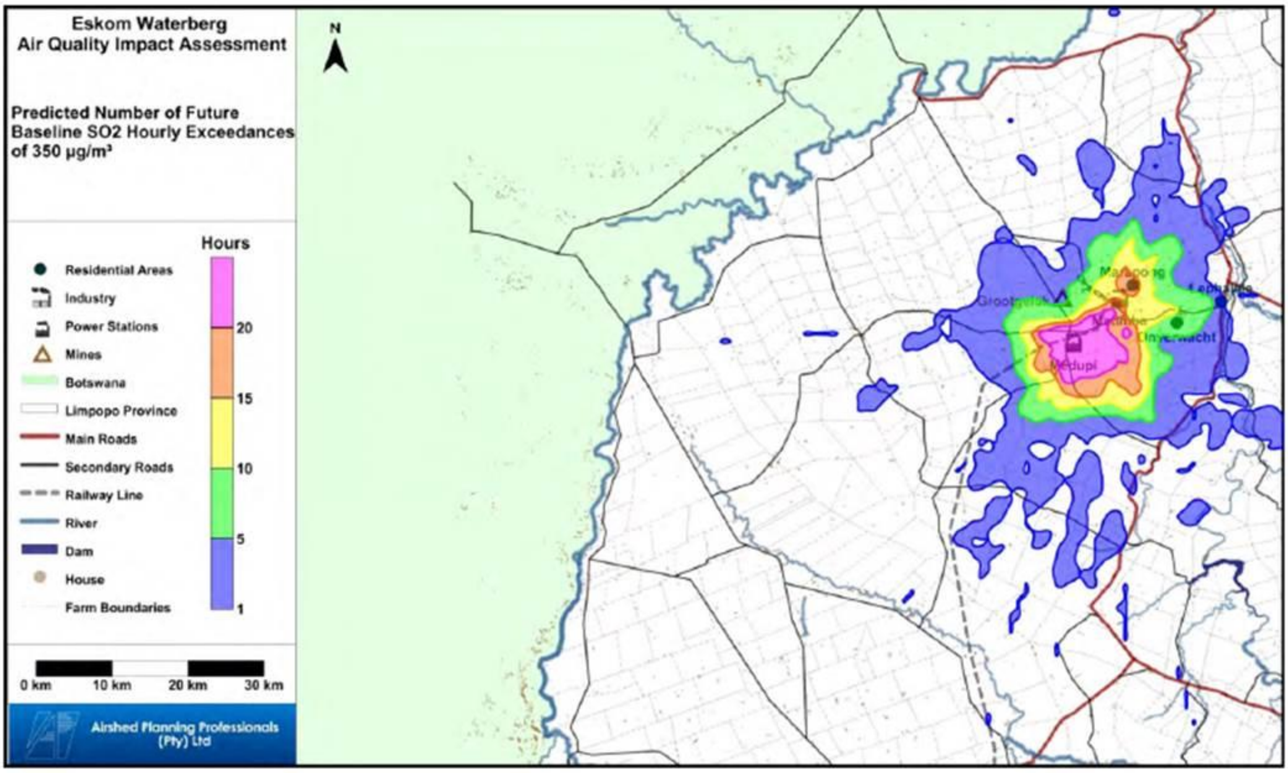


Figure 3. The number of hours per year over which the SO_2 hourly standard, 350 micrograms / m^3, is expected to be exceeded with both Matimba and Medupi power stations in operation (Feig 2010).

The Waterberg area is characterised by soils which originate from Waterberg sandstone and the Karoo Supergroup (Corbett *et al.* 2008). Within the location of this study the soils are predominantly sandy, with low clay content and a low buffering capacity (Cole 1986). This results in the soils reduced ability to absorb additional nutrients and increased acidity. In a study conducted in the Highveld grasslands, Bird (2011) found that soils with a lower clay content showed significant decreases in pH as a result of atmospheric deposition. In previous research conducted in areas of the Waterberg, soil pH levels were found to range from 3.84 to 4.98, depending on the location of the soils in relation to the power stations (Itzkin 2012). Decreases in air quality and possible resultant deposition of nitrogen and sulphur with soil acidification, may have greater impacts in these areas.

Plants which have evolved in these nutrient poor soils have adapted to these inherent nutrient poor and acidic soil conditions. The sensitivity of these plants to both nutrient accumulation and further increases in acidity is unknown; particularly the dominant species found within these areas. Dominant species maintain and exert pressures on community structure and ecosystem functioning (Emery and Gross 2007). The loss of dominant species, those species which are the most abundant or have the greatest biomass, results in a greater loss of productivity than the loss of rare species which Isbell *et al.* (2013) found to have little effect on the productivity of ecosystems. The regulation of ecosystem properties, such as productivity and mineralisation rates, is regulated by these dominant species (Lavorel and Garnier 2002). The loss of keystone species, species that do not necessarily have the largest biomass, but whose presence is essential in maintaining the structure and diversity of an ecosystem, will also impact ecosystem functioning (Mills *et al.*1993). If the dominant species and keystone species found within this landscape are found to be under threat, this will have a far greater impact on the functioning of ecosystems within the Waterberg than the loss of a rare species.

2.2.2 Global environmental change

Global environmental change, mainly as a result of anthropogenic activities, results in climate change, stratospheric ozone depletion, changes in biogeochemical cycles, and changes within ecosystems, as a result of biodiversity loss (Chapin III *et al.* 2002).

Globally there has been an increase in mean temperatures over the last century and this has been attributed to anthropogenic greenhouse gas emissions (MacKeller *et al.* 2014).

The future impacts of climate change in South Africa include; increases of 3 - 6°C over the interior by 2100, changes in rainfall patterns which will in turn have an impact on water availability, and increases in extreme weather events (Niang *et al.* 2014). Research has shown that in the past, minimum temperatures have increased relatively more than increases in maximum temperatures (New *et al.* 2006). Delays in seasonal rains are also expected in the future, and precipitation is expected to increase in daily intensity followed by increases in the frequency of droughts, which will have implications for soil run-off (Niang *et al.* 2014).

Climate change poses a significant risk for South Africa’s water availability, the health of its population, food security, ecosystem services and biodiversity. These changes in climate will have an impact on terrestrial ecosystems and are likely to result in biome shifts. In savanna and grassland biomes a net increase in the woody vegetation has been seen as a result of increased carbon dioxide (CO_2) concentrations, increases in nitrogen deposition and increasing temperatures. These drivers are all responsible for increases in woody biomass, with these increases being dependent on which of these three drivers are prevalent within an area at a certain time (Wigley *et al.* 2010; Buitenwerf *et al.* 2012).

In the Limpopo Province climatic trends show that over the past 50 years there have been decreases in the number of rain days a year, with there being a decrease of 16 rain days in a 50 year period. There have also been increases in maximum temperatures during winter months and an approximate increase in mean maximum temperatures of 1°C over a 50 year period. The Limpopo Province has also seen increases in minimum temperatures in winter and summer months, with a 0.011°C increase in the minimum temperatures annual mean. Seasonal rainfall has also been found to occur later in the year and is accompanied by an increased dry spell (MacKeller *et al.* 2014).

2.2.3 Biodiversity in South Africa

South Africa is a biologically diverse country; with nine biomes, ranging from deserts to grasslands, over 10% of the world's plant population, 24 000 plant species, high levels of endemism and genetic diversity, and three globally recognised biodiversity hotspots (Driver *et al.* 2012). An ecosystem assessment was conducted by both the South African National Biodiversity Institute (SANBI) and the Department of Environmental Affairs and Tourism (DEAT) in 2004, where it was found that of South Africa's terrestrial ecosystems, 34% were threatened, with 13% being endangered, most of which include savanna and grassland biomes (DEAT 2005).

In South Africa the National Environmental Management: Biodiversity Act (NEMBA) (Act 10 of 2004) became effective as of the 1 September 2004. Chapter 4, Part 1 of the Biodiversity Act (NEMBA 2004) refers to the protection of threatened or protected ecosystems. The third category refers to vulnerable ecosystems, which are explained as being ecosystems that are not endangered or critically endangered, but are, as a result of human intervention, in danger of changing in form, function and composition (DEA 2004). In 2011 SANBI, according to NEMBA, identified and compiled a list of natural terrestrial ecosystems that were threatened and therefore in need of protection. From this list 9.5% of South Africa's remaining natural terrestrial ecosystems are deemed to be threatened, 5.4% of South Africa's threatened ecosystems fall within the Limpopo Province (Table 2) (DEA 2011).

Table 2. The amount and percentage of natural terrestrial ecosystems under threat in South Africa in total and in the Limpopo Province (DEA 2011).

	Critical (CR)		**Endangered (EN)**		**Vulnerable (VU)**		**Total**	
	000 ha	%	000 ha	%	000 ha	%	000 ha	%
Limpopo	9	0.1	123	1.0	536	4.3	668	5.4
South Africa	903	0.7	2392	2.0	8252	6.8	11547	9.5

Of South Africa's land surface only 6.5% is nationally and provincially protected (Driver *et al.* 2012). According to the Protected Areas Act 2003, protected areas in South Africa are formally protected by the law mainly for the conservation of biodiversity. These include nature reserves and protected environments, world heritage sites, specially protected forested areas and mountain catchment areas. These areas do not have to be state owned, and can be private or communal land whereby the owner is recognised as the managing authority (Driver *et al*. 2012). The savanna biome is the largest biome within South Africa, with 32.6% of the total area of the country, of which only 8.9% is formally protected (Table 3) (DEAT 2006). Within biomes that are seemingly well protected, there may be discrepancies between ecosystem types. For example arid savannas are well protected by the Kgalagadi Transfrontier Conservation Area, and Lowveld savannas are well protected as a result of the Kruger National Park, but central savannas found in Central and Western Limpopo are less protected (Driver *et al.* 2012). In many areas throughout South Africa the savanna biome is being protected informally by the conversion of farmlands to private game reserves and conservation areas, and are managed and protected by the current owners of these properties. This form of protection within various areas in South Africa is not formally protected by the law and therefore these lands are still vulnerable (Driver *et al.* 2012).

Table 3. The types of biomes found within South Africa and their formal protection (DEAT 2006).

Biome	Area (km^2)	% of total area of the country	% remaining	% protected
Desert	8 548	0.7	93.4	12.5
Succulent Karoo	85 207	6.7	96.5	3.1
Fynbos	84 580	6.7	70.2	11
Nama Karoo	250 069	19.7	98.4	0.6
Grassland	373 984	29.5	70.8	1.9
Savanna	412 753	32.6	86.1	8.9
Albany Thicket	30 256	2.4	91.9	6.3
Forest	4 730	0.4	94.7	39.6
Woodland	16 790	1.3	92.1	4.6

2.2.4 Definitions and measurements of biodiversity

Biological diversity, more commonly known as biodiversity, refers to the variety between living organisms, from the level of genes to ecosystems, groups of living organisms and biotic processes (Noss 1990). The Convention on Biological Diversity was established in 1992 at the Earth Summit in Rio de Janeiro with three main goals; the conservation of biodiversity, the sustainable use of this biodiversity, and equitable sharing of the gains from these genetic resources (Secretariat of the Convention on Biological Diversity 2010). The conservation of biodiversity requires natural resource management, and this in turn requires quantitative baseline measurements of these resources in order for future comparisons to be made (Swingland 2013).

Different components of biodiversity are measured and described by diversity indexes. In this research three different types of alpha, as well as beta diversity indexes were used. The first alpha diversity index was species richness which describes the number of different species present within a site, but does not take into account species proportion or distribution. Secondly, species diversity was used, and in this case the Shannon diversity index, whereby the proportion of species relative to the total number of species is calculated, and then multiplied by the natural logarithm of this proportion, in order for species abundance within a site to be calculated (Magurran 2004; Swingland 2013). Past research shows that areas with greater species diversity and higher productivity were more susceptible to the impacts of pollution, as competition between species increases in an already competitive environment (Zvereva *et al.* 2008; Zvereva and Kozlov 2012). This is in contrast with research by Chapin III *et al.* (1997) whereby greater species diversity resulted in increased ecosystem stability and research by Loreau *et al.* (2001) where high species diversity is important for maintaining ecosystem processes in changing environments. Lastly species evenness was calculated to determine the relative abundance of different species within a site, with sites that have a more equal abundance of species showing greater diversity. Pielou's evenness index (J) was used, which uses the Shannon diversity index divided by the total number of species within the site (Magurran 2004; Swingland 2013).

Beta diversity shows the changes in species diversity between sites at a local level, with the emphasis on the degree to which one species is replaced by another. The Whittaker index is used to calculate beta diversity, with lower beta diversity indices showing higher similarity between sites, and higher beta diversity indices showing greater species variation between sites (Whittaker 1972; Trubina and Vorobeichik 2012; Swingland 2013). It has been found that the beta diversity between areas increases as air pollution levels increase, as it has been found that increased pollution levels result in a decrease in populations of species rather than eliminating them (Trubina and Vorobeichik 2012). The abundance of species within a site is another important measurement and was determined by grass and forb biomass, as well as tree density in order to observe trends over time (Magurran 2004).

Past research by Solbrig *et al*. (1996) found that savanna biomes have higher species diversity than biomes such as grasslands and woodlands. The average number of species found in savannas in South Africa is 67 species per 0.1 ha, however this is variable and that the number of species increases as the mean annual precipitation increases, and can fluctuate from 50 to 100 species (Cowling *et al*. 1989; Shakelton 2000).

2.2.5 Phenotypic elasticity and functional types

Phenotypic elasticity can be defined as the ability of genotypes to express different phenotypes with a changing environment (Bazzaz 1997). This phenotypic elasticity can present itself during a lifetime, or over generations in phenology, behaviour, growth patterns, physiology and life patterns (Miner *et al*. 2005). Functional types are groups of species categorised together based on their function, which vary in their functional traits along environmental gradients (Nicotra *et al*. 2010). Plant functional types are useful as a concept to allow a prediction of the future nature and function of species assemblages in ecosystems with changing environmental conditions, with functional groups assumed to react in the same manner to these changes (Lavorel and Garnier 2002).

Three different photosynthetic pathways are found in vegetation – C3 photosynthesis, C4 photosynthesis and Crassulacean Acid Metabolism (CAM), all of which are examples of functional types (Díaz and Cabido 1997). The C3 photosynthetic pathway gets its name from the initial product of carbon fixation, which is a 3-carbon compound (phosphoglyceric acid, PGA). The C4 photosynthetic pathway differs from the C3 pathway in its initial carboxylating enzyme (phosphoenolpyruvate (PEP) carboxylase) and initial product being a 4-carbon acid. In the CAM photosynthetic pathway stomata open at night, whereby carbon dioxide (CO_2) combines with PEP carboxylase and forms malate, which is stored in vacuoles to be used during the day (Chapin *et al.* 2002). Globally C3 photosynthetic vegetation is dominant. C4 plants evolved under low levels of carbon dioxide (CO_2), and C4 photosynthesis is characteristic of vegetation that is adapted to high light, and arid to moist environments, where trees are not the dominant vegetation. CAM photosynthesis allows certain plants to gain carbon under extremely dry, high light conditions (Chapin *et al.* 2002).

In C4 photosynthetic vegetation a decrease in water loss and a high efficiency of Ribulose-1,5-bisphosphate carboxylase/ oxygenase, more commonly known as Rubisco is seen, therefore less nitrogen is needed (Chapin III *et al*. 2002). Increases in CO_2, increase the photosynthetic rates of plants with C3 photosynthetic pathways, particularly when other nutrients are not limiting. A plant's photosynthetic capacity is positively correlated to nitrogen concentration with Rubisco accounting for 25% of the nitrogen found in the leaves (Vitousek 1994; Chapin III *et al*. 2002).

It has been found in long-term studies in Minnesota grassland plots by Wedin and Tilman (1996) that nitrogen enrichment resulted in a decrease in native, dominant C4 grasses and eventually the loss of these, whereas nitrogen enrichment increased the dominance of non-native, less dominant C3 grasses. The elevation of CO_2 in these same plots over periods of time resulted in no change to plant diversity but an increase in productivity (Isbell *et al.* 2013). Different responses are expected in the Waterberg as the grasses are C4 grasses rather than C3 grasses, growing on nutrient poor substrates.

Species that are functionally similar and have diversity within their functional group, yet respond differently to environmental disturbances, have a greater resilience to environmental change. However species that come from different functional groups and respond differently to environmental disturbances can make ecosystems more vulnerable to change. This may result in a shift of competition, causing an imbalance between the functional groups, ultimately resulting in a change from one biome to another (Chapin III *et al.*1997). The substitution of one functional group for another will alter ecosystem properties and ultimately ecosystem resilience to future impacts (Loreau *et al.* 2001).

With the expected increase in the levels of both nitrogen and sulphur deposition, and the resulting acidification of the soils as a result of the operation of power stations in the Waterberg area, it is expected that there will be an impact on the vegetation diversity. Baseline data of the current vegetation in the Waterberg area needs to be assessed for future studies. Predictions of what these impacts will be and the magnitude of these impacts to the vulnerability of the plants through physiological mechanisms will be determined experimentally.

2.2.6 The importance of baseline data

Baseline studies require that quantifiable data are collected, to assess whether biodiversity is changing and to allow the rate of change to be measured. In order for this to occur, a state of reference is needed for future comparisons to be made (Vackar *et al.* 2012).

This will enable long-term monitoring of selected sites, whereby comparisons can be made between the present data, where Matimba is only operational, and future data, where both Matimba and Medupi will be operational, thus allowing for the evaluation of the impacts of the presently expanding industrial activity within the Waterberg area.

2.3 Soil acidification and nitrogen and sulphur deposition

2.3.1 The effects of pollution on plant growth

The degree to which air pollution impacts the ecosystem is dependent on a number of factors, namely; the source of the pollutant and its aerial distribution, biome type, plant structure within the biome, plant functional groups and the climatic conditions (Zvereva and Kozlov 2012).

Previous studies have found that herbaceous and woody plants that grow near pollution points differ in their responses. A pattern has been seen whereby vegetation that is larger in size is affected before smaller vegetation to pollution induced changes, with trees being affected first, then taller shrubs and finally smaller shrubs and herbs (Zvereva and Kozlov 2012). Abundance in woody plants was found to decrease to a greater extent than the growth of herbaceous plants, mainly as a result of air-borne pollutants rather than the availability of soil nutrients (Zvereva and Kozlov 2012). In herbaceous plants the above ground mass was found to remain the same as a decrease in leaf size was offset by an increase in the number of leaves, but there was a decrease in the below ground mass. The difference in the response of woody plants and herbaceous plants to pollution was found to be a result of the positioning of the buds (Zvereva *et al.* 2010). Tree buds are positioned at the growing tips of the branches making trees more vulnerable to the exposure of environmental stressors; pollution induced as well as natural stressors such as drought and frost (Rapport *et al.* 1985). Tree abundance was found to decrease in soils that increased in either acidity or alkalinity, whereas the abundance of shrubs and herbs were found to only decrease in more acidic soils (Zvereva and Kozlov 2012). Past research has found that grasses under air pollution did not change in abundance to increasing soil acidity or alkalinity (Zvereva and Kozlov 2012). This may be as a result of a number of factors including; the smaller size of grasses including their growth form, a greater number of grasses being acid tolerant, many grasses being pioneer species and therefore benefitting from a decline in competition with other plants, and the ability of grasses to rapidly develop a tolerance to increases in heavy metals (Zvereva *et al.* 2008).

Pollution effects are increased in areas with higher temperatures and increased precipitation, as this enhances the mobility of pollutants into the soil and therefore their toxicity, as well as gaseous pollution uptake (Zvereva *et al.* 2010). Therefore as climate changes, existing pollution loads will become even more harmful to ecosystems, and the dangers of future pollution will be increased. Precipitation within the study area is low and variable, resulting in dry deposition having more of an impact in this area.

2.3.2 The importance of water and nitrogen in plant functioning

Water has numerous essential roles in the functioning of plants, as a result of both its chemical and physical properties. The most important of these is that water is the universal solvent in which charged ions and molecules dissolve. Water is also the medium in which biological reactions take place and biological molecules are transported (Hopkins and Hüner 2009). Water controls the duration of processes such as growth, nitrogen mineralisation, carbon assimilation and the uptake of other nutrients, which are all essential determinants in the productivity of ecosystems, particularly savanna systems (Scholes and Walker 1993).

The rainfall within the study site is low and variable, between 400 mm – 600 mm a year (Claasen *et al.* 2010). This variability is shown in the rainfall patterns between the months of March 2013 and March 2014 (Figure 4).

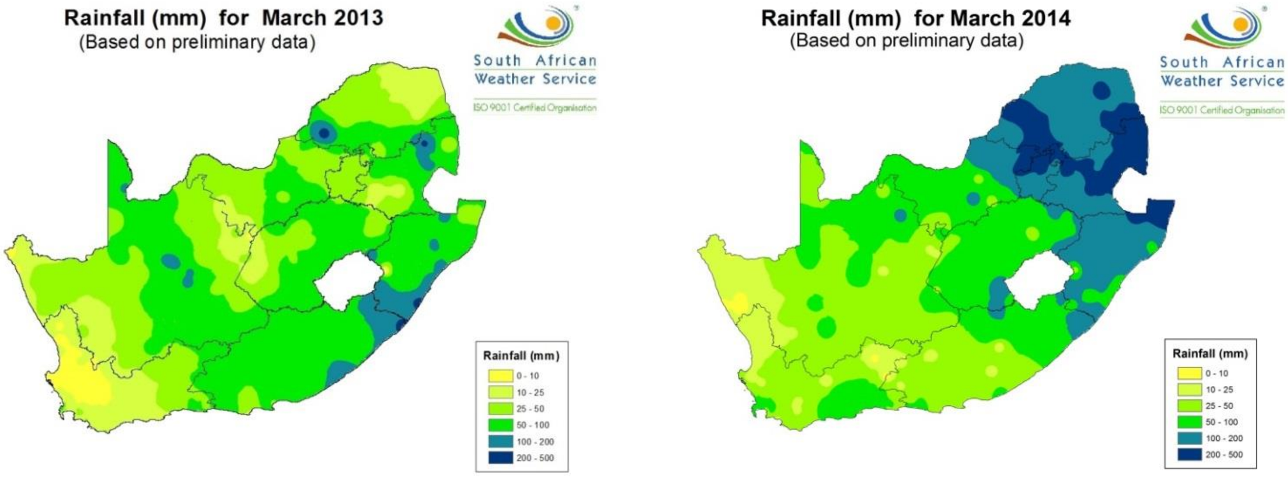


Figure 4. The differences between rainfall (mm) in March 2013 and March 2014 (South African Weather Service 2014).

Extreme weather events, such as droughts and floods, increase plant tolerance to pollution by resulting in stomatal closure which restricts pollution uptake (Zvereva *et al.* 2010).

The availability of nutrients in an ecosystem also plays a large role in determining vegetation composition, with nitrogen often being the limiting nutrient (Vitousek 1994; Bobbink *et al*. 1998).

2.3.3 Biogeochemical processes

Biogeochemical processes include the flux and flow of nutrients within the ecosystem. This study focuses on the cycling of nitrogen and sulphur.

2.3.3.1 Nitrogen and sulphur in coal and their transformations in the atmosphere

Coal is a resource that is abundant in certain areas in South Africa, and is burnt by Eskom to generate approximately 93% of South Africa's primary energy. This remains South Africa's cheapest source from which electricity is generated. Coal is mainly composed of carbon, nitrogen, sulphur and ash (particulates). During the combustion of coal, carbon dioxide, sulphur dioxide, nitrogen oxides and mercury compounds are released.

The sulphur dioxide and nitrogen oxides react with water vapour in the atmosphere and form sulphuric and nitric acid, which is more commonly known as acid rain, which poses an environmental threat far from the actual source of emission. Rainfall data collected at Louis Trichardt, an area north-east of the study sites, over the past 20 years, shows that the average pH of rainfall in the area is 4.9 (Mphepya *et al*. 2004).

According to Eskom (2014) their coal-fired power stations emit between 200 – 250 Mt $yr^{-1}$of carbon dioxide (CO_2). They also emit between 1800 – 1860 kT yr^{-1} of sulphur dioxide (SO_2) and 900 – 1000 kT $yr^{-1}$of nitrogen oxide (NO_x). The ratio of SO_2: NO_x is therefore approximately 2:1 (Eskom 2014).

2.3.3.2 The nitrogen cycle

The atmosphere has the largest pool of nitrogen (N) in the form of dinitrogen (N_2) (3.9 – 4 x 10^9 Tg N) (Reeburg 1997). Dinitrogen is transformed to biologically available N through the processes of either nitrogen fixation (90 – 130 Tg N yr^{-1}) or lightning (3 – 5 Tg N yr^{-1}) (Galloway 1995). The terrestrial pool of N is approximately 3.5 x 10^4 Tg N (Reeburg 1997). Plants take up N in the form of either ammonium (NH_4^+) or nitrate (NO_3^-) through microbial processes whereby organic matter is broken down into ammonia (NH_3) and ammonia to nitrate. Immobilisation is another process whereby microbes access inorganic N through the decomposition of dead material (Chapin III *et al.* 2002). The processes of volatilisation and denitrification result in fluxes of ammonia (NH_3), and nitrous oxides (NO_x) or nitrogen dioxide (NO_2) respectively, leaving the terrestrial system (Scholes and Walker 1993). Annual fires are another flux whereby N is lost from terrestrial systems in the form of either N_2 or nitrous oxide (N_2O); however the amount of N lost is dependent on the temperature of the fire (Scholes and Walker 1993). Nitrous oxides are an important greenhouse gas, and destroy stratospheric ozone (Scholes and Walker 1993; Schlesinger 2009). The average global estimate flux of N_2O is approximately 4 Tg N yr^{-1}(Schlesinger 2009). The transformations and cycling of N is shown in a simplified diagram in Figure 5.

Anthropogenic activities have almost doubled the amount of N entering the global N terrestrial cycle (150 Tg N yr^{-1}) (Schlesinger 2009). Increases in energy production, increases in fertiliser production, through the Haber-Bosch process, and the cultivation of nitrogen fixing crops are responsible for these increases in N (Chapin III *et al.* 2002; Galloway *et al.* 2004; Schlesinger 2009). Emissions of NO_x, an important component of acid rain, have resulted in a flux of 21 – 25 Tg NO_x y^{-1}, as a result of fossil fuel burning (Hewitt 2001). By 2050 it is predicted that anthropogenic activities will result in 270 Tg N yr^{-1} of N entering the global N cycle.

Nitrogen saturation results when the amount of available nitrates and ammonium is greater than the needs of both plants and soil microbes (Grantz *et al.* 2003; Scholes *et al.* 2003). This results in both NH_4^+ and NO_3^- being susceptible to leaching, particularly NO_3^- , as a result of its negative charge. Decreases in soil fertility occur due to the resultant leaching of cations. This results in increased concentrations of N in streams and groundwater, resulting in eutrophication of these systems, and a decrease in water quality (Grantz *et al.* 2003; Galloway *et al.* 2004).

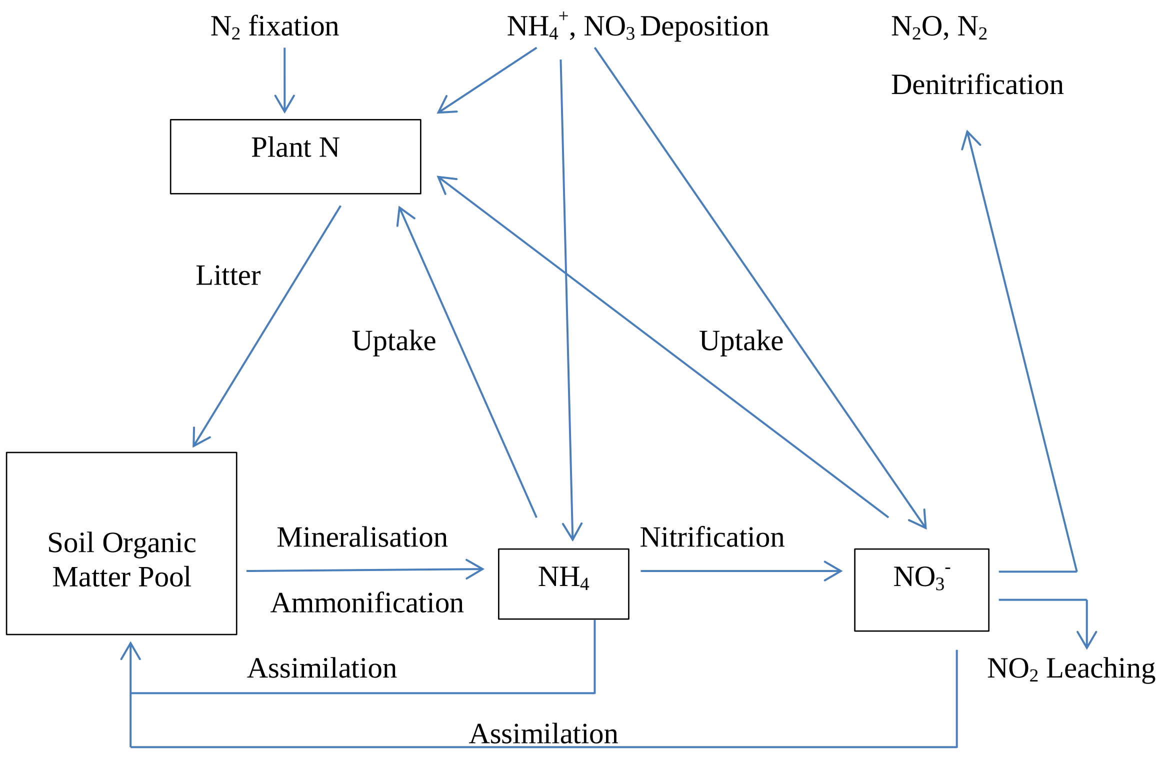


Figure 5. A simplified diagram showing the transformations of N in the terrestrial N cycle (reproduced from Nadelhoffer and Fry 1994).

2.3.3.3 The sulphur cycle

Sulphur (S) is released naturally into the atmosphere through a number of processes including volcanic eruptions (10 Tg S yr^{-1}), biogenic processes (2.5 Tg S yr^{-1}) and terrestrial dust (20 Tg S yr^{-1}). The vast majority of S however is released through anthropogenic processes, mainly fossil fuel emissions (93 Tg S yr^{-1}) (Reeburg 1997). The S cycle is similar to the N cycle, with exchanges occurring between forms of organic and inorganic forms of S by the processes of mineralisation, immobilisation, and oxidation and reduction processes (Figure 6) (Edwards 1998).

At least 90% of S released during the burning of fossil fuels becomes sulphur dioxide (SO_2) (65 Tg SO_2 yr^{-1}) (Hewitt 2001). Emissions of SO_2 results in acid rain, with areas downwind from the source being impacted the most, as well as increases in the concentration of sulphate aerosols (Vitousek 1994).

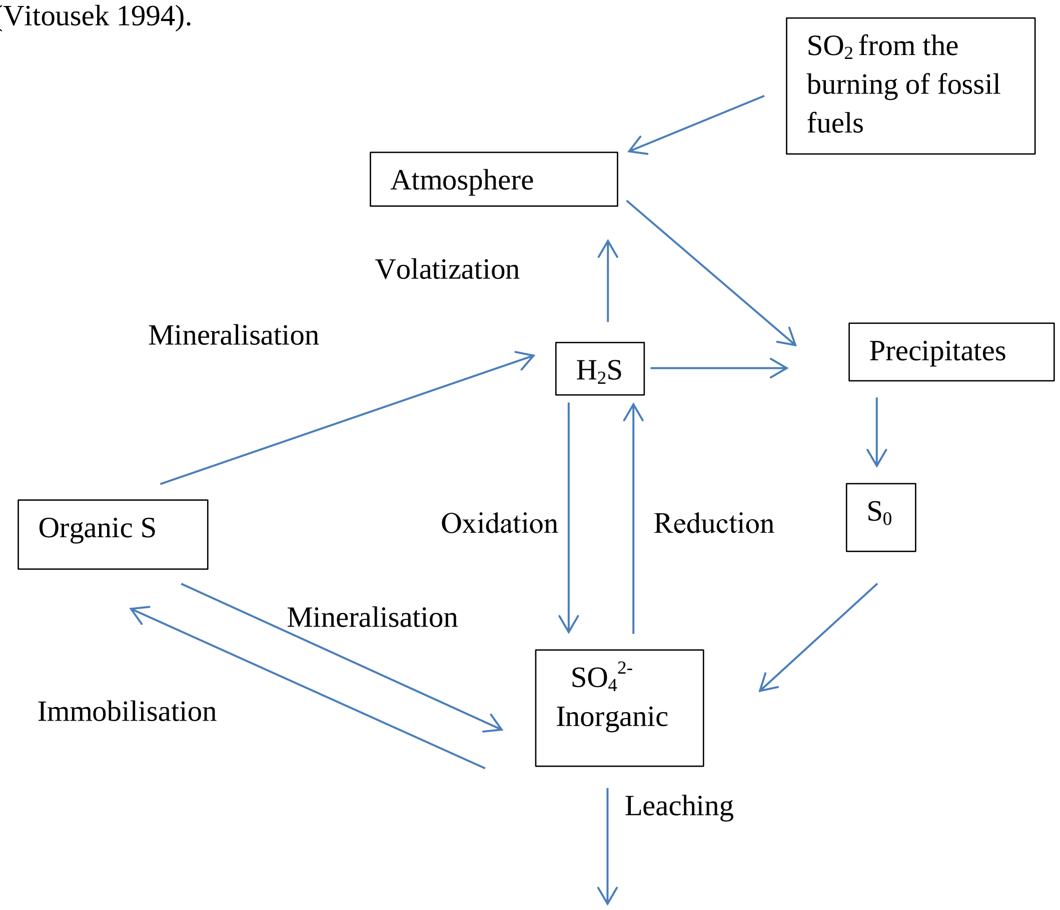


Figure 6. A simplified, schematic diagram representing the processes of the terrestrial S cycle (reproduced from Edwards 1998).

2.3.4 Nitrogen and sulphur deposition

Nitrogen and sulphur deposition occurs by three processes:

- Wet deposition results when particles are dissolved within precipitation, and is a function of the concentration of the ambient pollutants and the amount of precipitation.
- Dry deposition occurs when particulates are delivered onto all surfaces by either dust or aerosols, and occurs closer to emission sources.
- Cloud-water deposition occurs when gaseous pollutants are dissolved in water droplets of fog or mist (Chapin III *et al.* 2002; Grantz *et al.* 2003).

A large proportion of dry deposition is found to accumulate in arid ecosystems, whereas greater amounts of wet deposition are found in areas with higher rainfall (Chapin III *et al.* 2002). It is therefore expected that in the study site dry deposition will be more prevalent and a greater risk than wet deposition.

Pollution from nutrients, in this case nitrogen and sulphur, results in a global threat to the biodiversity of ecosystems. Both nitrogen and sulphur deposition results in an increase in the acidity of soil, resulting in an increase in toxic heavy metals, decreased buffering ability, the leaching of essential cations from the soil and the inhibition of root growth (Marschner 1995; Bobbink *et al.* 1998; Grantz *et al.* 2003).

2.3.5 Deposition in South Africa

The acidification of ecosystems resulting from atmospheric pollution is a concern for developing countries. The experiences of developed countries show that damage to vegetation downwind from coal-fired power stations and other pollution sources, resulting from acid rain, could be repeated in developing countries as pollution emissions increase in the future (Josipovic *et al.* 2010). Atmospheric pollution is removed from the atmosphere by three pathways including; chemical transformations, wet deposition and dry deposition (Josipovic *et al.* 2011).

The Mpumalanga Highveld has a large concentration of coal reserves and subsequently has nine coal-fired power stations in the area. Josipovic *et al.* 2010 found that areas centrally located around the industrial Highveld and downwind from the pollution sources had the highest concentration of total acidic deposition, whereas background areas and those upwind from pollution sources had lower concentrations of total acidic deposition. Peak sulphur deposition has been calculated at 30kg S ha^{-1} yr^{-1}, lower than the historical estimates of 50 – 80kg S ha^{-1} yr^{-1}. Over the greater Highveld area the wet and dry deposition was calculated to be > 8kg S ha^{-1} yr^{-1}. Maximum sulphur rates in this area have increased from 5kg ha^{-1} yr^{-1} in 1948 to > 35kg ha^{-1} yr^{-1} in 2007, whereas sulphur deposition is predicted to be approximately 1kg S ha^{-1} yr^{-1} in background areas within South Africa (Blight *et al.* 2009). Nitrogen deposition was calculated to be between 6.7kg ha^{-1} yr^{-1} and 13.1kg ha^{-1} yr^{-1} (Collett *et al.* 2010). Nitrogen rates have increased from 0.5kg ha^{-1} yr^{-1} in 1948 to > 15kg ha^{-1} yr^{-1} in 2007 (Blight *et al.* 2009).

Sulphur deposition in Nylsvley, which is upwind from emission sources, is estimated to be 5.7kg S ha^{-1} yr^{-1} for wet deposition and 8.2kg S ha^{-1} yr^{-1} for dry deposition (Scholes *et al.* 2003). The nitrogen deposition for savanna systems in Nylsvley and Zimbabwe are estimated to be between 1.66 – 4.01kg N ha^{-1} yr^{-1} (Scholes *et al.* 2003). The nitrogen deposition in the grasslands in Sabie, Mpumalanga, was calculated to be 25kg N ha^{-1} yr^{-1}, and in the forested areas 71.2kg N ha^{-1} yr^{-1}. The difference in the calculated depositions were a result of an increase in dry deposition in the forested areas resulting from rougher surfaces and an increase in cloud-water deposition as a result of a greater efficiency in capturing more mist and fog than the grasslands (Lowman 2003). The nitrogen deposition calculated for a semi-arid savanna in Skukuza in the Kruger National Park, which is downwind from emission sources in Mpumalanga, was calculated to be 21.6kg N ha^{-1} yr^{-1} (Woghiren 2002).

The only deposition rate that has been calculated in the Waterberg is in Thabazimbi where the combined acidic deposition of both nitrogen and sulphur was estimated to be 5.30kg ha^{-1} kg^{-1} over a two year period, between 2005 and 2007 (Josipovic *et al.* 2011). The deposition of nitrogen and sulphur for the entire Waterberg area, and in particular Lephalale, is currently unknown.

2.3.6 The impacts of deposition on vegetation diversity and growth

Initially increases in nitrogen may improve plant growth, however this will only be for a period, as other resources become limiting. The addition of nitrogen has the greatest impact on nutrient poor environments, resulting in species which benefit from the additional nitrogen, out-competing those species that do not benefit (Secretariat of the Convention on Biological Diversity 2010). Species diversity within an area may actually increase, due to an increase in alien species with increases in nitrogen, however the species diversity initially found within an area will be lost (Bobbink *et al*. 1998).

Increases in nitrogen concentrations within vegetation tissue, resulting in increases in the content of amino acids, increases the vegetation's susceptibility to pests, and changes in vegetation development and physiology may influence the vegetation's vulnerability to frost, drought, and pathogens (Marschner 1995; Crawley 1997; Bobbink *et al*. 1998).

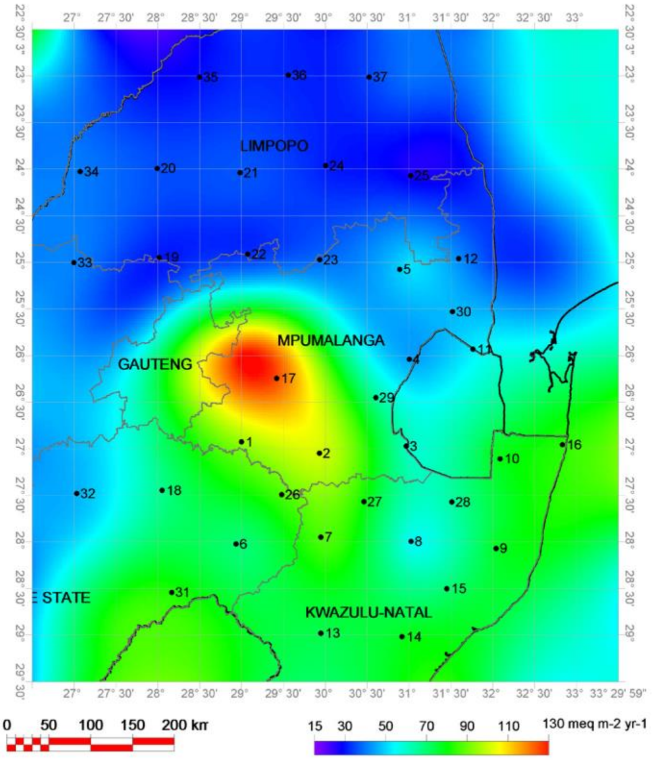
Nitrogen and sulphur deposition result in the acidification of soils. Vegetation that is resistant to acidic soils will be able to out-compete other species which are less resistant, thereby changing the species composition of an ecosystem (Bobbink *et al*. 1998).

2.3.7 The effects of nitrogen and sulphur deposition on different ecosystems

Much research about the effects of nitrogen and sulphur deposition has occurred in grasslands where a loss of species diversity has been seen as a result of faster growing species, these often being C3 grasses, being able to out-compete other native C4 grasses in nitrogen rich conditions (Bobbink *et al.* 2010; Porter *et al.* 2012). The critical load of nitrogen, whereby effects are seen in vegetation species in semi-natural grasslands, has been found to be between 15-20kg N ha^{-1} y^{-1} (Bobbink *et al.* 2010). In a study by Stevens *et al.* (2004), in acidic grasslands in the United Kingdom, it was found that an increase of 2.5kg N ha^{-1} y^{-1} resulted in the loss of one vegetation species (Stevens *et al.* 2004). Kraaij and Ward (2006) found that the addition of nitrogen to savannas in the Northern Cape resulted in an increase in the growth of C4 grasses which in turn had a negative effect on the growth and establishment of tree seedlings. This may have occurred as grass growth increased, a reduction in another resource needed for tree growth, such as space, was evident, or that even with the increased nitrogen, another element needed for additional tree growth was still limiting (Kraaij and Ward 2006). In 1988 the Convention on Long-range Transboundary Air Pollution developed the critical load concept which refers to an estimated quantity of a pollutant or pollutants whereby exposure below these quantities does not result in any harmful effects to sensitive elements of the environment (Nilsson and Grennfelt 1988). The critical threshold for damage is unknown in our environments especially in the selected site.

Acidic deposition impact on the environment is dependent on the interaction between the amount of deposition and the sensitivity of the ecosystem, and therefore the vulnerability of changes in structure and function of these ecosystems (Kuylenstierna *et al.*1995). Research conducted by Josipovic *et al.* (2011) focused on assessing the sensitivity of areas according to their soil attributes and the total estimated amount of acidic deposition in these areas, with critical loads being exceeded once the soils buffering capacity to increased acidity is saturated. Figure 7 shows the total estimated deposition rates in an area within South Africa. The highest deposition rates are in and surrounding Mpumalanga, as a result of the operation of the nine power stations in that area. Figure 8 represents ecosystems that are at risk as the net acidic deposition exceeds critical loads. Net acidic deposition takes into account both the total wet and dry acidic deposition minus the total wet and dry base cation deposition. The ecosystems at risk are predominantly grasslands in the Mpumalanga area (Figure 8). Acidification of these grasslands and changes within the terrestrial and aquatic ecosystems has already occurred or is currently happening.

The grey areas in Figure 8 represent areas in which no critical loads are exceeded (Josipovic *et al.* 2011). Presently ecosystems within the Limpopo fall within the grey areas within Figure 8 and are not at risk of exceeding the critical load. However this may actually be because no previous measurements have been taken within this region and all previous studies have focussed on the Vaal Triangle and Mpumalanga. Bird (2011) found that there was an increase in soil acidity in the Highveld grasslands as a result of atmospheric deposition, particularly in areas with low clay content and therefore a low buffering capacity.



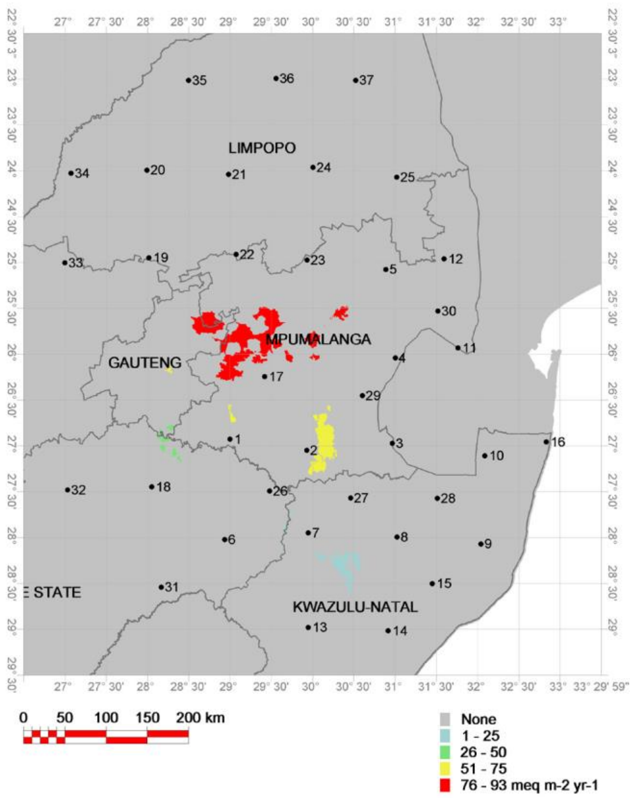


Figure 7. Total acidic deposition rates (meq/m^2/yr) (Josipovic *et al.* 2011).

Figure 8. The exceedance of acidity critical loads, taking into account the sensitivity of areas according to the area's soil attributes (Josipovic *et al*. 2011).

2.3.8 Co-deposition of cations

During certain anthropogenic processes such as agricultural practices and the combustion of fuel, including coal and wood, base cations are emitted as particulates into the atmosphere. Base cations include the ions Sodium (Na^+), Potassium (K^+), Calcium (Ca^+) and Magnesium (Mg^+). Base cation deposition needs to be taken into account as it counteracts the acidification effects of nitrogen and sulphur deposition, by adding alkalinity to the soil (Lovblad *et al.* 2004). There is currently no accurate data on base cation deposition within the Waterberg, and in particular the study site.

Chapter 3: Materials and Methods

3.1 Vegetation biodiversity

3.1.1 Study site

The vegetation diversity assessment took place in an area of the Waterberg in the Limpopo Province. Winds blow predominantly from a north easterly direction. A transect was chosen around an environmental gradient, with downwind areas expected to be more impacted regarding air pollution, and upwind areas less impacted (Figure 9). Transect sites that had similar land use and which were all privately owned farmland, were selected.

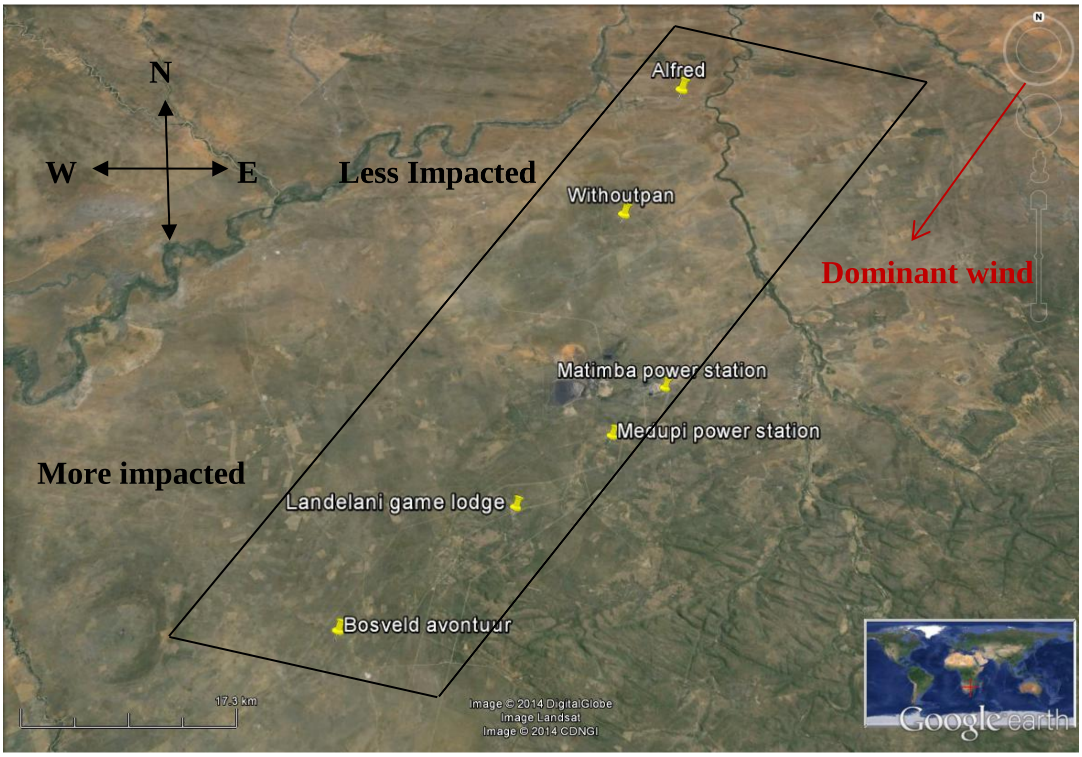


Figure 9. The belt transect along an environmental gradient in the Waterberg, representing the positions of the four sites; Alfred, Withoutpan, Landelani game lodge and Bosveld avontuur and the two power stations; Matimba and Medupi (Google Earth 2005).

All four sites fall into the Mucina and Rutherford's (2006) broad vegetation type of Limpopo Sweet Bushveld, characterised by short open woodland with dominant tree species such as *Combretum apiculatum*, *Acacia nigrescens*, *A. erubescens* and *Commiphora* species: and dominant shrub species including *Grewia flava*, *G. monticola* and *Euclea undulata*. *Acacia mellifera*, *A. erubescens* and *Dichrostachys cinerea* are found in areas which have been disturbed.

The sites that are found downwind from the currently operating power station Matimba, and under construction Medupi, are Bosveld avontuur (23°52′36.31″S; 27°20′16.87″E) and Landelani game lodge (23°46′51.57″S; 27°28′54.34″E). Both these sites are thought to be currently impacted by air pollution from Matimba and further impacted once Medupi starts operating. Bosveld avontuur is a cattle farm, private reserve and hunting farm, with all three elements included within the selected site. Landelani game lodge is also a private reserve and hunting farm, although within the selected site larger animals were excluded. The upwind sites from both power stations include Withoutpan (23°30′15.90″S; 27°35′7.80″E) and Alfred (23°21′24.98″S; 27°39′10.51″E). Withoutpan is a private reserve and hunting farm and the selected area included both these aspects. Alfred was similar with the site selected being both a private reserve and hunting area, however the sampled area within this site was found to be close to a river. Changes of topography between sites and within sites will have an impact on soil texture within these areas. The distances from each site to each power station vary, with Landelani being the closest site to both the Medupi and Matimba power stations (Table 4).

Table 4. The distance from each site to the currently operating Matimba and under construction Medupi.

		Power stations	
	Sites	**Medupi**	**Matimba**
Downwind	Bosveld avontuur	29.24 km	35.88 km
	Landelani game lodge	10.88 km	17.74 km
Upwind	Withoutpan	23.60 km	19.63 km
	Alfred	41.03 km	35.82 km

The Waterberg is unique in that it has four main landscape features which include the Waterberg Plateau, which dominates the area, the Transvaal Plateau Basin, the Pietersburg Plain and the Limpopo Depression. The area around Lephalale and the study sites consists of large open plains, and is surrounded by areas characterised by hills and lowlands with hills (Claasen *et al.* 2010). The underlying geology within the Waterberg area is composed of sediments and volcanics from the Waterberg Group and the Karoo Super Group (Figure 10) (Claassen *et al.* 2010). The Waterberg Group is found to the South and is characterised by quartzite sandstone, conglomerates and grits. Igneous intrusions of pre-Karoo diabase are found within the Waterberg rocks. The Karoo Supergroup, which is subdivided into the Dwyka, Ecca, Beaufort and Stomberg groups, is found in the Northern and South Eastern areas of the Waterberg and is rich in coal reserves (Corbett *et al.* 2008; Claassen *et al.* 2010). The substrate over the selected sites, within the belt transects, was standard, and falls within the Waterberg Group and the Karoo Super Group.

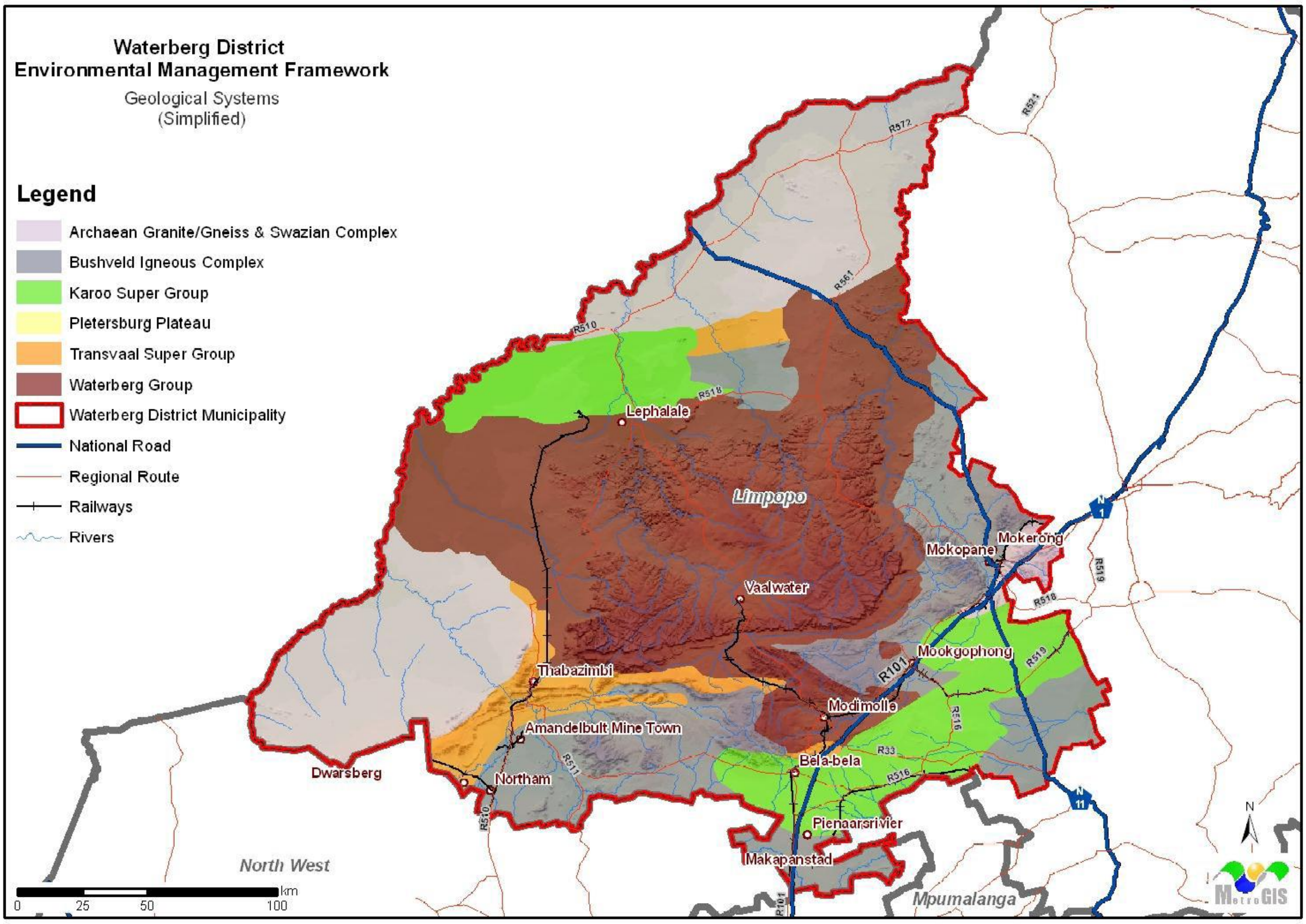


Figure 10. The geology of the Waterberg area (Claasen *et al*. 2010).

3.1.2 Vegetation identification and measurements

The first vegetation assessment took place at the end of October 2013, a period when the majority of vegetation should have been at various stages of growth and easily identifiable, however the area had not received any rain, and the sites were both dry and devoid of vegetation. The second vegetation assessment took place in March 2014, ten days after the region had experienced approximately 400 mm of rain, resulting in a completely transformed landscape, with vast amounts of growth.

At each site the first one of six 100 meter transects was randomly selected, and from this transect line five other transects were set up equidistantly in a parallel and then perpendicular orientation (Figure 11). Each transect was five meters wide. The different types of vegetation along and within these transects were identified and analysed using the following methods:

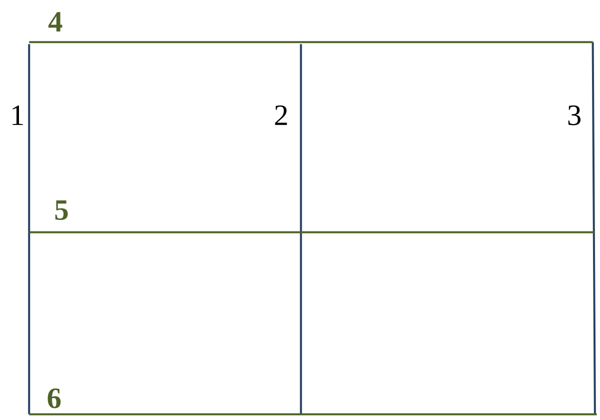


Figure 11. A diagram representing how the six transects were set up, with transects one, two and three parallel to one another, and transects four, five and six perpendicular to these.

3.1.2.1 Grasses and forbs

Quadrats, which were 0.5 x 0.5 meters, were placed every ten meters on alternating sides of each of the six transect lines. At each quadrat the GPS co-ordinates were established as a record and source of information for future monitoring.

Dry weight rank method and comparative yield method

The following methods were used to determine species composition and cover of the grasses and forbs, along the transect line within the allocated quadrats.

Each quadrat was ranked from 0 to 5, with rank 5 containing the highest dry matter yield; rank 1 the lowest dry matter yield, and rank 0 containing bare ground. Five of each ranked quadrats were randomly selected, and the above-ground vegetation from a height of two centimeters from ground level, was harvested and dried, in order for the total biomass of the quadrat to be estimated (Haydock and Shaw 1975).

The vegetation composition was estimated using the dry-weight rank method, whereby species were ranked first, second or third depending on the cover of the grass or forb species within the quadrat. A multiplier was used for each ranking with species in the first rankings being multiplied by 0.7, the second by 0.24 and the third by 0.06. These results were summed giving a species-weighting from which the percentage of vegetation composition was determined (t' Mannetje and Haydock 1963).

3.1.2.2 Trees

All trees that fell within an area of five meters on each side of the six transect lines were identified and assessed using the following methods.

Density

The distance from the transect line to each tree within the designated area was measured by pacing in approximately meter steps, and each tree species was identified. From this the total tree density of the area was determined, from which the total tree density per hectare was calculated. The calculation used was: Density (total trees per ha) = number of trees in the site * 10 000 m^2/ site area (m^2)

Allometry

The stem diameter of each tree was measured at breast height, approximately 1.3m, with a centimeter tape measure, as well as the tree height which was measured using two recorders, one person as the height standard and the other person at a fixed distance from the tree. Two allometric equations were used to determine the standing biomass of each tree species. The first allometric equation was for trees under four meters, and the other was for trees over four meters.

Tree biomass < 4m (tonnes/ha): log (shrub dry mass) = 2.320 (log (circumference)) - 2.30 (Shakelton and Scholes 2011).

Tree biomass > 4m (tonnes/ha): log (tree dry mass) = 2.397 (log (circumference)) - 2.441 (Shakelton and Scholes 2011).

3.1.3. Data analyses

At each site, all vegetation species were identified; alpha diversities were calculated using the Shannon diversity index to measure species diversity:

$H' = \sum pi \log pi$

Pielou's evenness index was used to measure species evenness within each site:

$J = {H'}/{lnS}$; H′ = Shannon diversity index, S = species richness.

Species richness was calculated by the actual number of different species seen within a site

Beta diversities between sites were calculated using Sorensen's similarity index:

$\beta = {2c}/{S1 + S2}$; c = number of species common to both sites, S1 = total number of species in site 1, S2 = total number of species in site 2.

Tree density was calculated per site, as well as tree biomass using Shakelton and Scholes (2011) allometric equations and its variances.

The estimated yields per hectare for grasses and forbs, both in October and again in March, were calculated by multiplying the mass of each ranked quadrat by the number of these ranked quadrats in the site and summing these values. This sum was divided by the number of quadrats per site to get a mass value per quadrat and then multiplied by 40 to get a value in kg/ha:

$$\text{Estimated yield (kg/ha)} = \left(\frac{\sum(\text{mass of ranked quadrat X number of ranked quadrat})}{\text{number of quadrats per site}}\right) \text{ x } {}^{*}40$$

$$\text{With } {}^{*}40 = \left(\frac{40000 \text{ quadrats/ha}}{1000\text{g/kg}}\right)$$

The data were found to be not normally distributed, and as a result they were log transformed. Differences in tree density and biomass per hectare, and grass and forb density and biomass per hectare, both in October and in March, between the four sites and between individual tree and grass and forb species were tested by using a two way analysis of variance (ANOVA) with no replication. It was not possible to examine the interaction between the four sites and the individual tree or grass and forb species as there was no replication of the data. The data were analysed using both Excel and R statistical software.

3.2 Deposition and Acidity

3.2.1 Experimental design

Initially seeds from a number of trees species were collected from the Waterberg and were placed in near boiling water overnight before planting in soil collected from the Bosveld avontuur site. Soil from this site was used as it falls within the predetermined transect in the Waterberg, in an area which is expected to be most sensitive to acidic deposition. The seeds were under optimal growing conditions in the greenhouse, however it can be seen that the germination percentage of all four species was extremely low (Table 5).

Table 5. The type of tree species planted and the number of seeds that germinated over the course of four months.

Tree species	Seeds planted	Seeds germinated	Germination (%)
Combretum apiculatum	172	0	0.00
Combretum molle	255	3	1.18
Dichrostachys cinerea	628	9	1.43
Terminalia sericea	476	2	0.42

The low germination percentage of all four species may be an indication of the inability of seed germination of various tree species at various sites within the study area, or may indicate that seed germination is low either as a result of predation from either insects or ants, or in built dormancy during unfavourable conditions, due to higher pollution levels, nutrient availability or drought.

Panicum maximum and *Eragrostis rigidior* tillers were also collected in the field and planted in soil from the Bosveld site. The growth of both the tree seeds and the grass tillers were minimal and were not sufficient for conducting the experiment.

Poor growth of these tree seeds and grass tillers from the field resulted in two C3 tree species, *Acacia sieberiana* and *Combretum erythrophyllum*, as well as two C4 grass species *Panicum maximum* and *Eragrostis curvula* having to be purchased. *Panicum maximum* is a shade loving grass which generally grows in fertile soils, whereas *Eragrostis curvula* is found in disturbed areas and mainly in sandy or loam soils (Van Oudtshoorn 2012). Both the tree species and the grass species were planted in 15cm pots, in the soil collected from the Bosveld site, and grown in the greenhouse at the University of the Witwatersrand for a period of approximately nine months. Each grass pot was watered every second day during summer with 100ml of distilled water and each tree pot with 150ml of distilled water every second day. This period was increased to every three days during winter.

After four months, once the plants had established and were in a healthy condition, fifteen random pots of each grass species, nine pots of *Acacia sieberiana* and 14 pots of *Combretum erythrophyllum* were harvested, in order to determine a relationship between height and above and below ground mass, and to leave 36 plants per species to be treated. The remaining 36 pots per species were then treated on a monthly basis depending on the responses seen in the plants, in order to ensure that they were not being over treated resulting in death. The treatments consisted of a 100ml solution containing one of the nine treatments, given over a two day period in two 50ml amounts (Table 6). In total the plants were treated three times over a period of five months. During this period on three occasions all the tree species were sprayed with Efekto Malathion, as scale insects had started negatively impacting their growth.

Table 6. The experimental design of the nine treatments that were given to both tree and grass species.

Solution of Potassium sulphate (K_2SO4)		Solution of Potassium nitrate (KNO_3): Distilled water	Solution of Potassium nitrate (KNO_3): 0.14g N (14 % wt/vol)	Solution of Potassium nitrate (KNO_3): 0.28g N (28% wt/vol)
	Distilled Water	**1** 4 reps of each grass species 4 reps of each tree species (100ml of distilled water)	**2** 4 reps of each grass species 4 reps of each tree species (80ml of distilled water + 20ml of N solution)	**3** 4 reps of each grass species 4 reps of each tree species (60ml of distilled water + 40ml of N solution)
	0.32g S (32% wt/vol)	**4** 4 reps of each grass species 4 reps of each tree species (80ml of distilled water + 20ml of S solution)	**5** 4 reps of each grass species 4 reps of each tree species (60ml of distilled water + 20ml of N solution + 20ml of S solution)	**6** 4 reps of each grass species 4 reps of each tree species (40ml of distilled water + 40ml of N solution + 20ml of S solution)
	0.64g S (64% wt/vol)	**7** 4 reps of each grass species 4 reps of each tree species (60ml of distilled water + 40ml of S solution)	**8** 4 reps of each grass species 4 reps of each tree species (40ml of distilled water + 20ml of N solution + 40ml of S solution)	**9** 4 reps of each grass species 4 reps of each tree species (20ml of distilled water + 40ml of N solution + 40ml of S solution)

An average height measurement of each grass species and each tree species was taken per pot before any treatments began. If any of the plants died during the experiment each plants height was measured again and the dead plant was harvested with the above and below ground biomass being recorded. At the completion of the research, both the tree species and the grass species were harvested in order to determine the above and below ground biomass of each.

The pH of the soil was determined before the experiment using the 1:2 dilution method. The same method was used to determine the pH of the soil at the end of the experiment for each of the treatments. During the course of the experiment the pH of each solution for each treatment was determined before every application.

3.2.2 Data analyses

All the data were averaged over each treatment, for each measurement, and the standard error was calculated. The data were analysed for normality using the Shapiro Wilk test, and all data were found to be normally distributed. The data were then assessed using multi-factorial Analysis of Variance (ANOVA) to determine if the addition of nitrogen and sulphur had caused a significant change in the height or biomass of any of the vegetation, as well as changes in the above or below ground mass. Post-hoc Tukey HSD tests were performed on treatments that showed a significant difference. Regression analysis was used to determine whether a relationship existed between pH and biomass of each species. The change in soil pH was found to be normally distributed and as a result the data were analysed using the two way analysis of variance (ANOVA) with replication in order to assess whether the changes in soil pH of the trees and grass species were significantly different. The data were analysed using both Excel and R statistical software.

Chapter 4: Results

The results are divided into two main sections; vegetation biodiversity and the effects of deposition and acidity on plant growth in four plant species.

4.1 Vegetation biodiversity

The first section of the results focuses on the number and type of species identified in each sampling site, as well as the alpha diversities within each site and beta diversities between these sites. This is followed by the biomass of trees, and grasses and forbs in each site.

4.1.1. Species identification, dominant species and species richness in each site

The dominant (number per site) tree, forb and grass species in each site are shown in Table 7. It can be seen that there are no common dominant species which are shared in any of the sites. This indicates that the sites are inherently variable and that drawing conclusions from just four sites must be carefully considered. It can be seen that whilst there are no common dominant tree species, the dominant tree species within a site is found in at least one of the other sites (Table 8). *Acacia caffra, Dichrostachys cinerea* and *Grewia monticola* are three tree species that are found in all four sampled sites (Table 8).

There are no common dominant forb and grass species between sites, however the dominant forb and grass species in the two downwind sites and the upwind, Withoutpan site, are found in at least one other site (Table 9). However the dominant forb species, *Geigeria burkei*, and dominant grass species, *Enneapogon scoparius* are both unique to the Alfred site and not shared by any of the other sites (Table 9). The grass species *Aristida congesta, Eragrostis chloromelas, Schmidtia pappophoroides* and *Urochloa mosambicensis* are shared in all four sites, as is the forb species *Ipomoea bolusii* (Table 9).

Table 7. The dominant species found in each of the downwind sites, Bosveld and Landelani, and the upwind sites, Withoutpan and Alfred.

	Downwind sites		**Upwind sites**	
	Bosveld	**Landelani**	**Withoutpan**	**Alfred**
Dominant tree species	*Commiphora pyracanthoides*	*Grewia flava*	*Terminalia sericea*	*Acacia mellifera*
Dominant forb species	*Indigofera hilaris*	*Dicoma anomala*	*Tephrosia rhodesica*	*Geigeria burkei*
Dominant grass species	*Aristida junciformis*	*Eragrostis chloramelas*	*Urochloa panicoides*	*Enneapogon scoparius*

The identified tree species within each site can be seen in Table 8. This is followed by the identified grass and forb species found within each site in Table 9.

Table 8. The identification and absolute number per hectare of each tree species found at the downwind sites, Bosveld and Landelani, and the upwind sites, Withoutpan and Alfred.

Functional type	Family name	Species identification	Downwind sites		Upwind sites	
		Scientific name	Bosveld	Landelani	Withoutpan	Alfred
Tree species	Mimosaceae	*Acacia caffra*	50	13	3	7
	Mimosaceae	*Acacia galpinii*		7		
	Mimosaceae	*Acacia karoo*			3	30
	Mimosaceae	*Acacia mellifera*	5	5		225
	Mimosaceae	*Acacia nigrescens*		30		
	Mimosaceae	*Acacia robusta*				30
	Mimosaceae	*Acacia tortilis*	202	2		85
	Capparaceae	*Boscia albitrunca*			3	
	Caesalpiniaceae	*Burkea africana*		2		
	Combretaceae	*Combretum apiculatum*		57	17	
	Combretaceae	*Combretum molle*			137	
	Burseraceae	*Commiphora pyracanthoides*	475			8
	Mimosaceae	*Dichrostachys cinerea*	168	38	43	78
	Apocynaceae	*Diplorhynchus condylocarpon*		17		
	Boraginaceae	*Ehretia rigidi*	30			
	Ebenaceae	*Euclea schimperi*			7	7
	Ebenaceae	*Euclea undulata*		15	85	
	Sparrmanniaceae	*Grewia flava*		145	33	202
	Sparrmanniaceae	*Grewia monticola*	260	122	7	5
	Sparrmanniaceae	*Grewia occidentalis*				5
	Celastraceae	*Gymnosporia buxifolia*			3	
	Celastraceae	*Gymnosporia heterophylla*			3	
	Anacardiaceae	*Lannea discolor*		7		
	Capparaceae	*Maerua angolensis*			3	
	Chrysablanaceae	*Parinari curatellifolia*		8		
	Anacardiaceae	*Sersia dentata*			17	
	Anacardiaceae	*Sersia pyroides*		35	251	2
	Euphorbiaceae	*Spirostachys africana*			55	
	Strychinaceae	*Strychos madagascarrensis*		93		
	Combretaceae	*Terminalia sericea*		23	428	
	Rhaminaceae	*Ziziphus mucronata*		6	2	6
Totals (/ha)			**1190**	**625**	**1100**	**690**

There was no significant difference in tree density between all four sites ($F = 1.12$, $P > 0.05$), however the number of trees per hectare do vary between 625 and 1190 across the sites.

The two downwind sites, Bosveld and Landelani, and the upwind site Withoutpan all have a larger percentage of broad-leaved trees to fine-leaved trees, with the upwind site, Withoutpan being composed of over 90% of broad-leaved trees (Figure 12). Alfred, the other upwind site is the only site which is dominated by fine-leaved trees (plant family Mimosaceae), with this area having over 65% of fine-leaved trees (Figure12). This may be an indication of Alfred being a nutrient rich site as a river runs perpendicular to the sampled area.

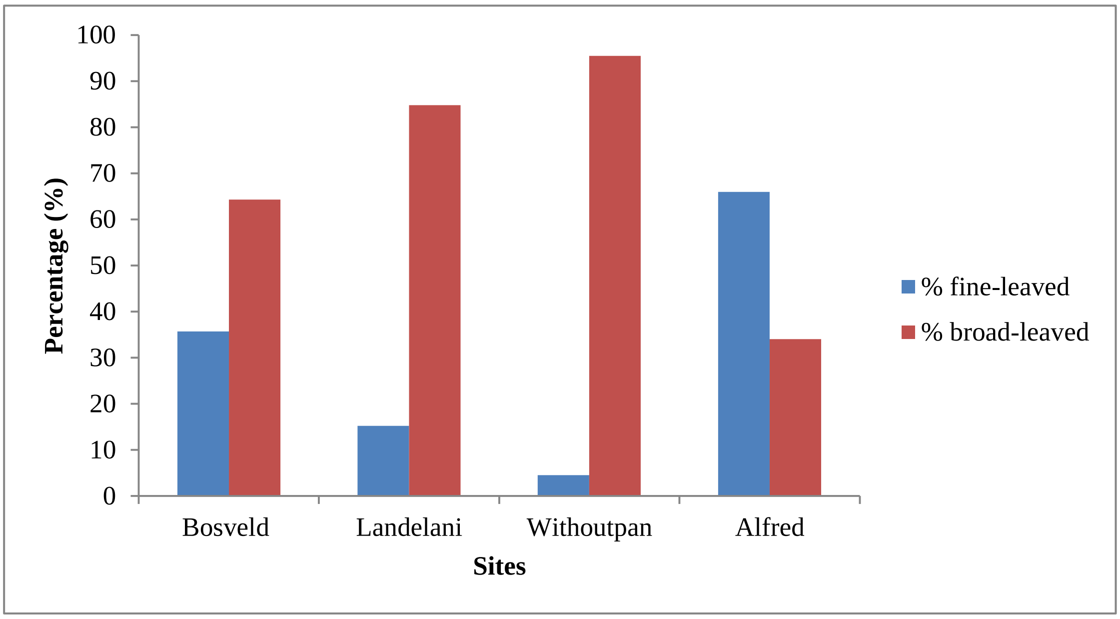


Figure 12. The percentage of fine-leaved and broad-leaved trees in each sampled area in each of the four sites.

Table 9. The identification and absolute number per hectare of each grass and forb species found at the downwind sites, Bosveld and Landelani, and the upwind sites, Withoutpan and Alfred.

Functional type	Family Name	Species identification	Downwind sites		Upwind sites	
		Scientific name	Bosveld	Landelani	Withoutpan	Alfred
Grass and forb species	Amaranthaceae	*Achyranthes aspera*				7
	Poaceae	*Aristida adscensionis*				5
	Poaceae	*Aristida bipartita*			18	2
	Poaceae	*Aristida congesta*	13	15	5	33
	Poaceae	*Aristida junciformis*	63	5	5	
	Asparagaceae	*Asparagus laricinus*				7
	Asteraceae	*Berkheya species*			7	3
	Nyctaginaceae	*Boerhavia diffusa*				7
	Poaceae	*Chloris virgata*	5			
	Capparaceae	*Cleome angustifolia*				3
	Combretaceae	*Combretum molle*			7	
	Burseraceae	*Commiphora pyracanthoides*	5			
	Cupressaceae	*Cypres species*	3	2		2
	Asteraceae	*Dicoma anomala*	2	13		
	Poaceae	*Ehretia rigida*	8			
	Poaceae	*Enneapogon cenchroides*				15
	Poaceae	*Enneapogon scoparius*				43
	Poaceae	*Eragrostis chloromelas*	33	107	22	2
	Poaceae	*Eragrostis trichophora*		30	28	12
	Euphorbiaceae	*Euphorbia species*			2	8
	Euphorbiaceae	*Euphorbia species*				2
	Asteraceae	*Felicia filifolia*	8			
	Asteraceae	*Felicia mossamedensis*	2			
	Asteraceae	*Geigeria burkei*				47
	Sparrmanniaceae	*Grewia flava*			2	2
	Sparrmanniaceae	*Grewia monticola*	13	25	5	
	Asteraceae	*Helichrysum species*				2
	Boraginaceae	*Heliotropium steudneri*				8
	Malvaceae	*Hermannia burkei*				2
	Malvaceae	*Hermannia cristata*		3		
	Malvaceae	*Hermannia species*				2
	Malvaceae	*Hermannia species*			5	
	Malvaceae	*Hibiscus trionum*				3

Table 9 (continued). The identification and absolute number per hectare of each grass and forb species found at the downwind sites, Bosveld and Landelani, and the upwind sites, Withoutpan and Alfred.

Functional type	Family name	Species identification	Downwind sites		Upwind sites	
		Scientific name	Bosveld	Landelani	Withoutpan	Alfred
Grass and forb species	Fabaceae	*Indigofera hilaris*	10	2	8	
	Convolvulaceae	*Ipomoea bolusii*	2	3	2	2
	Rubiaceae	*Kohautia species*				2
	Chenopodiaceae	*Lophiocarpus polystachyus*				3
	Lamiaceae	*Leonotis ocymifolia*	2			
	Poaceae	*Melinis repens*	17	20		3
	Poaceae	*Panicum maximum*	2	22		
	Lamiaceae	*Plectranthus fruticosus*	5	8		
	Poaceae	*Schmidtia pappophoroides*	25	7	5	2
	Poaceae	*Setaria pumila*			8	3
	Malvaceae	*Sida cordifolia*			7	7
	Malvaceae	*Sida dregei*				3
	Fabaceae	*Tephrosia rhodesica*			25	7
	Poaceae	*Urochloa mosambicensis*	22	2	10	3
	Poaceae	*Urochloa panicoides*		2	82	
	Malvaceae	*Waltheria indica*			18	5
Total (/ha)			**240**	**266**	**271**	**257**

No significant differences between the grass and forb numbers in all four sites can be seen ($F = 2.31$, $P > 0.05$). However a significant difference does exist between the number of each individual grass and forb species across the sites ($F = 1.84$, $P < 0.05$).

4.1.2 Alpha and beta diversities

The three alpha diversities that were calculated and analysed in each site were species richness, Shannon diversity index (H') and Pielou's evenness index (J) (Table 10). Species richness represents the number of different species found within a site. The highest tree species richness were found at the downwind Landelani and upwind Withoutpan sites, sites closest to both power stations, with each site having 18 different tree species. The downwind Bosveld site had the least number of different tree species, with only seven species found within the sampled area. The upwind site, Alfred had almost double the amount of grass and forb species found in any of the other three sites (Table 10).

The Shannon diversity index (H') measures species diversity within a site, with the higher the value indicating the greater the diversity. The tree species diversity in isolation, and tree, grass and forb species diversity was greatest in the site closest to the power stations, the downwind site Landelani. The grass and forb species diversity was found to be greatest in the upwind Alfred site (Table 10).

Pielou's evenness index (J) measures the relative abundance of species within an area, with sites with a higher species evenness value having a more equal abundance of species and therefore greater species diversity. Overall the species evenness was most similar between the four sites with regards to the grasses and forbs. The highest overall species evenness can be seen in the downwind site closest to the power stations, Landelani, reinforcing that this site has the greatest species diversity (Table 10).

Table 10. The alpha diversities, of both trees, and grasses and forbs, in each sampling site.

Diversity indexes	Functional type	Diversity indexes	Downwind sites		Upwind sites	
			Bosveld	**Landelani**	**Withoutpan**	**Alfred**
Alpha diversities	**Trees**	**Species richness**	7	18	18	13
		Shannon diversity index (H')	1.52	2.3	1.85	1.78
		Pielou's evenness index (J)	0.78	0.8	0.64	0.69
	Grasses and forbs	**Total Species richness**	19	16	20	33
		Shannon diversity index (H')	2.42	2.06	2.46	2.85
		Pielou's evenness index (J)	0.82	0.74	0.82	0.82
	Trees, grasses and forbs	**Species richness**	26	34	38	46
		Shannon diversity index (H')	2.13	2.84	2.47	2.65
		Pielou's evenness index (J)	0.65	0.80	0.68	0.69

Beta diversity reflects species diversity between sites, with a high value indicating greater species variation between sites, and a low value showing higher species similarity between sites. The two downwind sites, Bosveld and Landelani, showed the highest value (0.60) and therefore greatest species variation between these sites, with Bosveld and Withoutpan having the lowest value (0.34) and therefore having the highest species similarity (Table 11). There is almost double the species variation between the two downwind sites and the Bosveld and Withoutpan site. Beta diversity values between Bosveld and Withoutpan, Bosveld and Alfred, and Landelani and Alfred are more similar than the species variation seen in Bosveld and Landelani, Landelani and Withoutpan, and Withoutpan and Alfred.

Table 11. The beta diversities between the four sampling sites.

Sites		Beta diversity (β_w)
Downwind sites	Bosveld and Landelani	0.60
Downwind and upwind site	Bosveld and Withoutpan	0.34
Downwind and upwind site	Bosveld and Alfred	0.36
Downwind and upwind site	Landelani and Withoutpan	0.53
Downwind and upwind site	Landelani and Alfred	0.40
Upwind sites	Withoutpan and Alfred	0.52

4.1.3 Tree, grass and forb, biomass

The Bosveld site had the largest number of individual trees, with 1190 trees per hectare (Table 8), however this site also had the lowest tree biomass with 11.04 tonnes per hectare (Table 12). The dominant tree species in the sampled area within Bosveld was *Commiphora pyracanthoides*, which all had a small and shrub-like growth form (Table 7). Withoutpan had the next largest number of individual trees, with 1100 trees per hectare (Table 8). The tree biomass in this site was the largest, with 69.95 tonnes per hectare (Table 12). The individual trees in this site were larger, well established and more mature than those found in Bosveld. Both of the sites that are closer to the power stations, Landelani and Withoutpan, had more tree biomass, than those sites which were further away, Bosveld and Alfred (Table 12). The number of trees and tree biomass within each sampled area was variable across all four sites.

Table 12. Tree biomass (kg) per hectare of each tree species found in each site.

Functional Type	Family name	Species Identification	Downwind sites		Upwind sites	
		Scientific name	Bosveld	Landelani	Withoutpan	Alfred
Tree species	Mimosaceae	*Acacia caffra*	2971.5	8728.5	214.2	100.4
	Mimosaceae	*Acacia galpinii*		358.8		
	Mimosaceae	*Acacia karoo*			23.3	2318
	Mimosaceae	*Acacia mellifera*	1086.4	6.5		4722.5
	Mimosaceae	*Acacia nigrescens*		1510.8		
	Mimosaceae	*Acacia robusta*				2218.8
	Mimosaceae	*Acacia tortilis*	3789.9	41.8		3966.6
	Capparaceae	*Boscia albitrunca*			51.9	
	Caesalpiniaceae	*Burkea africana*		77.6		
	Combretaceae	*Combretum apiculatum*		1596.9	1342.3	
	Combretaceae	*Combretum molle*			2521.5	
	Burseraceae	*Commiphora pyracanthoides*	68.88			26
	Mimosaceae	*Dichrostachys cinerea*	532.3	757.8	562.1	2217.9
	Apocynaceae	*Diplorhynchus condylocarpon*		6.7		
	Boraginaceae	*Ehretia rigidi*	41.9			
	Ebenaceae	*Euclea schimperi*			4.2	1232.8
	Ebenaceae	*Euclea undulata*		268.6	38.9	
	Sparrmanniaceae	*Grewia flava*		7644.8	489.6	2829
	Sparrmanniaceae	*Grewia monticola*	2546.7	6810.4	349.1	39.9
	Sparrmanniaceae	*Grewia occidentalis*				5.2
	Celastraceae	*Gymnosporia buxifolia*			799.6	
	Celastraceae	*Gymnosporia heterophylla*			3.5	
	Anacardiaceae	*Lannea discolor*		222.8		
	Capparaceae	*Maerua angolensis*			502.2	
	Chrysablanaceae	*Parinari curatellifolia*		23.8		
	Anacardiaceae	*Sersia dentata*			1132	
	Anacardiaceae	*Sersia pyroides*		5534.4	26664.8	2.7
	Euphorbiaceae	*Spirostachys africana*			32461.1	
	Strychinaceae	*Strychos madagascarrensis*		9247.7		
	Combretaceae	*Terminalia sericea*		3311.1	1078.5	
	Rhaminaceae	*Ziziphus mucronata*		487.4	1662.8	334.3
Total (kg/ha)			**11037.6**	**46636.4**	**69901.6**	**20014.1**
Total (tonnes/ha)			**11.04 ± 4.65**	**46.64 ± 1.01**	**69.9 ± 2.89**	**20.01 ± 1.41**

A significant difference in tree biomass between the four sites can be seen (F = 3.19, P < 0.05). However the tree biomass of each individual tree species across sites was not significantly different (F = 1.55, P > 0.05).

The grass and forb biomass was calculated before the rains in October 2013 (Table 13) and again after the rains in March 2014 (Table 14).

Table 13. Grass and forb biomass (kg), in October 2013, per hectare of each species found in each site.

Functional type	Family name	Species identification	Downwind sites		Upwind sites	
		Scientific name	Bosveld	Landelani	Withoutpan	Alfred
Grass and forb species	Amaranthaceae	*Achyranthes aspera*				5.36
	Poaceae	*Aristida adscensionis*				4.02
	Poaceae	*Aristida bipartita*			7.14	1.34
	Poaceae	*Aristida congesta*	6.68	17.83	1.95	26.81
	Poaceae	*Aristida junciformis*	31.75	5.94	1.95	
	Asparagaceae	*Asparagus laricinus*				5.36
	Asteraceae	*Berkheya species*			2.59	2.68
	Nyctaginaceae	*Boerhavia diffusa*				5.36
	Poaceae	*Chloris virgata*	2.51			
	Capparaceae	*Cleome angustifolia*				2.68
	Combretaceae	*Combretum molle*			2.59	
	Burseraceae	*Commiphora pyracanthoide*	2.51			
	Cupressaceae	*Cypres species*	1.67	1.98		1.34
	Asteraceae	*Dicoma anomala*	0.84	15.85		
	Poaceae	*Ehretia rigidi*	4.18			
	Poaceae	*Enneapogon cenchroides*				12.07
	Poaceae	*Enneapogon scoparius*				34.86
	Poaceae	*Eragrostis chloramelas*	16.71	126.77	8.43	1.34
	Poaceae	*Eragrostis trichophora*		35.65	11.03	9.39
	Euphorbiaceae	*Euphorbia species*			0.65	6.70
	Euphorbiaceae	*Euphorbia species*				1.34
	Asteraceae	*Felicia filifolia*	4.18			
	Asteraceae	*Felicia mossamedensis*	0.84			
	Asteraceae	*Geigeria burkei*				37.54

Table 13 (continued)**.** Grass and forb biomass (kg), in October 2013, per hectare of each species found in each site.

Functional type	Family name	Species identification	Downwind sites		Upwind sites	
		Scientific name	Bosveld	Landelani	Withoutpan	Alfred
Grass and forb species	Sparrmanniaceae	*Grewia flava*			0.65	1.34
	Sparrmanniaceae	*Grewia monticola*	6.69	29.71	1.95	
	Asteraceae	*Helichrysum species*				1.34
	Boraginaceae	*Heliotropium steudneri*				6.70
	Malvaceae	*Hermannia burkei*				1.34
	Malvaceae	*Hermannia cristata*		3.96		
	Malvaceae	*Hermannia species*				1.34
	Malvaceae	*Hermannia species*			1.95	
	Malvaceae	*Hibiscus trionum*				2.68
	Fabaceae	*Indigofera hilaris*	5.01	1.98	3.24	
	Convolvulaceae	*Ipomoea bolusii*	0.84	3.96	0.65	1.34
	Rubiaceae	*Kohautia species*				1.34
	Chenopodiaceae	*Lophiocarpus polystachyus*				2.68
	Lamiaceae	*Leonotis ocymifolia*	0.84			
	Poaceae	*Melinis repens*	8.36	23.77		2.68
	Poaceae	*Panicum maximum*	0.84	25.75		
	Poaceae	*Plectranthus fruticosus*	2.51	9.90		
	Poaceae	*Schmidtia pappophoroides*	12.53	7.92	1.95	1.34
	Poaceae	*Setaria pumila*			3.24	2.68
	Malvaceae	*Sida cordifolia*			2.59	5.36
	Malvaceae	*Sida dregei*				2.68
	Fabaceae	*Tephrosia rhodesica*			9.73	5.36
	Poaceae	*Urochloa mosambicensis*	10.86	1.98	3.89	2.68
	Poaceae	*Urochloa panicoides*		1.98	31.78	
	Malvaceae	*Waltheria indica*			7.14	4.02
Total (kg/ha)			**120.33**	**314.94**	**105.08**	**205.10**
Total (tonnes/ha)			**0.12 ± 0.001**	**0.31 ± 0.01**	**0.11 ± 0.005**	**0.21 ± 0.005**

A significant difference between grass and forb biomass in all four sites during October, before the rains, can be seen (F = 3.13, P < 0.05). There was also a significant difference in the biomass of each individual grass and forb species across the sites during this time (F = 1.81, P < 0.05) (Table 13).

Table 14. Grass and forb biomass (kg), in March 2014, per hectare of each species found in each site.

Functional type	**Family name**	**Species identification**	**Downwind sites**		**Upwind sites**	
		Scientific name	**Bosveld**	**Landelani**	**Withoutpan**	**Alfred**
Grass and forb species	Amaranthaceae	*Achyranthes aspera*				17.76
	Poaceae	*Aristida adscensionis*				13.32
	Poaceae	*Aristida bipartita*			37.16	4.44
	Poaceae	*Aristida congesta*	46.11	60.15	10.14	88.79
	Poaceae	*Aristida junciformis*	219	20.05	10.14	
	Asparagaceae	*Asparagus laricinus*				17.76
	Asteraceae	*Berkheya species*			13.51	8.88
	Nyctaginaceae	*Boerhavia diffusa*				17.76
	Poaceae	*Chloris virgata*	17.29			
	Capparaceae	*Cleome angustifolia*				8.88
	Combretaceae	*Combretum molle*			13.51	
	Burseraceae	*Commiphora pyracanthoide*	17.29			
	Cupressaceae	*Cypres species*	11.53	6.68		4.44
	Asteraceae	*Dicoma anomala*	5.76	53.47		
	Poaceae	*Ehretia rigida*	28.82			
	Poaceae	*Enneapogon cenchroides*				39.96
	Poaceae	*Enneapogon scoparius*				115.43
	Poaceae	*Eragrostis chloramelas*	115.26	427.73	43.92	4.44
	Poaceae	*Eragrostis trichophora*		120.3	57.43	31.08
	Euphorbiaceae	*Euphorbia species*			3.38	22.2
	Euphorbiaceae	*Euphorbia species*				4.44
	Asteraceae	*Felicia filifolia*	28.82			
	Asteraceae	*Felicia mossamedensis*	5.76			
	Asteraceae	*Geigeria burkei*				124.31
	Sparrmanniaceae	*Grewia flava*			3.38	4.44
	Sparrmanniaceae	*Grewia monticola*	46.10	100.25	10.14	
	Asteraceae	*Helichrysum species*				4.44
	Boraginaceae	*Heliotropium steudneri*				22.20
	Malvaceae	*Hermannia burkei*				4.44
	Malvaceae	*Hermannia cristata*		13.37		
	Malvaceae	*Hermannia species*				4.44
	Malvaceae	*Hermannia species*			10.14	
	Malvaceae	*Hibiscus trionum*				8.88

Table 14 (continued)**.** Grass and forb biomass (kg), in March 2014, per hectare of each species found in each site.

Functional type	Family name	Species identification	Downwind sites		Upwind sites	
		Scientific name	Bosveld	Landelani	Withoutpan	Alfred
Grass and forb species	Fabaceae	*Indigofera hilaris*	34.58	6.68	16.89	
	Convolvulaceae	*Ipomoea bolusii*	5.76	13.37	3.38	4.44
	Rubiaceae	*Kohautia species*				4.44
	Chenopodiaceae	*Lophiocarpus polystachyus*				8.88
	Lamiaceae	*Leonotis ocymifolia*	5.76			
	Poaceae	*Melinis repens*	57.63	80.2		8.88
	Poaceae	*Panicum maximum*	5.76	86.88		
	Poaceae	*Plectranthus fruticosus*	17.29	33.42		
	Poaceae	*Schmidtia pappophoroides*	86.45	26.73	10.14	4.44
	Poaceae	*Setaria pumila*			16.89	8.88
	Malvaceae	*Sida cordifolia*			13.51	17.76
	Malvaceae	*Sida dregei*				8.88
	Fabaceae	*Tephrosia rhodesica*			50.68	17.76
	Poaceae	*Urochloa mosambicensis*	74.92	6.68	20.27	8.88
	Poaceae	*Urochloa panicoides*		6.68	165.55	
	Malvaceae	*Waltheria indica*			37.16	13.32
Total (kg/ha)			**829.88**	**1062.64**	**547.32**	**679.24**
Total (tonnes/ha)			**0.83 ± 0.001**	**1.06 ± 0.008**	**0.55 ± 0.003**	**0.68 ± 0.01**

In March, after the rains, there was no significant difference between the grass and forb biomass in the four sites ($F = 2.47$, $P > 0.05$). However a significant difference does exist in the grass and forb biomass of each individual species across sites during March ($F = 1.67$, $P < 0.05$) (Table 14).

Large increases in grass and forb biomass were seen after the rains, with an increase in water availability (Figure 13). The largest grass and forb biomass can be seen in Landelani, with 0.31 tonnes per hectare in October and 1.06 tonnes per hectare in March, and the least grass and forb biomass in Withoutpan with 0.11 tonnes per hectare in October and 0.55 tonnes per hectare in March. Both the downwind sites had a larger biomass in March than either of the upwind sites. The greatest increase in grass and forb biomass with an increase in rain was seen in Bosveld with an increase of 6.9 times the initial biomass (Figure 13).

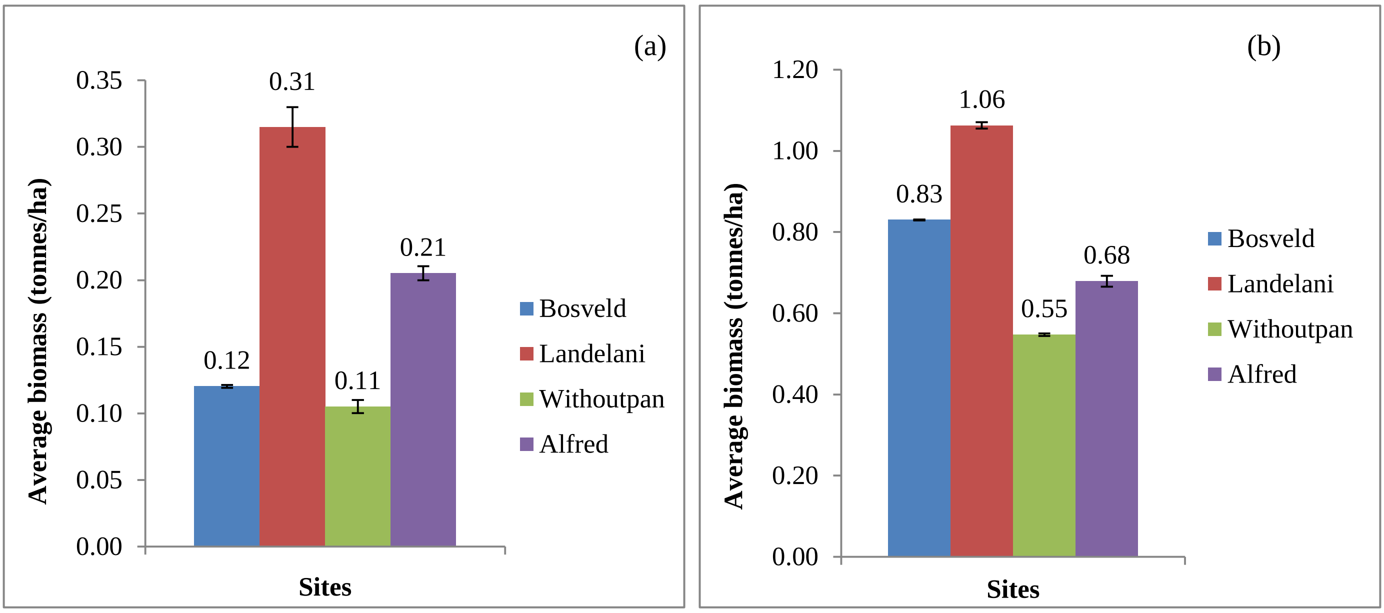


Figure 13. The average (Mean ± SE) biomass of grass and forbs in each downwind site, Bosveld and Landelani, and each upwind site, Withoutpan and Alfred, before the rains, in October 2013 (a) and after the rains in March 2014 (b).

4.2 Nitrogen and sulphur deposition and soil acidification

This section of the results looks at the increase or decrease in growth of all four plant species, with regard to height, above ground mass, below ground mass, and above and below ground mass in combination, over the nine treatments. This is followed by examining whether or not there is a relationship between these increases or decreases in growth, with changes in the soil pH.

4.2.1 The average increase or decrease in height in all four plant species, over the nine treatments

The height of all four plant species were measured before the treatments began, and then again at the end of the experimental growth period. A decrease in the height of all four plant species was seen when dieback occurred resulting in the tip of the plant dying first. The average gain in height (final height – initial height) of *Acacia sieberiana* in all treatments in which either nitrogen or sulphur, or a combination of both, were added, resulted in a decrease in height in comparison to treatment one, distilled water (Figure 14). The height of *Acacia sieberiana* was seen to be sensitive to both nitrogen and sulphur additions, but the increase or decrease in height was not statistically different with the addition of nitrogen ($F = 1.103$, $P > 0.05$), or sulphur ($F = 1.216$, $P > 0.05$), or nitrogen and sulphur in combination ($F = 1.363$, $P > 0.05$). In *Combretum erythrophyllum* the height was increased with the addition of nitrogen alone, treatment two and treatment three, however the addition of sulphur to all other treatments decreased the average height comparative to treatment one (Figure 14). The height of *Combretum erythrophyllum* was therefore found to be sensitive to sulphur, and responded to nitrogen alone. No significant difference was seen in the increase or decrease in the height of *Combretum erythrophyllum* with the addition of nitrogen ($F = 0.032$, $P > 0.05$), or sulphur ($F = 3.351$, $P > 0.05$) or the interaction between nitrogen and sulphur ($F = 0.814$, $P > 0.05$).

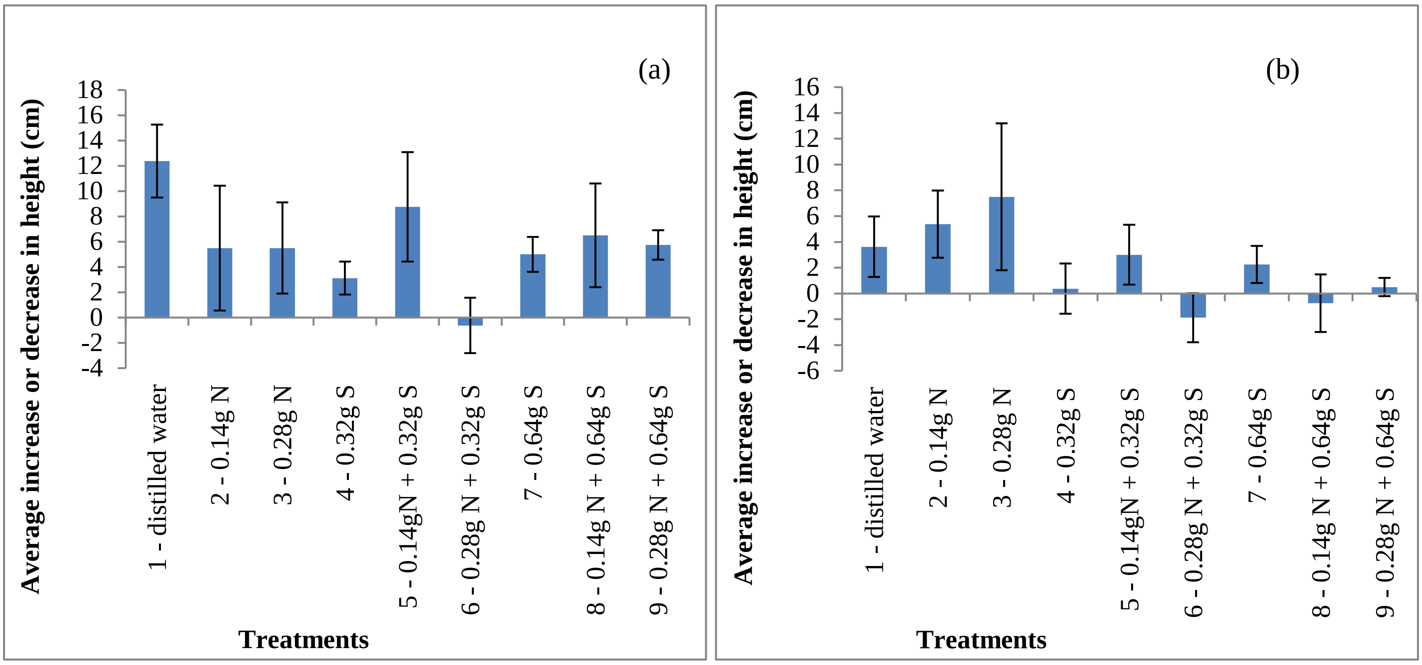
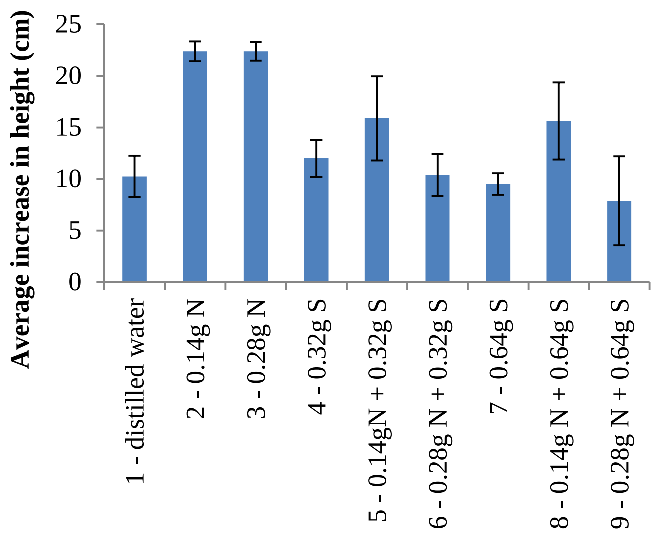


Figure 14. The average (Mean ± SE) increase or decrease in height of *Acacia sieberiana* (a) and *Combretum erythrophyllum* (b) across all nine treatments (N = nitrogen and S = sulphur).

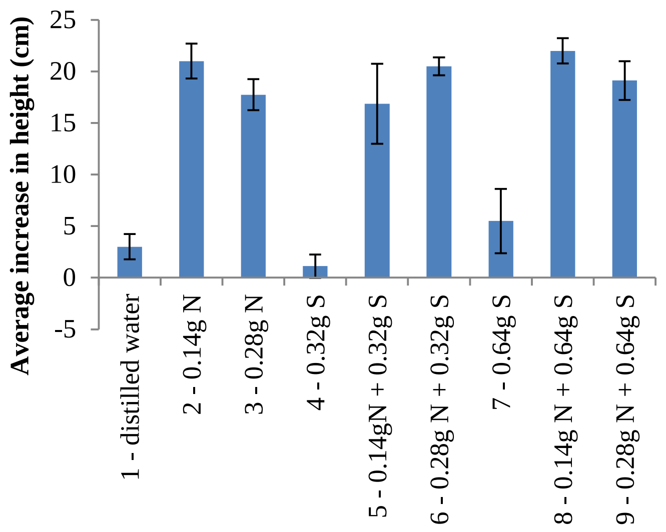
The response seen in trees was different to the response seen in grasses, with increases in grass height being far greater than the increases in tree height. The average gain in height of *Eragrostis curvula* increased in all treatments except treatments seven and nine, compared with treatment one. Both treatments seven and nine contained the highest level of sulphur addition, of 0.64g (Figure 15). The height of *Eragrostis curvula* responded to nitrogen and low levels of sulphur; however it was sensitive to the higher sulphur levels. A number of the increases in height of *Eragrostis curvula* were significantly different (Figure 15). The average gain in height of *Panicum maximum* increased in all treatments that contained nitrogen. Therefore the only two treatments that resulted in a decrease in average height were treatments four and seven, which both consisted of sulphur alone (Figure 15). The height of *Panicum maximum* was found to be sensitive to sulphur alone, however the response of *Panicum maximum* to nitrogen counteracted this sensitivity and resulted in increased height. A number of significant increases in height resulting from the addition of nitrogen and sulphur in *Panicum maximum* can be seen (Figure 15).

(a)



Treatments	P value
1 and 2	P < 0.01
1 and 4	P < 0.05
1 and 7	P < 0.01
2 and 7	P < 0.05
2 and 9	P < 0.05
3 and 7	P < 0.05
3 and 9	P < 0.05

(b)



Treatments	P value
1 and 2	P < 0.001
1 and 3	P < 0.001
1 and 5	P < 0.01
1 and 6	P < 0.001
1 and 8	P < 0.001
1 and 9	P < 0.001
2 and 4	P < 0.001
2 and 7	P < 0.001
3 and 4	P < 0.001
3 and 7	P < 0.01
4 and 5	P < 0.001
4 and 6	P < 0.001
4 and 8	P < 0.001
4 and 9	P < 0.001
5 and 7	P < 0.05
6 and 7	P < 0.001
7 and 8	P < 0.001
7 and 9	P < 0.01

Figure 15. The average (Mean ± SE) increase in height of *Eragrostis curvula* (a) and *Panicum maximum* (b) across all nine treatments (N = nitrogen and S = sulphur). The significant differences in the height of both *Eragrostis curvula* and *Panicum maximum* can be seen in the figure.

4.2.2 The average increase or decrease in above ground dry mass in all four plant species, over the nine treatments

In order to have 36 pots per species to treat, any additional pots per species were harvested after four months of initial growth. A relationship between height and above and below ground mass was established, and this was used to determine the initial above and below ground mass of the remaining plants still under treatment. The initial mass values were used in calculating the average increase or decrease in above and below ground mass, by subtracting this value from the above and below ground mass obtained after treatments and at final harvesting in order for comparative values to be obtained.

The average above ground mass (final above ground mass – initial above ground mass) in *Acacia sieberiana* increased only in treatment four (sulphur = 0.32g). Any further additions of sulphur or the addition of nitrogen resulted in a decrease in the average above ground mass in all the other treatments, compared to treatment one (Figure 16). The above ground mass of *Acacia sieberiana* was seen to respond to low levels of sulphur additions alone; however it was sensitive to any further sulphur additions and nitrogen additions. The only significant difference in the above ground mass of *Acacia sieberiana* is between treatment two (N = 0.14g) and treatment four (S = 0.32g) ($F = -0.295$, $P < 0.05$). In comparison to treatment one the average above ground mass of *Combretum erythrophyllum* increased in treatments two, three, five, seven and eight, with decreases seen in treatments four, six and nine, all of which contain sulphur (Figure 16). The above ground mass of *Combretum erythrophyllum* responded to nitrogen alone, however it was sensitive to sulphur both alone and in combination with nitrogen. No significant difference was seen in the increase or decrease in the above ground mass of *Combretum erythrophyllum* with the addition of nitrogen ($F = 1.509$, $P > 0.05$), or sulphur ($F = 0.132$, $P > 0.05$) or the interaction between nitrogen and sulphur ($F = 2.865$, $P > 0.05$).

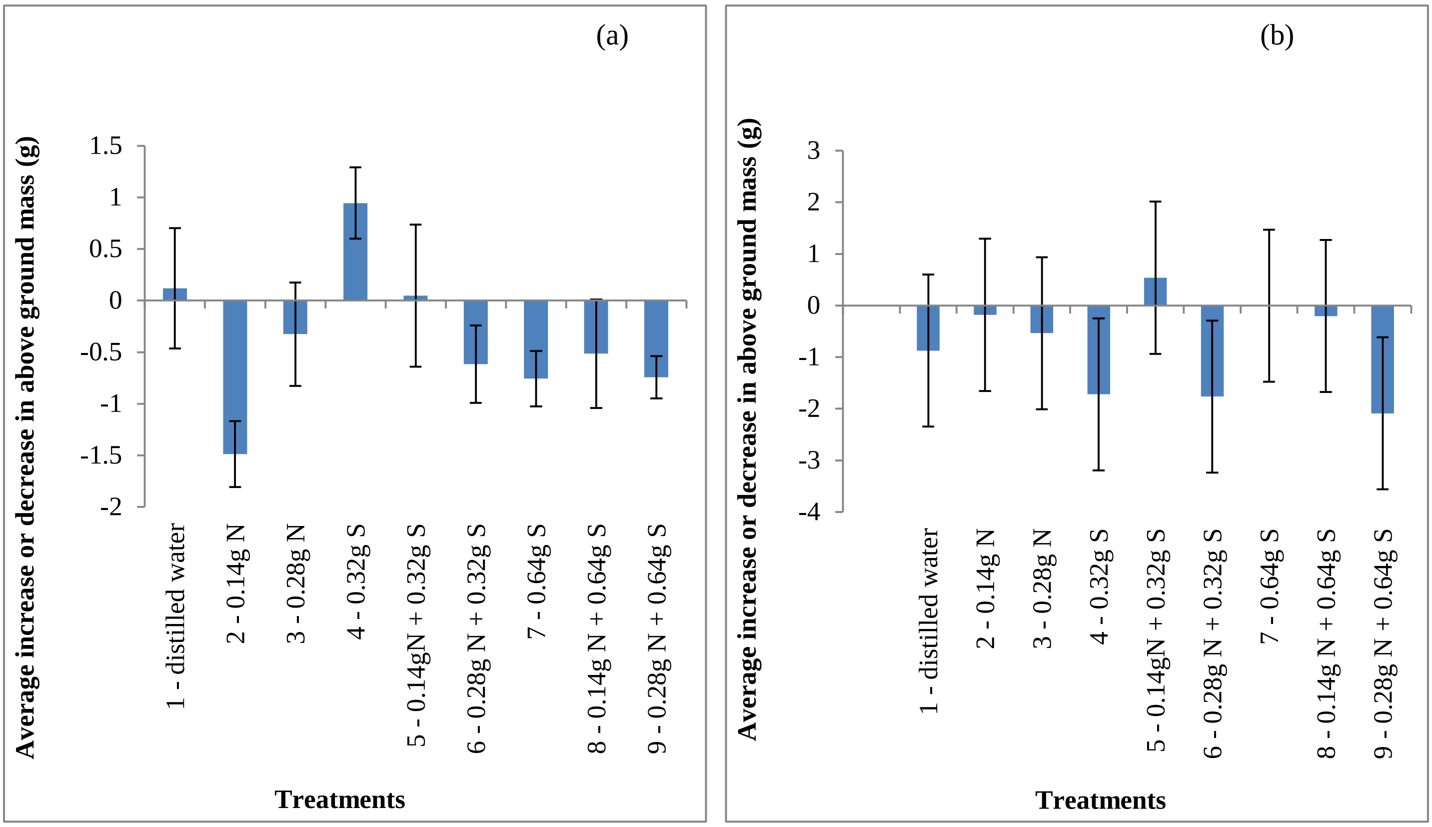
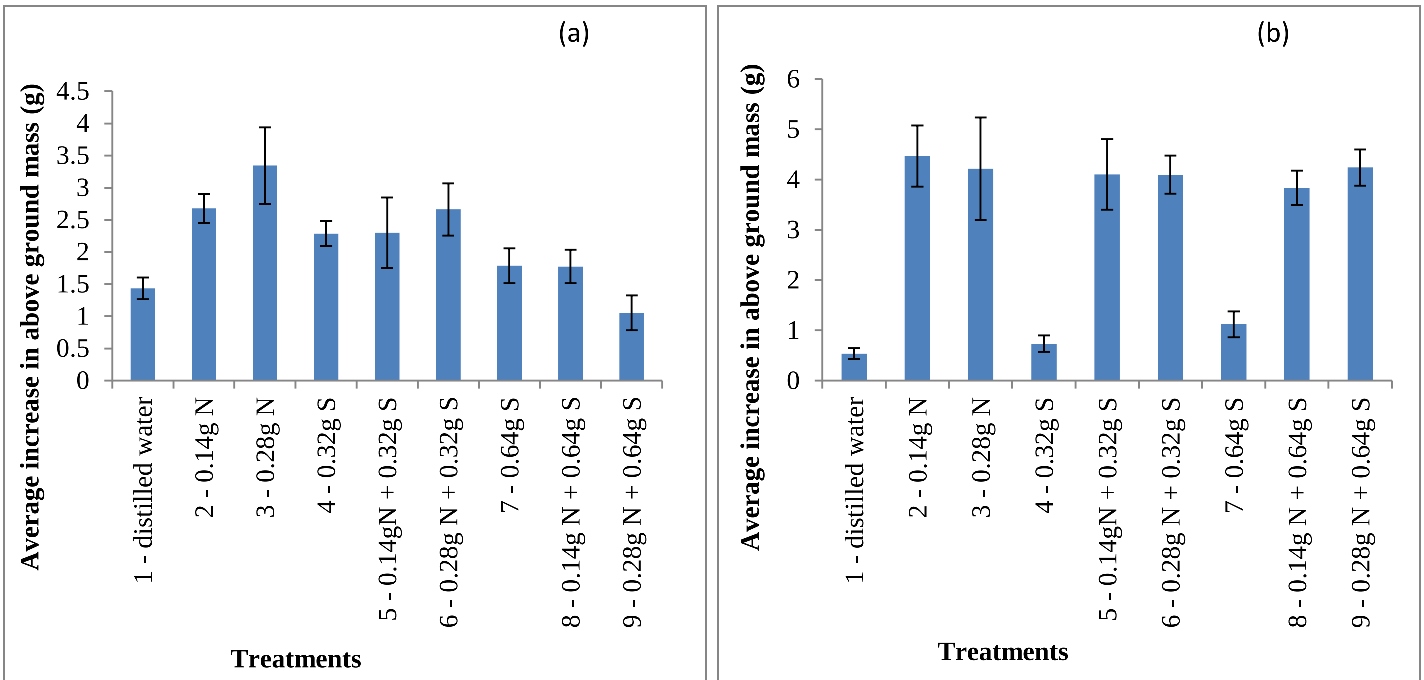


Figure 16. The average (Mean ± SE) increase or decrease of above ground dry mass in *Acacia sieberiana* (a) and *Combretum erythrophyllum* (b) across all nine treatments (N = nitrogen and S = sulphur).

The average above ground mass in *Eragrostis curvula* increased with all treatments except treatment nine, which contained the greatest amounts of both nitrogen (0.28g) and sulphur (0.64g), compared to treatment one (Figure 17). The above ground mass of *Eragrostis curvula* was responsive to nitrogen and sulphur; however it was sensitive to the higher levels of sulphur and nitrogen in combination. Significant differences were seen between treatment one (distilled water) and treatment three (N = 0.28g) ($F = -0.207$, $P < 0.05$), and treatment three (N = 0.28g) and treatment nine (N = 0.28g + S = 0.64g) ($F = 3.995$, $P < 0.01$). The average above ground mass in *Panicum maximum* increased with all treatments in comparison to treatment one, however the greater increases can be seen in those treatments containing nitrogen (Figure 17). The above ground mass of *Panicum maximum* is highly responsive to both nitrogen alone, and in combination, with a slight response seen with sulphur addition alone.

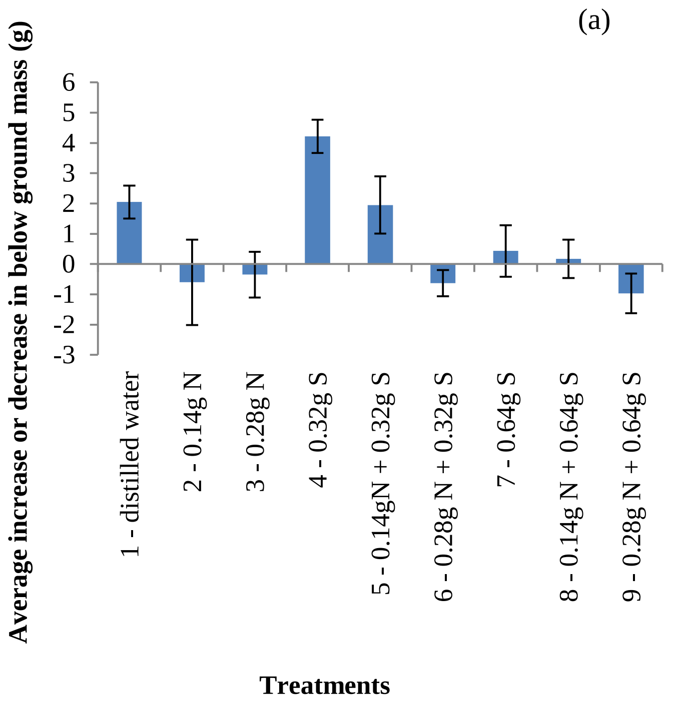


Treatments	P value
1 and 2	P < 0.001
1 and 3	P < 0.001
1 and 5	P < 0.01
1 and 6	P < 0.01
1 and 8	P < 0.01
1 and 9	P < 0.001
2 and 4	P < 0.001
2 and 7	P < 0.01
3 and 4	P < 0.01
3 and 7	P < 0.01
4 and 5	P < 0.01
4 and 6	P < 0.01
4 and 8	P < 0.01
4 and 9	P < 0.01
5 and 7	P < 0.01
6 and 7	P < 0.01
7 and 8	P < 0.05
7 and 9	P < 0.01

Figure 17. The average (Mean ± SE) increase in above ground dry mass in *Eragrostis curvula* (a) and *Panicum maximum* (b) across all nine treatments (N = nitrogen and S = sulphur). The significant differences in above ground mass in *Panicum maximum* can be seen in the figure.

4.2.3 The average increase or decrease in below ground dry mass in all four plant species, over the nine treatments

The average below ground mass (final below ground mass – initial below ground mass) in *Acacia sieberiana* increased in treatment four in comparison to treatment one, and decreased in all eight other nitrogen and sulphur treatments (Figure 18). The below ground mass of *Acacia sieberiana* was found to respond to low levels of sulphur addition alone, however it was sensitive to all other treatments containing nitrogen and sulphur. The average below ground mass of *Combretum erythrophyllum* increased in all treatments, except for decreases in treatment four and six, which both consisted of 0.32g of sulphur, compared to treatment one (Figure 18). The below ground mass of *Combretum erythrophyllum* responded to high levels of sulphur addition and all treatments containing nitrogen, and was found to be sensitive to low levels of sulphur addition. No significant difference was seen in the below ground mass of *Combretum erythrophyllum* with the addition of nitrogen ($F = 0.768$, $P > 0.05$), or sulphur ($F = 0.994$, $P > 0.05$) or the interaction between nitrogen and sulphur ($F = 0.168$, $P > 0.05$).



Treatments	P value
1 and 2	$P < 0.05$
1 and 3	$P < 0.001$
2 and 4	$P < 0.01$
3 and 4	$P < 0.05$
4 and 7	$P < 0.05$
4 and 8	$P < 0.05$
4 and 9	$P < 0.01$

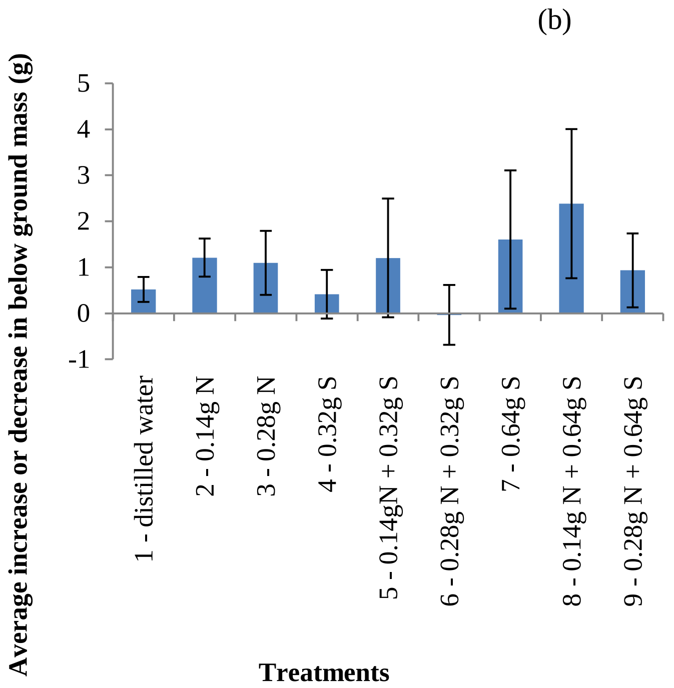
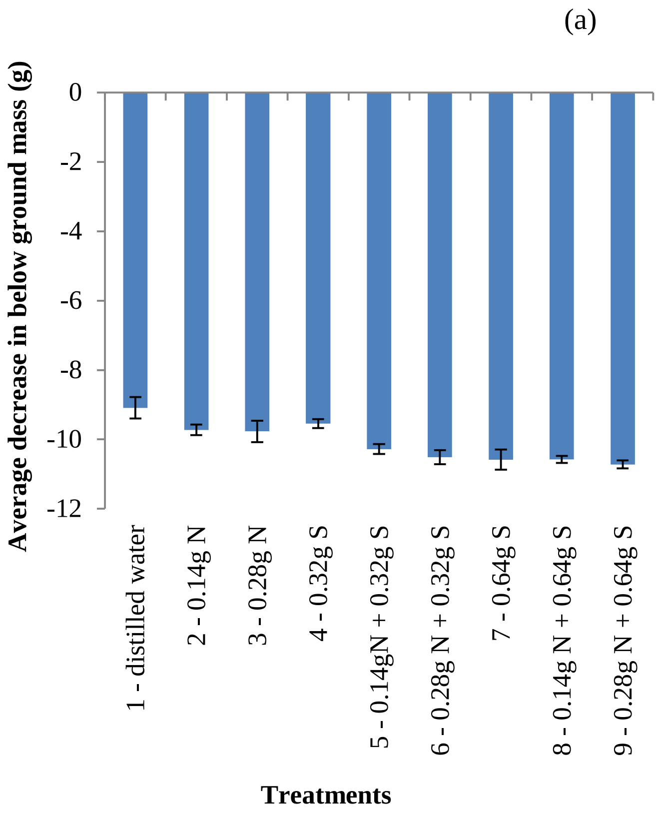
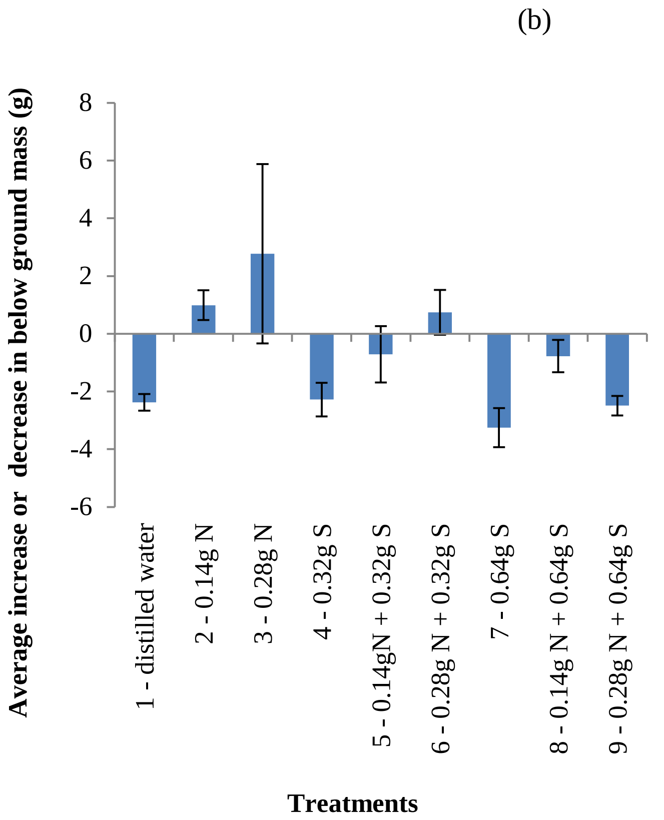


Figure 18. The average (Mean ± SE) increase or decrease in below ground dry mass in *Acacia sieberiana* (a) and *Combretum erythrophyllum* (b) across all nine treatments (N = nitrogen and S = sulphur). Significant differences in below ground mass for *Acacia sieberiana* can be seen in the figure.

All treatments added to *Eragrostis curvula* resulted in a decrease in average below ground mass compared to treatment one (Figure 19). The below ground mass of *Eragrostis curvula* was found to be sensitive to both nitrogen and sulphur additions. Compared with treatment one the average below ground mass in *Panicum maximum* increased in all treatments, except treatments seven and nine, both of which contained 0.64g of sulphur (Figure 19). The below ground mass of *Panicum maximum* was found to respond to nitrogen and low levels of sulphur, and was sensitive to high levels of sulphur addition.



Treatments	P value
1 and 2	P < 0.05
1 and 3	P < 0.01
1 and 4	P < 0.01
1 and 5	P < 0.05
1 and 6	P < 0.01
1 and 7	P < 0.001
1 and 8	P < 0.001
1 and 9	P < 0.001
2 and 9	P < 0.05
4 and 8	P < 0.05
4 and 9	P < 0.05



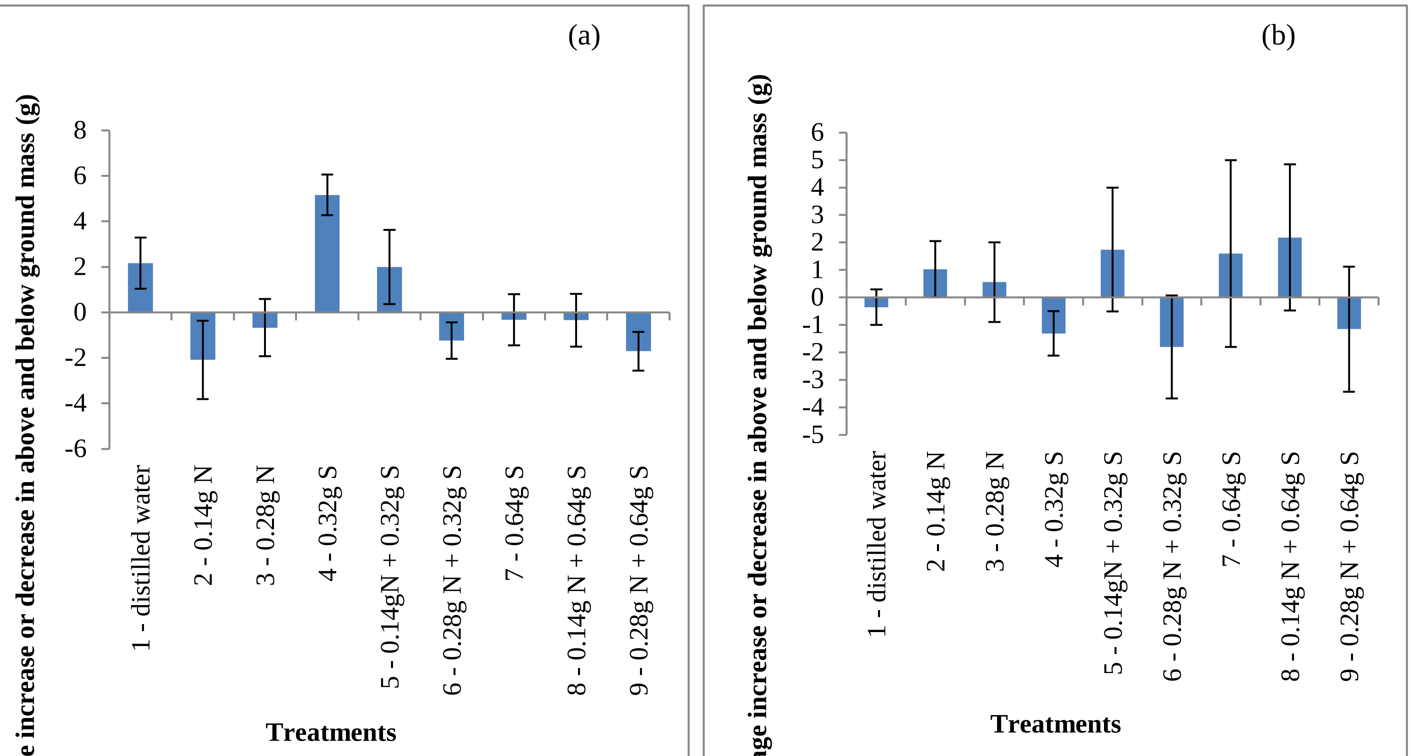
Treatments	P value
1 and 2	P < 0.05
1 and 3	P < 0.05
1 and 7	P < 0.05
3 and 7	P < 0.05

Figure 19. The average (Mean ± SE) increase or decrease in below ground dry mass in *Eragrostis curvula* (a) and *Panicum maximum* (b) across all nine treatments (N = nitrogen and S = sulphur). Significant differences in below ground mass of *Eragrostis curvula* and *Panicum maximum* can be seen in the figure.

4.2.4 The average increase or decrease in above and below ground dry mass in all four plant species, over the nine treatments

The above and below ground mass was added, with an average increase or decrease being calculated by subtracting the initial above and below ground mass from the final above and below ground mass.

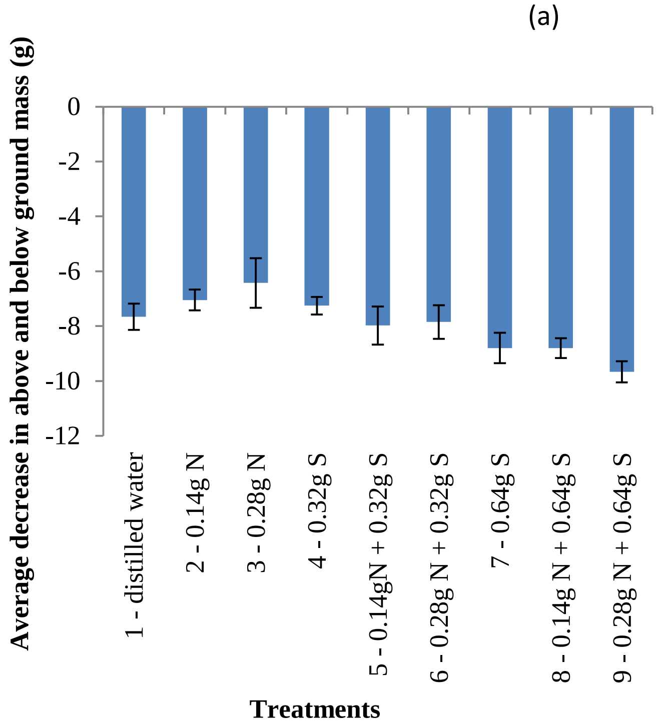
An average increase in below and above ground mass in combination (final above and below mass – initial above and below mass) can only be seen in treatment four in *Acacia sieberiana*. Any additional sulphur, or the addition of nitrogen resulted in a decrease in the total biomass, compared to treatment one (Figure 20). The total biomass of *Acacia sieberiana* responded to low levels of sulphur alone, however it was sensitive to all nitrogen additions and to higher levels of sulphur addition. The average below and above ground mass increased in *Combretum erythrophyllum* in treatments two, three, five, seven and eight, and decreased in treatments four, six and nine, all of which contained sulphur, in comparison to treatment one (Figure 20). The total biomass of *Combretum erythrophyllum* was variable but was found to be sensitive to sulphur additions. The below ground losses were largely offset by the increase in above ground mass. No significant difference was seen in the below ground mass of *Combretum erythrophyllum* with the addition of nitrogen ($F = 1.252$, $P > 0.05$), or sulphur ($F = 0.365$, $P > 0.05$) or the interaction between nitrogen and sulphur ($F = 0.354$, $P > 0.05$).



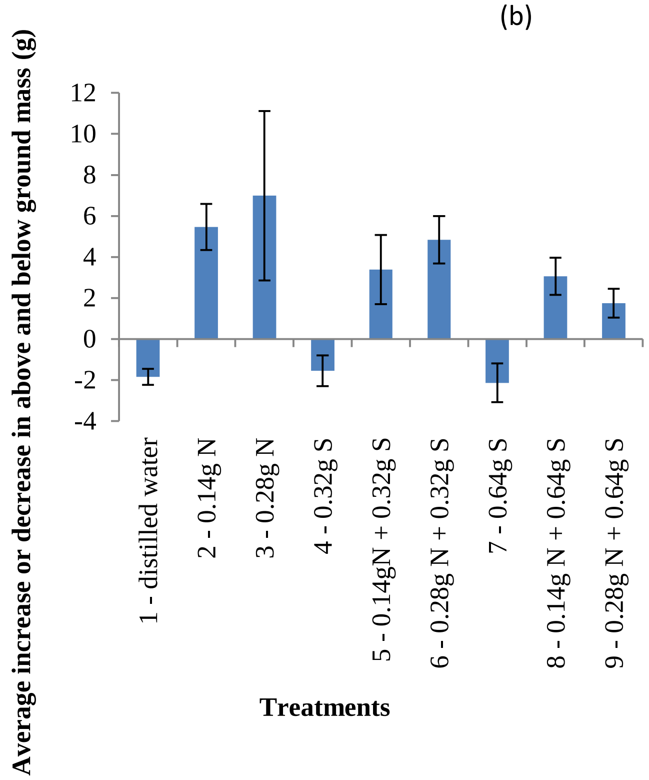
Treatments	**P value**
1 and 2	$P < 0.05$
1 and 3	$P < 0.01$
2 and 4	$P < 0.01$
3 and 4	$P < 0.05$
4 and 6	$P < 0.05$
4 and 7	$P < 0.05$
4 and 8	$P < 0.05$
4 and 9	$P < 0.01$

Figure 20. The average (Mean ± SE) increase or decrease in above and below ground dry mass in *Acacia sieberiana* (a) and *Combretum erythrophyllum* (b) across all nine treatments (N = nitrogen and S = sulphur). Significant differences in *Acacia sieberiana* can be seen in the figure.

In *Eragrostis curvula* the loss in below ground mass was far greater than the increase in the above ground mass, resulting in an overall decrease in total biomass. Treatments two and three resulted in less of a decrease, in the above and below ground mass compared to treatment one. The remaining treatments increased the decrease in total biomass (Figure 21). The total biomass of *Eragrostis curvula* was responsive to nitrogen alone and sensitive to any treatment levels that contained sulphur additions. The total biomass in *Panicum maximum* increased in all the treatments, except for treatment seven which contained the greater amount of sulphur (S = 0.64g) addition. Below ground loss in *Panicum maximum* was offset by increases in above ground mass. The below and above ground mass of *Panicum maximum* was responsive to all treatments containing nitrogen, and sensitive to sulphur when added alone. The greater increases were seen in treatment two and three which only contained nitrogen (Figure 21). There were a number of significant differences in total biomass resulting from the addition of nitrogen and sulphur in *Panicum maximum* (Figure 21).



Treatments	P value
1 and 7	P < 0.001
1 and 9	P < 0.05
2 and 8	P < 0.05
2 and 9	P < 0.01
3 and 7	P < 0.01
3 and 8	P < 0.01
3 and 9	P < 0.001
4 and 7	P < 0.001
4 and 9	P < 0.01



Treatments	P value
1 and 2	P < 0.05
1 and 3	P < 0.01
2 and 7	P < 0.05
3 and 4	P < 0.05
3 and 7	P < 0.01

Figure 21: The average (Mean ± SE) increase or decrease in above and below ground dry mass in *Eragrostis curvula* (a) and *Panicum maximum* (b) over all nine treatments (N = nitrogen and S = sulphur). Significant differences in the above and below ground mass of *Eragrostis curvula* and *Panicum maximum* can be seen in the figure.

4.2.5 Soil acidification in all four plant species, over the nine treatments

An increase in soil acidity is shown by a negative average difference in soil pH value, and a decrease in soil acidity is shown by a positive average difference in soil pH value. This was calculated by taking an initial average pH of the soil that all the plant species were grown in and subtracting this from the average pH obtained per plant species, per treatment, once the plants were harvested. Figure 22 shows the average difference in soil pH in all four plant species across the nine treatments. An increase in soil acidity is seen in some treatments in which the two tree species were grown, whereas a decrease in soil acidity was seen in some treatments in which grass was grown.

The results between treatments and between plant species were variable, but essentially in almost all cases there was a decrease in soil acidity (Figure 22). This decrease in soil acidity is very difficult to explain. The solutions that were added were acidic (pH 3.4 – 4.3) and the soil was sandy with very little buffering capacity (soil pH 5.3). Two possible explanations for this decrease in soil acidity could be that of the potassium which was the cation in the solution added, which may have acted to some extent as a buffer, and there may have been a differential uptake of the anions (nitrate and sulphate) from the soil solution into the plant, thereby leaving the soil solution more basic. Treatment one (distilled water) resulted in an increase in soil pH in all the plant species. The greatest increase in soil acidity was seen in *Combretum erythrophyllum* in treatment three (N = 0.28g).

The change in soil pH between treatments between the two tree species, *Acacia sieberiana* and *Combretum erythrophyllum*, was not significantly different ($F = 1.001$, $P > 0.05$), however the change in soil pH between treatments between the two grass species, *Eragrostis curvula* and *Panicum maximum*, was significantly different ($F = 39.90$, $P < 0.001$). For the majority of treatments between the two grass species a significant difference was seen. The significant changes in soil pH between treatments between the tree and grass species can be seen in Table 15. For the majority of treatments between the tree and grass species a significant difference was seen, excluding the treatments between *Acacia sieberiana* and *Eragrostis curvula*.

Table 15. The significant changes in soil pH over all nine treatments between the two tree species, *Acacia sieberiana* and *Combretum erythrophyllum*, and the two grass species, *Eragrostis curvula* and *Panicum maximum*.

Plant species	*Eragrostis curvula*	*Panicum maximum*
Acacia sieberiana	F = 1.183, P > 0.05	F = 8.523, P < 0.001
Combretum erythrophyllum	F = 3.238, P < 0.01	F = 8.678, P < 0.001

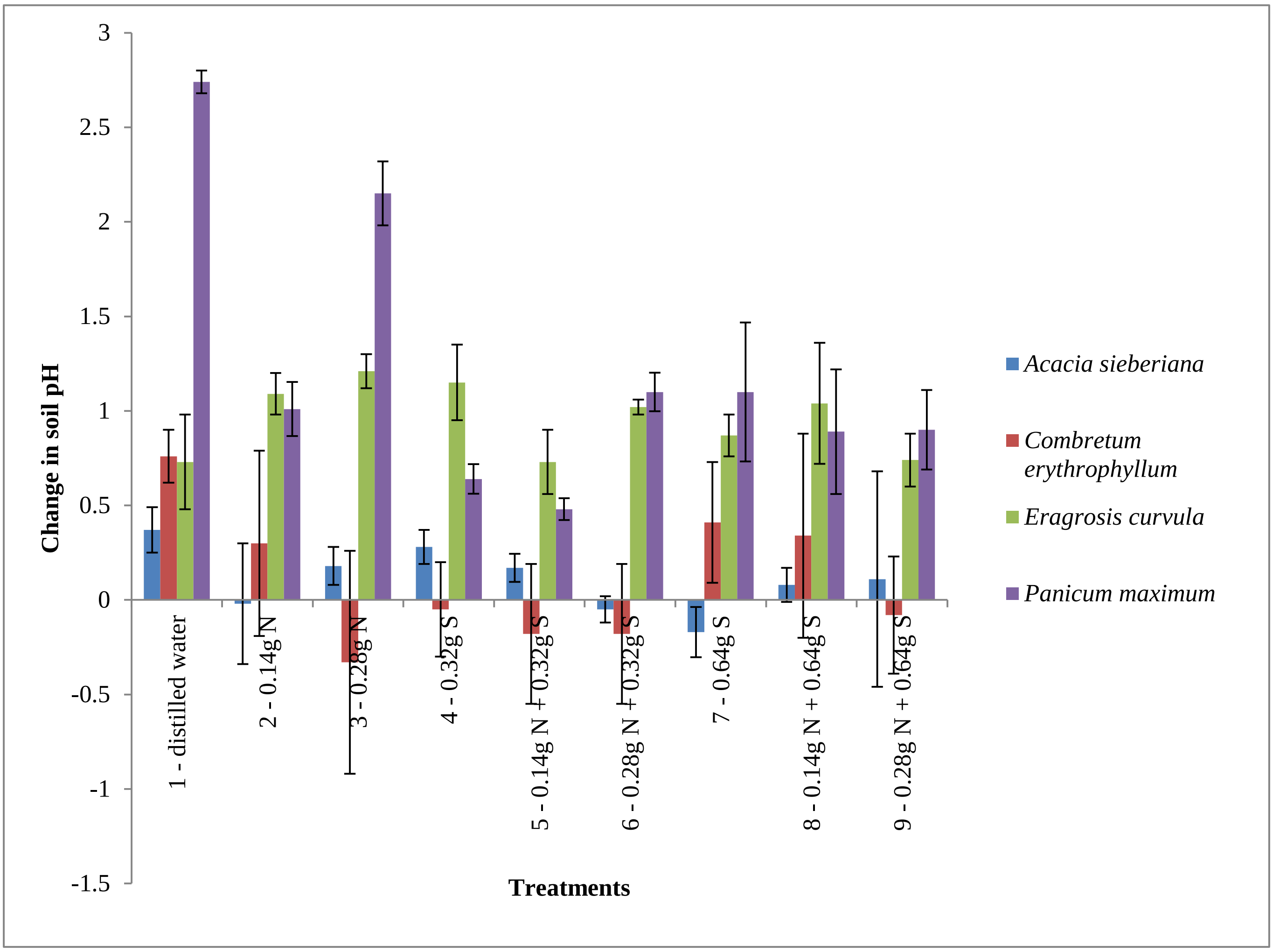


Figure 22. The change in soil pH (Mean ± SD), in each treatment, in each of the four plant species (N = nitrogen and S = sulphur). Negative values indicate increasing soil acidity and positive values decreasing soil acidity.

4.2.6 The average increase or decrease in above and below ground mass in all four plant species, across all nine treatments measured against the average change in soil pH

A relationship was seen to exist between the increase in the average above and below ground mass and a change in soil pH in *Acacia sieberiana* ($R^2 = 0.45$). This is a result of the average above ground mass and the average below ground mass both responding to decreases in the soil acidity. Therefore an increase in the average above and below ground mass is seen with a decrease in soil acidity (Figure 23). No relationship exists between the increase in the average above and below ground mass in *Combretum erythrophyllum* and a change in soil pH ($R^2 = 0.08$) (Figure 23).

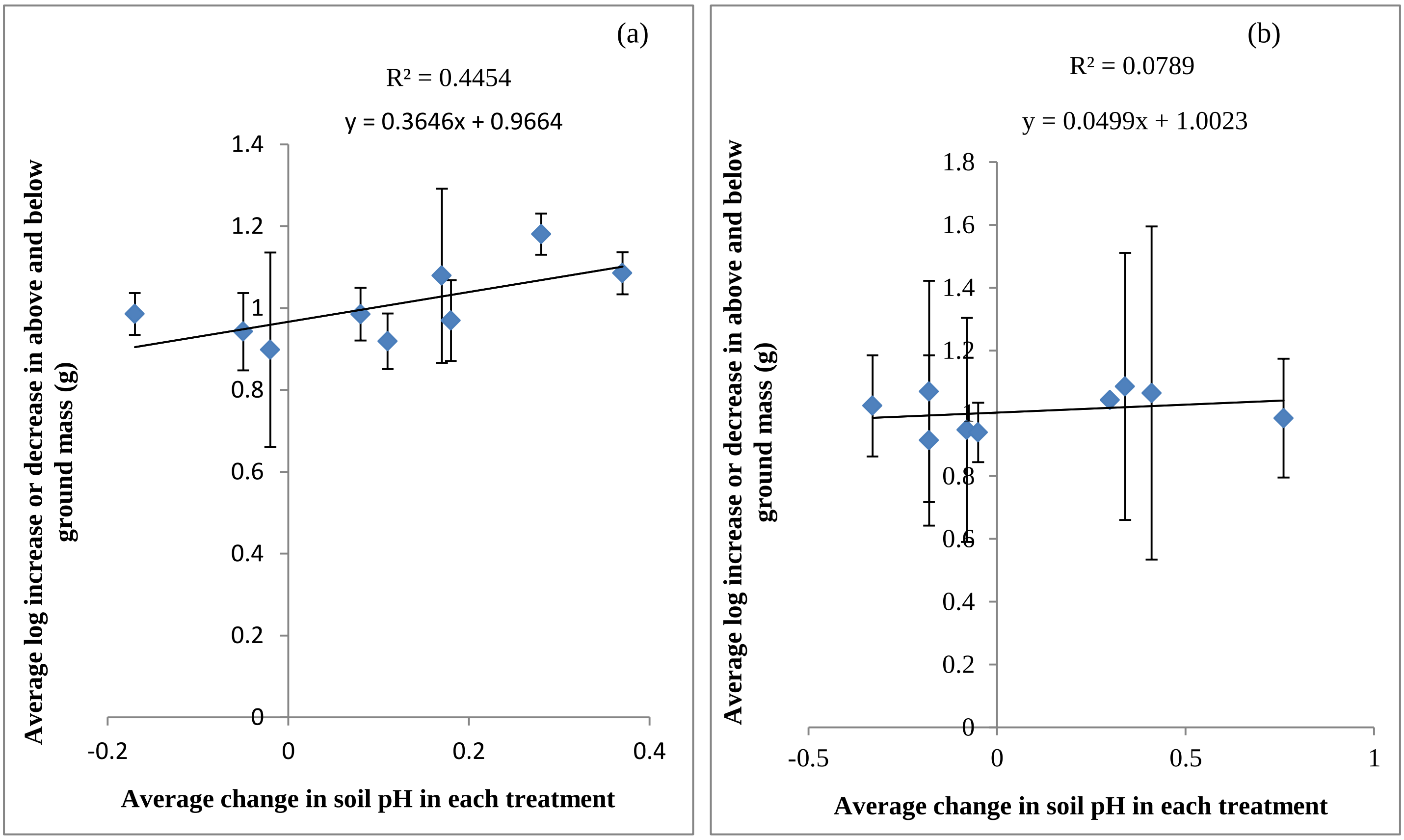


Figure 23. The relationship between the logged average increase or decrease in the above and below ground dry mass (final above and below ground mass – initial above and below ground mass) measured against the average difference in soil pH, in *Acacia sieberiana* (a) and *Combretum erythrophyllum* (b), across all nine treatments (Mean ± SE).

A positive relationship exists between the increase in average above and below ground mass in *Eragrostis curvula* and a change in soil pH (R^2 = 0.31). A slight increase in average above and below ground mass is seen with a decrease in soil acidity (Figure 24). This increase was mainly due to the increase in above ground mass with a decrease in soil acidity, rather than an increase in below ground mass. No relationship was found between the average increase in above and below ground mass in *Panicum maximum* and a change in soil pH (R^2 = 0.01) (Figure 24).

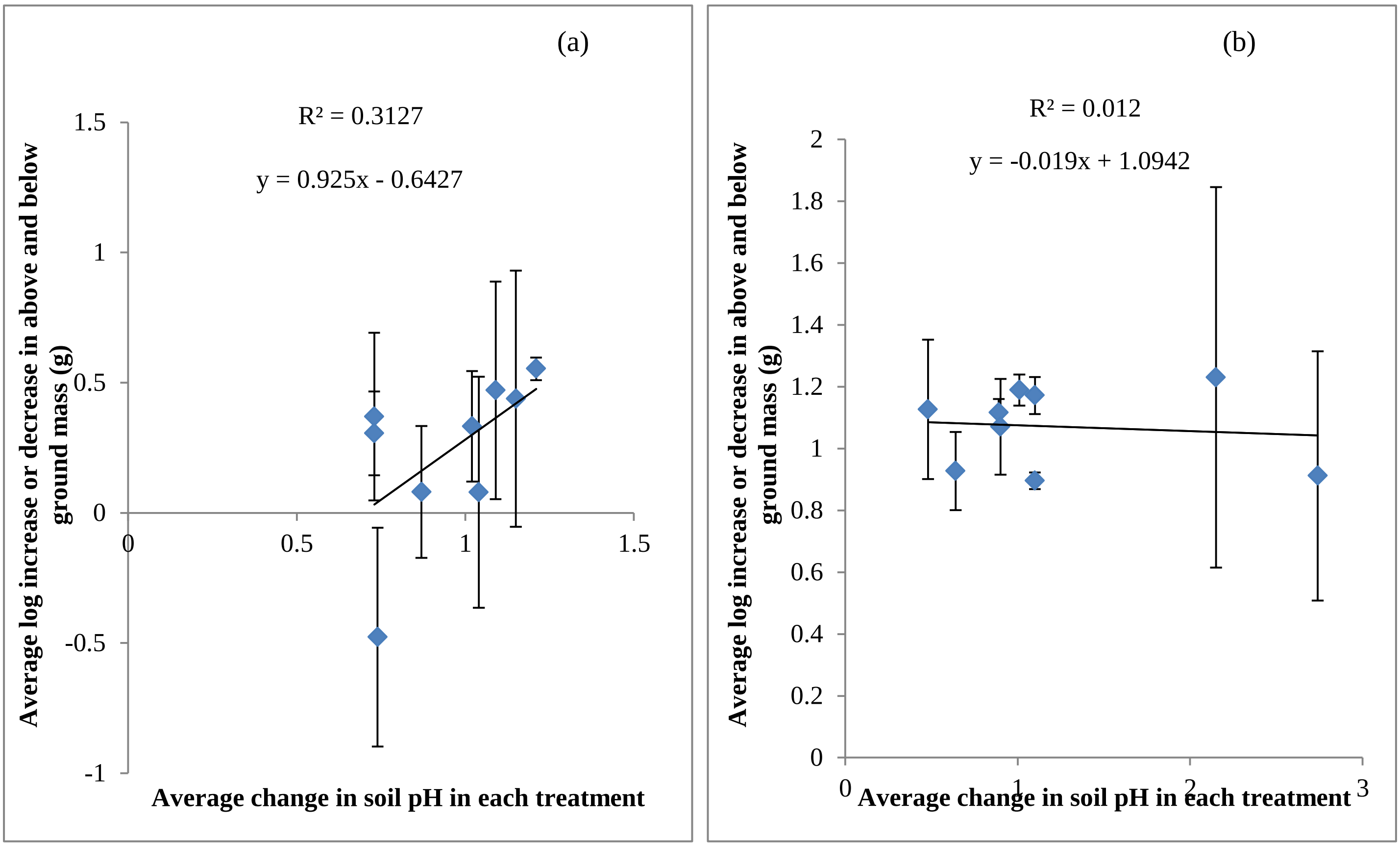


Figure 24. The relationship between the logged average increase or decrease in the above and below ground dry mass measured against the average difference in soil pH, in *Eragrostis curvula* (a) and *Panicum maximum* (b), across all nine treatments (Mean ± SE).

Chapter 5: Discussion

5.1 An assessment of vegetation biodiversity

A baseline vegetation assessment took place in four sites, two upwind of the power stations, Matimba and Medupi, and two downwind of the power stations, in the areas surrounding Lephalale in the Waterberg in the Limpopo Province.

It was found that the downwind site, Bosveld, had a high tree density, although it had low tree biomass. The dominant tree species in this site was *Commiphora pyracanthoides*, many of which were in the initial stages of growth with regards to their size. This is a fast growing and easily recruited tree species (Grant and Thomas 2000). Bosveld had the largest increase in grass and forb biomass with the rains; this is expected with this site's low tree biomass. This site also had the lowest overall species diversity and the lowest tree diversity, as well as having the lowest species richness. Landelani, the other downwind site and the site closest to both power stations, was found to have a low tree density with a high tree biomass. The individual trees in this site are mostly large species which are well established and fall within a similar age distribution. This site also had the largest grass and forb biomass both before and after the rains; however it had the lowest grass and forb diversity of all four sites. Kraaij and Ward (2006) found that additional nitrogen deposition in savanna systems in the Northern Cape resulted in increases in grass growth which negatively affected the establishment of tree seedlings. This may therefore negatively impact the establishment and growth of trees in these areas. Zvereva and Kozlov (2012) also found that tree abundance has been shown to decrease in more acidic environments. Trees in this site may be further vulnerable in the future with increases in soil acidity expected, as soil in downwind sites already show decreases in soil pH (Itzkin 2012). It has also been found that areas that have greater species diversity and higher productivity are more susceptible to pollution impacts as these environments are already competitive (Zvereva *et al*. 2008; Zvereva and Kozlov 2012). The Landelani site had the highest overall species diversity, as well as tree diversity of all four sites, making this site particularly vulnerable to future pollution impacts.

The beta diversity between the two downwind sites was the highest, showing greater species variation between these two sites. It would be assumed that sites which are closer to one another would share similar species, whereas those that are further apart would have dissimilar species. Past research by Trubina and Vorobeichik (2012) showed that beta diversity between areas actually increases as air pollution levels increase. This may imply that the two downwind sites are already impacted by the current pollution in the area, which will only be further impacted as these pollution levels increase in the future.

Withoutpan is the closest upwind site to both power stations. This site had the second highest tree density, and the highest tree biomass, with large, well established tree species. This site had the second highest overall species richness after the other upwind site, Alfred. Withoutpan was similar to Landelani with regards to tree biomass and tree species diversity. In the future we will be able to compare the impacts of increasing pollution levels on trees, in relation to upwind and downwind sites, with regards to changes in tree biomass and tree species diversity in the downwind Landelani and upwind Withoutpan sites. Withoutpan and the downwind site, Landelani had the next overall highest beta diversity, even though they are located next to one another, as well as the power stations, and may therefore also be already impacted.

The other upwind site, Alfred, had a low tree density, as well as low tree biomass. This site was dominated by fine-leaved trees, with over 65% of the site being composed of five different *Acacia* species. *Acacia mellifera* was the dominant tree species in this site. The greenhouse experiment showed that *Acacia sieberiana* was sensitive to sulphur and nitrogen additions, as well as soil acidification. If this is true for all *Acacia* species this site's trees may be particularly vulnerable by losing a large proportion of its tree density and biomass, even if it is found upwind of the power stations. Isabell *et al.* (2013) showed that the loss of a dominant species within an environment has a greater effect on ecosystem productivity than the loss of a rare species, and this will have impacts on the areas ecosystem functioning. Loss of the dominant *Acacia* species in this environment will result in changes to vegetation composition and structure and therefore ecosystem functioning. This site had the highest grass and forb species richness, as well as the highest grass and forb diversity. This site was unique in that the dominant grass species, *Enneapogon scoparius*, and the dominant forb species, *Geigeria burkei*, were not shared by any of the other sites.

Future monitoring in these four sites will need to be continued with close attention being paid to sites with lower tree density and higher tree biomass, such as Landelani and Alfred, as losses in these sites will have a large impact on the site's ecosystem functioning.

The average species richness in savannas in South Africa is 67 species per 0.1 ha, with variations found between 50 and 100 species depending on the amount of rainfall in the area (Cowling *et al.* 1989; Shakelton 2000). The species richness in all four sites are already found to be below this average, with the upwind Alfred site having the greatest species richness of 46 different plant species, whereas the downwind Bosveld site was far below this average with a species richness of 26 different plant species.

5.2 Vulnerability of plant species to nitrogen and sulphur addition, and increasing soil acidity

It was found that all the plant species showed a sensitivity of varying degrees to the sulphur additions. *Acacia sieberiana* was additionally found to be sensitive to all the levels of nitrogen addition, whereas *Combretum erythrophyllum* responded to nitrogen when it was added alone. The two grass species also responded to the nitrogen addition when it was added alone, however the positive effects of the nitrogen addition were negated by any sulphur addition in *Eragrostis curvula*. In *Panicum maximum* the positive response in height and above and below ground mass from nitrogen addition exceeded any negative effects from the sulphur addition. Even though a positive response in *Combretum erythrophyllum* and *Eragrostis curvula* to nitrogen added alone, and positive responses seen in *Panicum maximum* regardless of sulphur additions were seen, it has been found that increases of 2.5kg N ha^{-1} yr^{-1} actually result in the loss of one vegetation species (Steven *et al*. 2004).

Dispersion modelling simulations show that the SO_2 emissions from the operational Matimba power station result in ambient standards being exceeded, and this will be further exceeded once Medupi becomes operational (WDM 2009). Feig (2010) further predicted that the hourly accepted

SO_2 levels will be exceeded with both Matimba and Medupi power stations in operation. According to Eskom (2014) the current ratio of SO_2 : NO_x emissions are in the ratio of 2:1, therefore the only species that may be positively impacted by the additional sulphur and nitrogen deposition will be *Panicum maximum,* and decreases in both the tree species and *Eragrostis curvula* can be expected. Whether these decreases will be in the size of the species and therefore productivity, or will result in decreases in the absolute numbers of species, or will change the palatability of the species, remains to be seen.

The impacts of nitrogen and sulphur additions to the soil were variable and hard to explain. Soil from the Bosveld site was sandy and had very little buffering capacity (pH 5.3), whereas the solutions that were added were acidic (pH 3.4 – 4.3). An increase in soil pH, and therefore a decrease in soil acidity, was seen in both grass species, and most of the *Acacia sieberiana* treatments, although this was to a lesser degree. In most of the *Combretum erythrophyllum* treatments there was a decrease in soil pH and therefore an increase in soil acidity. This may have been a result of the solutions all containing potassium which may have had a buffering effect in the soils, or perhaps the differing absolute uptake of cations and anions from the soil solution by the different plant species.

Zvereva and Kozlov (2012) found that tree abundance decreased in soils which became more acidic, or more alkaline, whereas shrub and herb abundance only decreased in more acidic soils and grass abundance did not change in either more acidic or alkaline soils. In most of the *Acacia sieberiana* treatments the soil showed a slight decrease in soil acidity, whereas an increase in soil acidity was seen in five of the treatments of *Combretum erythrophyllum*.

The results regarding plant growth and soil acidification seen in this research were different between functional groups and within functional groups. *Acacia sieberiana* showed a decrease in above ground and below ground mass with increasing soil acidity, whereas there was little change in either the above or below ground growth of *Combretum erythrophyllum* with increasing soil acidity. The above ground mass of *Eragrostis curvula* was found to slightly increase with a decrease in soil acidity, while there was no change in below ground mass seen with increasing soil acidity. No change was seen in the above and below ground growth of *Panicum maximum* with increasing soil acidity. Chapin III *et al.* (1997) found that functionally similar species that had different sensitivities to changing environments actually increase ecosystem stability, whereas functionally different species that are different in their sensitivities to changing environmental conditions result in increases to ecosystem vulnerability. In this research species within the functional groups responded differently to the increase in soil acidity, thereby increasing ecosystem stability. However the results seen between functional groups showed that there was no or little response in the change of growth in either *Combretum erythrophyllum* or *Panicum maximum*; whereas a negative response in the change of growth was seen in *Acacia sieberiana* and *Eragrostis curvula.*

Studies by Zvereva and Kozlov (2012) have found that trees are negatively affected by pollution impacts before either shrubs or herbs, or grasses are affected. This was found to be mainly a result of air pollution rather than soil nutrient availability. Dry and wet deposition in the form of nitrogen and sulphur are components of air pollution. The precipitation in this area is low and variable, meaning that the impacts of dry deposition may play a larger role in impacting vegetation composition and structure than wet deposition.

5.3 Implications for ecosystem functioning and long-term monitoring

5.3.1 Ecosystem functioning

Ecosystems are essential in providing humans with a range of services. These ecosystem services include provisional, regulatory, cultural, recreational and aesthetic and supporting services (Secretariat of the Convention on Biological Diversity 2010). Changes in the functioning of these ecosystem services result because of environmental disturbances from human activities, including changes in biogeochemical cycles, which result in a decrease in the resilience of an ecosystem, thereby threatening these ecosystem functions (Chapin III *et al.* 2002). A way in which ecosystem resilience is increased is to conserve its biodiversity thereby decreasing any negative impacts of climate change and increasing pollution.

This study focused on photosynthetic pathways as the functional type, mainly C3 woody species and C4 grass species. Functional types are groups of species that have a similar response to changing environmental conditions. Diversity within a functional type increases the buffering ability of an ecosystem and therefore decreases the vulnerability of that functional type to environmental changes (Chapin III *et al.* 2002). It is therefore important to ensure that biodiversity within an area is conserved in order to ensure ecosystem stability.

5.3.2 Long term monitoring and measurements of impacts

There is a large amount of variability within the studied system resulting from a number of different sources. The Waterberg area is semi-arid, with high inter-annual climatic variability. The rainfall in this region is low and variable, with rainfall being found to occur later in the year with increased dry spells (Claasen *et al.* 2010; MacKeller *et al.* 2014). Over the period of the last 50 years there has been a decrease in rainfall of 16 rain days (MacKeller *et al*. 2014). There is also high temperature variability; both in daily temperature range from mild in the morning to very hot in the afternoon, as well as seasonal temperature variability. MacKeller *et al.* (2014) found that within this area, maximum temperatures in winter have increased, with a 1°C increase in maximum temperatures over a 50 year period. Minimum temperatures have also increased in this area both in summer and winter, with there being a 0.011°C increase in minimum temperatures per year. Another source of variability is the geological substrate found within the area. In general the area falls within the Waterberg Sandstone (Claasen *et al.* 2014), however there are a number of other substrates which at the microtopographical scale would lead to patches of nutrient rich and nutrient poor soils.

These sources of variability drive plant diversity. The four sites that were sampled showed inherent variability, with none of the sites having common dominant species, although these species were shared in at least one other site, and Alfred, the upwind site, having a unique dominant forb and grass species. These sites may also be on different naturally occurring landscapes with upwind sites falling within the Karoo group geology and downwind sites within the Waterberg group geology, or even occurring in different cantenal positions which may further explain the variability of the differing vegetation between the sites. Comparisons will still be able to made on changes within sites with further future pollutions and across sites. There is also an unresolved conflict as to whether the selected sites are background sites, which they are when future developments are considered, or sites which contribute to a transect of current pollution, which they also are when considering current developments. This research gave insights into the patterns of the plant diversity found within this area; however this needs to be addressed on a far greater scale in future. Larger baseline surveys will need to be conducted by using GIS and remote sensing in order map out heterogeneous and homogenous samples within the larger Waterberg area in order to expand the scope of this research. The data collected in these larger studies will enable future studies to determine what changes take place as a result of increased pollution pressures and the rate of those changes (Vackar *et al.* 2013).

Dominant species or keystone species in these areas also need to be identified and used as an indicator of change, as changes in these species rather than rare species, will result in greater changes to productivity, vegetation structure, and ecosystem properties, including goods and services and ecosystem resilience.

5.3.3. Future studies

There are still a number of areas in which future work is needed in order to have a better understanding of the pollution impacts on vegetation composition and structure, and therefore vegetation biodiversity. There is currently no research on base cation deposition in the area, which is occurring concurrently with acidic nitrogen and sulphur deposition. Base cation deposition needs to be taken into account when looking at changes in soil pH as these may counteract the acidifying effects of nitrogen or sulphur addition, or actually result in the soil becoming more alkaline (Lovblad *et al*. 2004). Future studies need to take base cations into consideration, particularly regarding dry deposition within the study site.

Josipovic *et al.* (2010) found that the highest total deposition concentrations were found in sites that were either centrally positioned within the industrial Highveld or were found downwind of the pollution sources. Experiences of developed countries have shown that acid rain results in vegetation damage, particularly downwind from pollution sources. This ecological damage can be repeated in developing countries, particularly with increases in pollution emissions in the future (Josipovic *et al.* 2010). Extensive further developments are expected within the Waterberg particularly with the development of the37GW power production facilities within South Africa and Botswana. There are currently no nitrogen and sulphur deposition data for the Waterberg and in particular Lephalale and the surrounding areas which are available to all researchers. However at present studies are being conducted in these areas to determine nitrogen and sulphur deposition data, which will need to be integrated into a study of this nature. Future studies on the impacts of plant growth to nitrogen and sulphur deposition and resulting soil acidification could be expanded by increasing the number of plant species tested or doing these experiments on site over an extended period.

5.3.4 Conclusion

Land in Lephalale, and the surrounding areas, is used for agriculture, game farming and hunting, cattle farming, conservation and eco-tourism. Changes in vegetation composition and structure in these areas surrounding the power stations will result in changes in land use. This will impact land owners and land users ability to use and manage their land and resources, ultimately impacting their capacity to generate an income, and therefore their livelihoods.

Increases in the deposition of nitrogen and sulphur, and resulting acidification of soils will result in changes in the composition and structure of vegetation within this area, which will have an unknown effect on ecosystem functioning, impacting ecosystem goods and services and impacting these ecosystems' resilience to future disturbances, climate change and increasing pollution impacts.

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