An initial investigation into key soil processes and associated influences on N and S cycles of a grassland site near a coal-fired power station, Mpumalanga, South Africa

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0700048T

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Science

DECLARATION

I declare that this Dissertation is my own, unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

29th day of October 2012

ABSTRACT

Nitrogen and sulfur deposition as a result of fossil fuel burning has affected numerous ecosystems globally. Documented impacts to terrestrial ecosystems include changes to soil chemical properties and processes, plant growth, plant productivity and surface water quality. The soils of the Mpumalanga Highveld grasslands in South Africa have been identified as sensitive to acidic atmospheric inputs. This study aimed to investigate nitrogen and sulfur cycling in the soils of a grassland site in the Sandspruit catchment (Mpumalanga) to assess the consequences of increased nitrogen and sulfur deposition on the potential for nitrate and sulfate leaching into nearby streams. Monthly vegetation, litter and soil sampling and analyses from October 2010 to May 2011 were used to construct nitrogen and sulfur input-output budgets that represented the inputs, cycling and outputs of nitrogen and sulfur in the Sandspruit system. The nitrogen and sulfur mineralization rates were found to range between -1.03 ± 0.15 and 1.05 ± 0.23 µg N g^{-1} soil day^{-1} and -1.06 ± 0.20 and 0.91 ± 0.21 µg S g^{-1} soil day^{-1} respectively. The nitrogen and sulfur input-output budgets showed sulfate leaching as the greatest threat to the Sandspruit system under the current and future modelled nitrogen and sulfur deposition rates.

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CHAPTER 1: INTRODUCTION

Humans have intentionally and unintentionally increased the amounts of nitrogen (N) and sulfur (S) in the atmosphere, on land and in water in an attempt to meet the demands of their population growth (Galloway *et al.*, 2004). Food and energy production have had to increase to sustain the human population growth rate. Together, these two anthropogenic activities have increased the release of reactive forms of N and S into the atmosphere since the industrial revolution (Galloway, 1995). Fertilizer use, due to volatilization can become a source of N and fossil fuel burning for electricity production emits both N and S into the atmosphere (Rodhe *et al.*, 1995). Fossil fuel burning releases N and S in the forms of nitrogen oxides (NO_x) and sulfur oxides (SO_x) and are often in reduced or partially oxidized states that may undergo a number of transformations in the atmosphere (Kennedy, 1986). The most common of these transformations convert NO_x and SO_x to nitrate (NO_3^-) and sulfate (SO_4^{2-}) respectively, which are deposited and, if they first react with water vapour in the atmosphere, are commonly referred to as acid deposition (Cowling & Linthurst, 1981; Van Breemen *et al.*, 1983; Fey & Guy, 1993; Galloway, 1995; Mphepya, 2002).

Known impacts of acid deposition fall into three main categories including changes in: soil chemical properties and processes; plant productivity and species diversity and surface water quality. Nitrogen and S deposition impacts soil chemical properties and processes by increasing the acid status of the soil, changing the amount and rate of leaching of base cations and changing the nutrient ratios and fluxes of the soil (Grennfelt & Hultberg, 1986). Following the changes to the soil, plant growth rates and plant species diversity are often negatively impacted in the area affected by deposition (Haddard *et al.*, 2000; Bobbink *et al.*, 2003; Clark & Tilman, 2008; Duprè *et al.*, 2010; Stevens *et al.*, 2010a). Changes to the pH and nutrient ratios of the soil alter the limiting resource for which plants compete,

intensifying the competition among plant species for the new limiting resource, often decreasing the plant species diversity (Jacobson *et al*., 2011; Stevens *et al*., 2011). Should the additional N and S from acid deposition not be immobilized by soil microorganisms or taken up by plants, the potential exists for increased surface run-off or leaching through the soil profile into nearby streams, negatively affecting surface water quality (Peierls *et al.*, 1991; Cole *et al.*, 1993; Vitousek *et al.*, 1997; Baron *et al.*, 2009).

Most of the research on the inputs, cycling and outputs of N and S, especially under moderate to severe acid deposition has taken place in the industrialized areas of the developed world, particularly forested regions of North America and Europe (Abrahamsen & Miller, 1984; Baron *et al.*, 2000; Takemoto *et al.*, 2001; Aber *et al.*, 2003; Fenn *et al.*, 2003a; Driscoll *et al.*, 2001). Strict environmental protection policies were put in place across North America and Europe following the realization of the hazardous effects S deposition was having on a number of ecosystems. The implementation of policies such as the Convention for Long-range Transboundary Air Pollution in Europe (Berge *et al.*, 1999; Reinds *et al.*, 2008) and the Clean Air Act in the United States (Butler *et al.*, 2001; Driscoll *et al.*, 2001; Likens *et al.*, 2001) has reduced S emissions and deposition. The Air Quality Act was introduced in South Africa on the 11th of September 2005 and was drafted to provide standards for air quality management and compliance (NEM: AQA 39 of 2004). These national ambient air quality standards, published for a number of air pollutants, were either adopted with immediate effect or will come into effect on the 1st of January 2015 (NEM: AQA 39 of 2004).

In a number of ecosystems in the US and Europe, N and S deposition rates have been recorded and modelled up to 36.0 kg N ha^{-1} yr^{-1} (Takemoto *et al.*, 2001; Stevens *et al.*, 2004) and 21.0 kg S ha^{-1} yr^{-1} (EPA, 2008) respectively. Although the

research focus has now shifted to the effects of N emissions and deposition, certain biomes (such as grasslands) remain sensitive to deposition and have not received the necessary attention to quantify the impacts of neither S nor N deposition.

In South Africa, coal is the most widely used fuel source for electricity production and coal-fired power stations provide approximately 90.0% of the country's electricity (Eskom, 2006). Eskom (Electricity Supply Commission), South Africa's primary electricity provider has built power stations near coal seams to keep production costs to a minimum (Eskom, 2006). Approximately 80.0% of South Africa's coal is located in seams within the Mpumalanga province (Mpumalanga DACE, 2003). This has lead to eight operational coal-fired power stations in the province and three currently undergoing recommissioning (Eskom, 2006). Since 1995, Eskom has supported the International Global Atmospheric Chemistry (IGAC) program DEBITS (Deposition of Biogeochemically Important Trace Species) that monitors important trace gas species in the atmosphere of the Mpumalanga Highveld region (Mphepya *et al.*, 2004).

The Mpumalanga Highveld grasslands receive levels of N and S deposition similar to a number of impacted ecosystems internationally as a result of fossil fuel burning emissions. Kuylenstierna *et al.* (1995) modelled and identified the soils sensitive to acid deposition in the developing world based on land cover, soil type and soil moisture as functions of precipitation and evapotranspiration. The Highveld grasslands were found to be moderately sensitive to acid deposition, a finding in agreement with a study by Bird (2011) that found sandy soils near the southern and eastern regions of the Highveld grasslands to be sensitive to additional acidic inputs. These sandy soils receive higher rainfall compared to the rest of the Highveld grasslands, do not have the buffering capacity to neutralize

acidic inputs, have low soil pH values, negative acid neutralizing capacity and high exchangeable acidity (Kuylenstierna *et al.*, 1995; Kuylenstierna *et al.*, 2001; Bird, 2011).

Considering the lack of research that has been conducted in sensitive South African grassland ecosystems that receive elevated N and S deposition, this study aimed to investigate the cycling of N and S in soils of a grassland site in the Sandspruit catchment, in the Mpumalanga Highveld grasslands of South Africa. It is vital to understand the drivers of the inputs, cycling and outputs of N and S in this system to make assessments on the consequences of N and S on the cycling and outputs of these nutrients in this system. The key questions in section 1.2 guided the study to achieve its aim.

1.1 Aim

The project aimed to investigate the cycling of N and S in the soils of a grassland site in the Sandspruit catchment.

1.2 Key Questions

1. What are the rates of N and S mineralization and immobilization in the soils of the Sandspruit catchment over the 2010-2011 growing season?
2. What are the effects of soil moisture on N and S mineralization rates in the soils of the Sandspruit catchment?
3. What are the N and S input-output budgets for the selected site in the Sandspruit catchment?
4. How does the input of atmospheric N and S pollutants affect the potential amounts of N and S available for leaching into nearby streams and rivers?

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The literature review commences with a conceptual framework to place the study in context and elaborates on how the system, with all its complexity, is best understood. An in-depth review of the terrestrial cycling and transformations of N, S, carbon (C) and phosphorus (P) follows the conceptual framework. The movement and changes that N and S undergo and the sources of inputs and outputs of the N and S pools in the vegetation and soil layers of the system are particularly important in this review. A brief description of acid deposition and its formation is followed by a comprehensive summary on the amounts of N and S deposition currently modelled and measured in a number of developed and developing countries globally. A breakdown of the N and S deposition modelled by Blight *et al.* (2009), the contribution of wet and dry components to total deposition and a brief overview of the effects of rainfall on N and S deposition on the Mpumalanga Highveld of South Africa are discussed. The final section of the literature review details the known impacts of N and S deposition globally on four key components of a grassland ecosystem including soil properties and processes, plant species diversity, plant growth and productivity and surface water quality.

2.2 Conceptual Framework (Figure 2.1)

A system is described as the complex interaction of all the individual elements that make up that system (Silvern, 1973). A systems approach, which allows a system to be understood and which will be required for this study, emphasizes all the individual parts that are required for the system to function as a whole (Silvern, 1973). For the purpose of understanding the system in this study, the focus will be upon the flow between individual parts as the inputs into the system, the cycling within the system and the outputs from the system. A number of key processes

contribute to the inputs, cycling and outputs of the system. The transfer between parts of the system is also known to occur at different rates. The most important parts in the system that will be studied include the atmosphere, the vegetation layer incorporating the above-ground biomass and litter and the soil layer. The faster the rate at which the nutrients in the ecosystem are transferred between the atmosphere, the vegetation and the soil layer, the greater the chance of a net loss of nutrients (leaking) from each part in the system (Brinkmann, 1985). The smaller and slower the transfer of nutrients between the different parts in an ecosystem, the smaller the chance of a net loss of nutrients from each part of the system (conservative) (Brinkmann, 1985). The interactions between key nutrients must also be considered as they may play an important role in understanding which part of the system will be impacted by inputs into the system (Austin *et al.*, 2003).

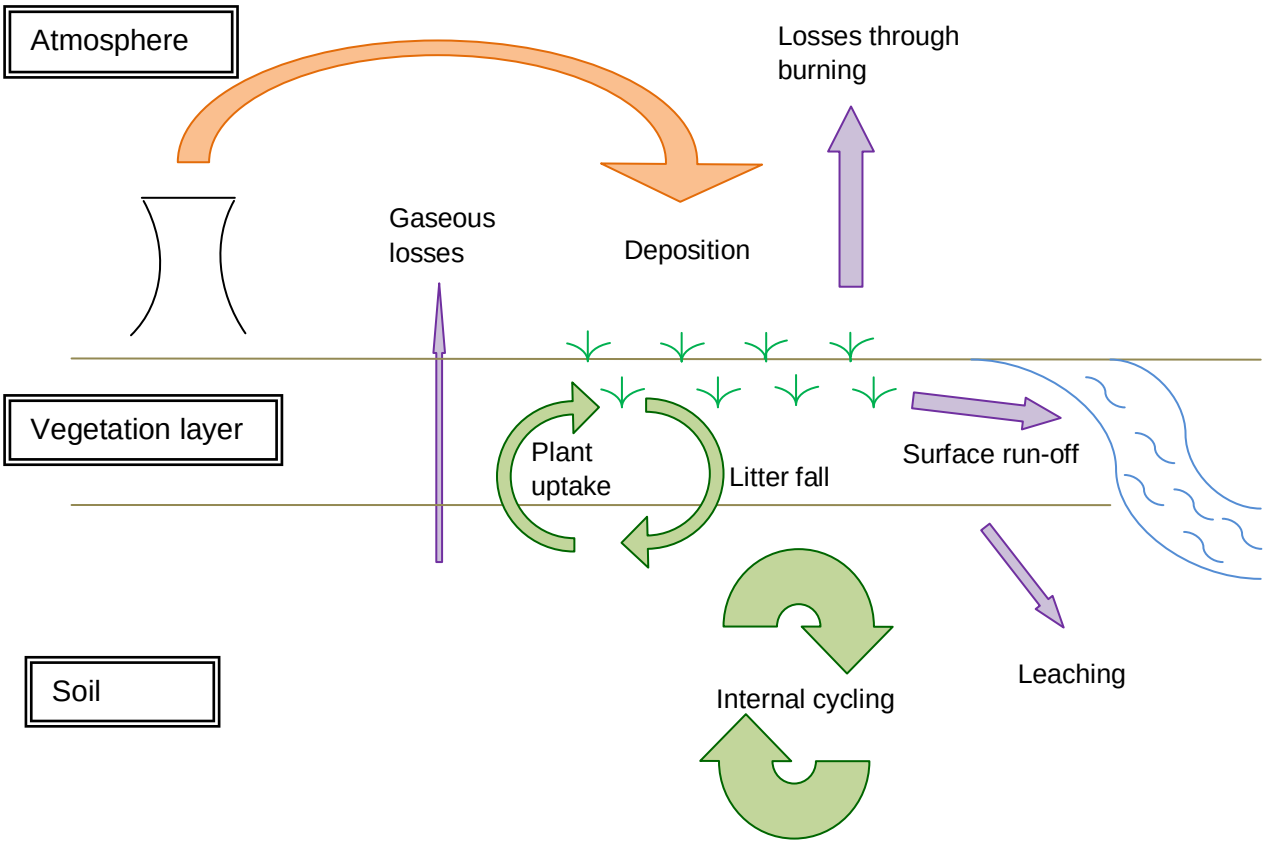

Figure 2.1: Conceptual diagram of the movement of N and S through the Sandspruit system. Arrow thickness represents the relative magnitude of N and S flux and arrow direction represents the direction of movement of N and S between the atmosphere, the vegetation layer and the soil. Orange arrows show N and S inputs, green arrows show N and S cycling between the vegetation and soil and purple arrows show N and S outputs.

The Sandspruit system is in a quaternary catchment of a grassland on the Mpumalanga Highveld of South Africa. Understanding the drivers of the inputs, cycling and outputs of N and S in the Sandspruit system is vital in assessing the consequences of additional N and S on the cycling and outputs of these nutrients in this system. A major source of N and S input into this system is N and S deposition from fossil fuel burning power stations in the province. Blight *et al*. (2009) modelled N and S deposition that estimated current maximum deposition rates of 6.2 kg N ha^{-1} yr^{-1} and 35.0 kg S ha^{-1} yr^{-1} for the grasslands in the

Mpumalanga Highveld. In the soils of a grassland ecosystem, such as the Sandspruit system, the major N and S pools exist in organic form. Various biological processes convert these nutrients into mineral forms and back to organic N and S over time. The process of mineralization is responsible for converting organic N and S into NH_4^+, NO_3^- and SO_4^{2-} respectively and is mediated by microorganisms. These processes provide a large input of NH_4^+, NO_3^- and SO_4^{2-} into inorganic soil pools that are available for plant uptake. Nitrate and SO_4^{2-} are absorbed by the roots and incorporated into grasses found growing in the system, especially during the growing season in the months from November to March.

Large amounts of N and S (between 50.0-75.0% of the N and S present in the above-ground biomass) are lost to the atmosphere when the standing grass biomass and litter are burnt. South African grasslands in general have an annual to triennial burning frequency during which a percentage of N and S is lost to the atmosphere (O'Connor & Bredenkamp, 2003; Bond, 1997). In the Sandspruit system however, this loss is not necessarily annual as the decision to burn is made by the farmer and depends upon the amount of standing biomass at the end of each growing season. Important potential losses of N and S from this system include denitrification, surface run-off and leaching of available NO_3^- and SO_4^{2-}. No direct measurements of these processes were made during this study.

Numerous consequences have been recorded worldwide as a result of N and S deposition and resultant changes to nutrient cycling in many ecosystems where acid deposition is less than in the Highveld grasslands. These consequences include a decrease in plant species diversity (Haddard *et al.*, 2000; Bobbink *et al.*, 2003; Clark & Tilman, 2008; Duprè *et al.*, 2010; Stevens *et al.*, 2010a), soil N saturation (Grennfelt & Hultberg, 1986; Van Miegroet *et al.*, 1992a; Baron *et al.*, 2000; Rueth & Baron, 2002; Fenn *et al.*, 2003b), an increase in the acid status of

the soil (Grennfelt & Hultberg, 1986; Horswill *et al.*, 2008), increased base cation leaching and aluminium ion (Al^{3+}) mobility (Hochman *et al.*, 1992; du Toit, 1993; Bowman *et al.*, 2008) and an increased NO_3^- concentration in surface water (Peierls *et al.*, 1991; Cole *et al.*, 1993; Vitousek *et al.*, 1997; Baron *et al.*, 2009).

The remainder of this literature review is dedicated to understanding the cycling of key nutrients and the known impacts of N and S deposition on a grassland ecosystem. The cycling of key nutrients is best understood by exploring N, S, C and P movement between the atmosphere, the vegetative layer and the soil. The potential impacts N and S deposition may have on this system will be explored by reviewing and comparing known impacts on key soil properties and processes, plant growth and productivity, plant species diversity and surface water quality in a number of ecosystems globally with local studies.

2.3 Cycling of Key Nutrients in a Grassland Ecosystem

Nutrient cycling is considered one of the most important processes occurring in any ecosystem and consists of both inputs in the form of atmospheric deposition and fixation and outputs in the form of gaseous losses and leaching (Jacobson *et al.*, 2011). Nitrogen, S, C and P are four key nutrients in the soil and in the vegetation of any ecosystem. In their various forms and combinations, they are responsible, amongst others, for soil fertility, plant productivity and plant diversity and a change in the availability of one of these nutrients may impact positively or negatively on the transformations and availability of the remaining three. Developing an understanding of the terrestrial N, S, C and P cycles will aid in predicting the fate of incoming N and S deposition and the impacts to be expected from these added nutrients in the Sandspruit system.

2.3.1 The Nitrogen Cycle

Nitrogen is an essential nutrient that is required for plant growth and is found in limited amounts in many ecosystems, controlling the productivity of the ecosystem (Van Miegroet *et al.*, 1992a; Vitousek, 1994; Bobbink & Roelofs, 1995; Vitousek *et al.*, 1997; Strong & Mason, 1999; Galloway *et al.*, 2004; Güsewell, 2004). Gaseous N (N_2) in the atmosphere is converted into reactive forms of N that are available for biological uptake (Miller & Donahue, 1990), among these conversions is biological nitrogen fixation (BNF). This conversion involves a specialized group of prokaryotes that convert atmospheric N_2 into NH_3 (Wagner, 2012) (Figure 2.2). These prokaryotes include certain aquatic organisms, free-living soil bacteria and bacteria that form an association with plants, such as the symbiotic relationship between *Rhizobium* and leguminous plants (Postgate, 1982). Microbial organisms are known to reduce N in soil organic matter (SOM) that is composed of dead plants, animals and soil organisms into mineral forms (Singer & Munns, 1987). Nitrogen is used by microbes in the soil, is converted to a number of different forms and can then be absorbed by plants (Lewis, 1986). The N cycle consists of processes that bring N into the soil, those that release N from the soil and a number of processes that change the form of N found in the soil. Precipitation, N fixation and the addition of fertilizers add N to the soil whilst denitrification and leaching remove N from the soil (Figure 2.2). The amount of N present in soil is thus a result of various processes including ammonification, nitrification, net mineralization and immobilization (Figure 2.2).

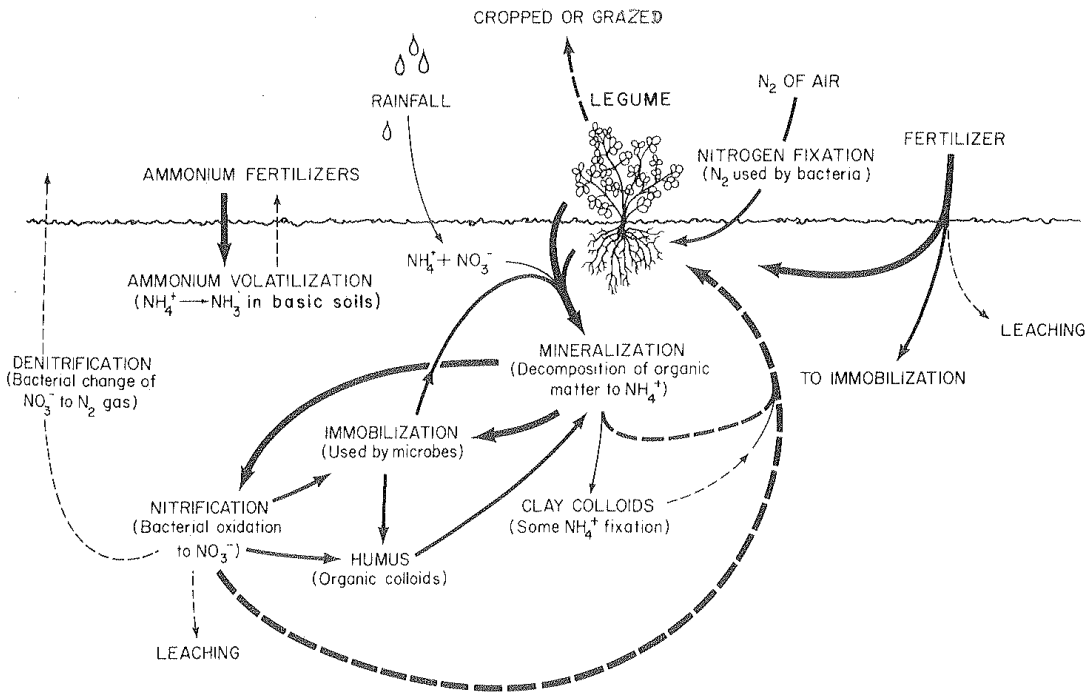

Figure 2.2: Flow diagram of the cycling and pathways involved in the transformations of N in the terrestrial N cycle (Miller & Donahue, 1990).

The concentration of N present in the soil at any one time is also partially dependent on the topography of the land, the soil texture and the soil depth (Callesen *et al.*, 2003). Studies have shown a higher N concentration at the bottom of slopes compared to the middle and top of the same slopes (Schimel *et al.*, 1985; Burke *et al.*, 1995) and an increased concentration of N with an increase in soil depth (Crowe *et al.*, 2004).

Ammonium and NO_3^- are the two important forms of mineral N focused upon in this study and are deposited in the forms of NH_3 and HNO_3 respectively (Reuss & Johnson, 1986; Strong & Mason, 1999; Driscoll *et al.*, 2001; Baisre, 2006). Nitrate is both highly soluble in the soil solution and weakly held by the soil (Abrahamsen & Miller, 1984; Reuss & Johnson, 1986; Groffman, 2000) and so is easily leached

and transported in surface runoff and precipitation (Groffman, 2000). The ease with which it is leached means it is easily absorbed in well aerated soils (Blackmer, 2000) and is often the cause of eutrophication in aquatic ecosystems (Groffman, 2000).

2.3.1.1 Nitrogen mineralization, ammonification and nitrification

Nitrogen mineralization is the process that breaks down organic forms of N into mineral, inorganic forms such as NH_4^+ and NO_3^-. If a positive rate of N mineralization occurs in soil, N is produced in mineral forms that are then available for plant uptake. If the decomposition rate is slowed and the C:N ratio is high, N immobilization occurs and more N is taken up by soil organic microbes than is being released into the soil.

Nitrogen mineralization is a two-step process including ammonification and nitrification. Heterotrophic organisms including actinomycetes, bacteria and fungi decompose organic material, serving as a source of N and C and excrete NH_4^+ as a waste product, completing the process of ammonification (Lewis, 1986). Nitrification is the process of microbial oxidation of reduced forms of N (nitrite (NO_2^-)) to less reduced forms of N (NO_3^-), with autotrophic nitrification appearing to dominate over heterotrophic nitrification in most soils (Robertson & Groffman, 2007). Autotrophic nitrification is also a two-step process involving two microbial nitrifiers including *Nitrosomonas* and *Nitrobacter* species. The conversion of NH_4^+ to NO_2^- in the presence of oxygen (O_2) is first completed by *Nitrosomonas* followed by *Nitrobacter* which quickly converts the available NO_2^- to NO_3^-:

Nitrosomonas: $NH_3^+ + 1½O_2 \rightarrow NO_2^- + H^+ + H_2O$

Nitrobacter: $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2é$ (reversible reaction)

The combined processes of ammonification and nitrification provide the net N mineralization rate that has been found to range between -0.60 and 0.52 µg N g^{-1} soil day^{-1} in the Highveld grasslands by Bird (2011). The net N mineralization rate measured in grassland soils in inner Mongolia was slightly higher and ranged between -0.10 and 1.03 µg N g^{-1} soil day^{-1} (Lui *et al.*, 2010).

2.3.1.2 Factors affecting nitrogen mineralization

Rainfall, temperature, soil pH and the decomposition rate are some of the factors that control the N mineralization rate. The N mineralization rate is known to approximately double with every 10.0 °C increase in temperature up to a certain temperature when microbes can not longer perform optimally (Rice & Havlin, 1994). Louw & Scholes (2002) found a positive relationship between the N mineralization rate and mean annual temperature. Louw & Scholes (2002) also found that relatively high levels of organic C coupled with low mean annual temperatures resulted in low N mineralization rates that slowed the annual N turnover in high altitude forest systems in South Africa. The rate of nutrient cycling varies, showing a faster turnover rate in labile pools and a much slower turnover rate in the more stable pools (Boone, 1994). The soil water content, the amount of aeration in the soil (Goncalves & Carlyle, 1994) and the concentration of P in the soil are also contributing factors that influence the N mineralization rate (Carlyle *et al.*, 1990).

The net N mineralization rate is also affected by soil pH (Harris, 1988). Decomposition and the breakdown of soil organic matter occur at faster rates in soil with a pH between 5.5 and 6.5 compared to more acidic soils as a result of the optimal pH range that favours microbial activity (Noble & Herbert, 1991). The optimum soil pH range for *Nitrosomonas* is between 7.8 and 8.0 and their growth is

known to be inhibited in soils with a pH below 6.5. The optimum soil pH range for *Nitrobacter* is between 7.3 and 7.5 (Holt & Hendricks, 1994; Grundmann *et al.* 2000).

Soil organic matter is composed of approximately 50.0% C, with lesser amounts of O_2 and H_2 as well as small amounts of N, P and S (Miller & Donahue, 1990). Soil organic matter is partly responsible for plant and microbial growth and is the primary source of N, S and P as essential nutrients for plant growth (Horwath, 2007). The production and decay of SOM are necessary processes that in turn regulate various atmospheric trace gases, especially carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). The process of decomposition in well aerated soils releases end products of CO_2, NH_4^+, NO_3^-, dihydrogen phosphates ions ($H_2PO_4^-$), SO_4^{2-} and water, with less desirable products like CH_4 being produced in less well aerated soils (Miller & Donahue, 1990).

Factors that affect decomposition include (Miller & Donahue, 1990):

- Temperature: colder periods slow or prevent plant growth and organic matter decay. Warm temperatures allow increased plant productivity but also promote a faster decomposition rate.
- Soil moisture: plant growth and decomposition require moisture. Extreme dry or anaerobic conditions reduce plant growth rate and microbial decomposition.
- Nutrients: a shortage of certain nutrients, especially N, reduces plant growth and can affect decomposition as soil microbes can take up N from dead organic matter before it becomes available to plant roots.
- Soil pH: most microorganisms become inhibited as soil pH drops below 4.5 and above 8.5.
- Soil texture: higher amounts of humus are normally retained in soils with a larger amount of clay particles as a result of the many attachment sites.

- Other factors: other factors that reduce the rate of decomposition include toxic levels of Al^{3+}. The specific plant type is also important to consider as legumes decompose more readily than grasses.

2.3.1.3 Denitrification

Denitrification is the process by which soil NO_3^- is reduced to N, N_2O and nitrogen dioxide (NO_2) by denitrifying bacteria present in the soil (Robertson & Groffman, 2007). Most of the bacteria that denitrify only use NO_3^- when O_2 is not available as a terminal electron acceptor during the process of respiration as NO_3^- is less efficient (Robertson & Groffman, 2007). In many soils, especially soils with a high clay content, the use of NO_3^- by bacteria only occurs after precipitation when the soil pores are saturated and O_2 diffusion into the soil microsites is slowed (Robertson & Groffman, 2007). Denitrification is also expected to occur faster when rain falls on soil that is already moist than when compared to soil that is dry (Groffman & Tiedje, 1988; Bergsma *et al.*, 2002). Although O_2 is the main limiter of denitrification, NO_3^- limits denitrification under saturated soil conditions as the nitrifiers that operate and provide NO_3^- are inhibited under low O_2 conditions (Robertson & Groffman, 2007). This means denitrification occurs under very slightly oxygenated conditions and at the sediment-water interface, where nitrifiers can oxidize NH_4^+ to NO_3^- (Robertson & Groffman, 2007). It can however be difficult to measure NO_3^- in constantly saturated soils as a result of a low nitrification rate and a close coupling between nitrifiers and denitrifiers (Robertson & Groffman, 2007). In unsaturated soils, it is often C that allows denitrification to occur as it provides donor electrons to denitrifiers and stimulates heterotrophic O_2 consumption (Robertson & Groffman, 2007).

Denitrification however is not only limited to anaerobic soils. Denitrification also occurs in the centre of soil aggregates and in decomposing plant litter. The centre of soil aggregates are anaerobic and provide an active area for denitrification to occur (Sexstone *et al.,* 1985). Denitrifying bacteria often only constitute up to 20.0% of the total soil microbial biomass. They are however important as denitrification is a large source of the important greenhouse gas N_2O and the process of denitrification can be used to remove NO_3^- from the soil before it enters streams and rivers (Tiedje, 1988; Robertson & Groffman, 2007).

2.3.2 The Sulfur cycle

Sulfur in the atmosphere occurs mainly as sulfide (S^{2-}), in the form of hydrogen sulfide (H_2S), carbon disulfide (CS_2), carbonyl sulfide (COS) and dimethyl sulfide ($(CH_3)_2S$), oxides in the form of sulfur dioxide (SO_2) and sulfur trioxide (SO_3) as well as SO_4^{2-} in precipitation (Seinfeld & Pandis, 1998). Hydrogen sulfide and SO_2 have been identified as common in the atmosphere of the Mpumalanga Highveld of South Africa with SO_2 being the major species of concern (Held *et al.*, 1994; Mphepya *et al.*, 2004). The main input of S into soils is soil weathering which increases SO_4^{2-} in the available soil SO_4^{2-} pool (Plante, 2007). Other inputs include plant residue inputs, S fertiliser, pesticides, irrigation water and S deposition. Any losses of S from the soil occur via plant uptake, long-term fixation into minerals, leaching of SO_4^{2-} through the soil profile and losses of volatile gaseous forms of S (Plante, 2007).

Similar to the N cycle, the S cycle is also made up of the inorganic and the organic pools, where organic S generally contributes a major portion to the total S of the soil (Roberts & Bettany, 1985; Reuss & Johnson, 1986). In humid to semi-humid regions, between 90.0 and 95.0% of the S in the soil is in organic form, although

the total soil S pool is important when considering S deposition (Reuss & Johnson, 1986). Inorganic S is generally in the form of SO_4^{2-}, the oxidized form of S (Tabatabai, 1982; Lewis, 1999). Sulfate is highly soluble and relatively mobile in the soil and so is easily leached through the soil profile in rainwater filtrate (Miller & Donahue, 1990). Soils with better drainage generally hold less water-soluble SO_4^{2-} in the upper soil horizons as water-soluble S will be leached through the soil profile into the lower horizons. The overall amount of water-soluble SO_4^{2-} is dependent on the SO_4^{2-} mineralization rate, amount of leaching, plant uptake of SO_4^{2-} and S deposition (Tabatabai, 1982). Plants mainly take up S in the form of SO_4^{2-} and measuring extractable SO_4^{2-} can give an accurate indication of the amount of S deposited on land from the atmosphere (Palomino *et al.*, 2005). The SO_4^{2-} anion can be kept in the soil by sorption to amorphous oxides as well as aluminium hydroxide ($Al(OH)_3$) and iron(II) oxide (FeO) and can be immobilized in the soil if it is integrated into the organic matter (Abrahamsen & Miller, 1984). Sulfate sorption in soils can reduce the ability of any atmospheric S inputs to cause leaching of ions from the soil solution (Singh & Johnson, 1986; Inskeep, 1989). This capacity of the soil to hold SO_4^{2-} is important to consider when determining the effects of acid deposition on an ecosystem (Singh & Johnson, 1986; Comfort *et al.*, 1992). It has also been shown that sub-soils have a greater capacity to hold SO_4^{2-} compared to top-soils as they generally have a larger clay component and a lower pH (Fey & Guy, 1993).

2.3.2.1 Sulfur mineralization and immobilization

Sulfates are formed in the soil by the process of mineralization which involves the conversion of organic S to SO_4^{2-} (Figure 2.3) (Edwards, 1998). Sulfur mineralization is less closely correlated to the degradation of C when compared to N mineralization (Plante, 2007). The S bonded to C can be mineralized through a number of different pathways:

- direct aerobic mineralization during the oxidation of C as an energy source,
- anaerobic mineralization of organic matter (desulfurization),
- incomplete oxidation of organic S into inorganic S compounds,
- biological oxidation of H_2S to SO_4^{2-} via elemental S and sulfite ($-SO_3^{2-}$),
- biological oxidation of tetrathionate to SO_4^{2-} via S^{2-},
- hydrolysis of cysteine desulfydrolase and
- indirect (enzymatic) mineralization when SO_4^{2-} esters are hydrolyzed by sulphatase (Lawrence, 1987).

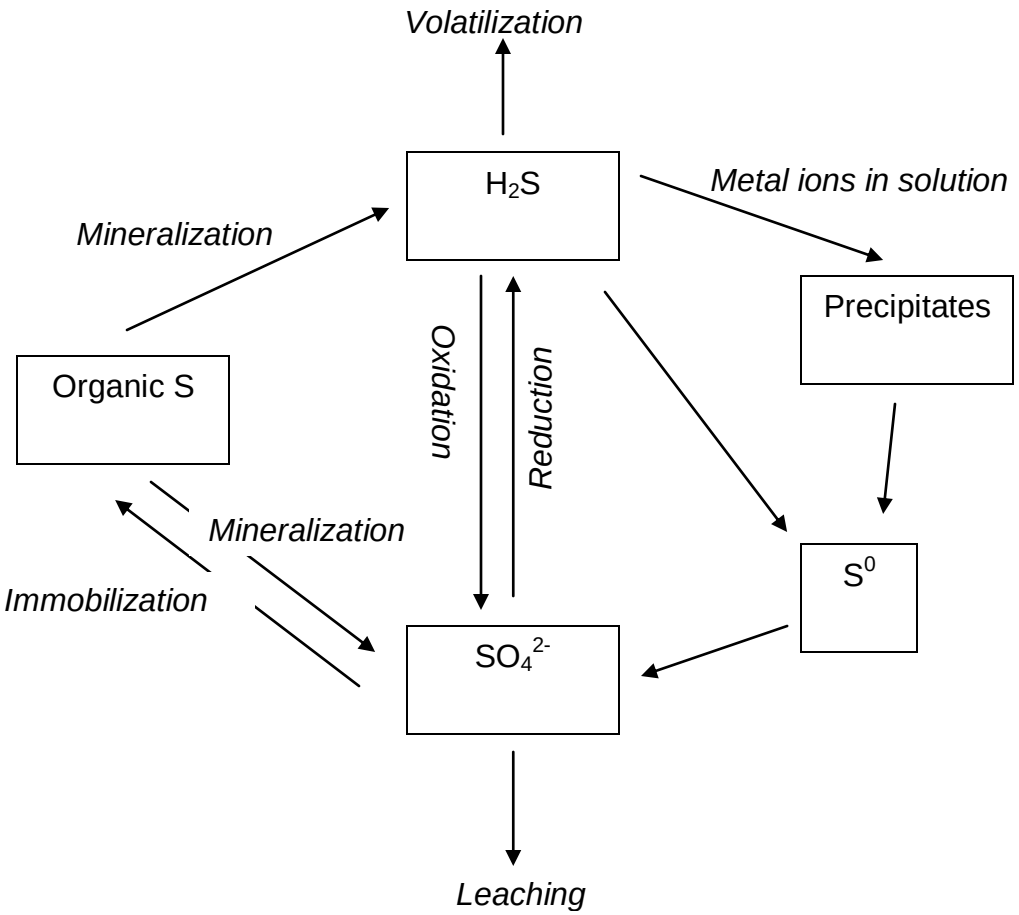

Figure 2.3: Simplified diagram representing the processes involved in the terrestrial S cycle (Edwards, 1998).

Immobilization, the reverse process of mineralization, converts SO_4^{2-} to organic S that is unavailable for plants to take up (Figure 2.3) (Singer & Munns, 1987). The microbial decomposition of plant matter with a C:S ratio of more than 400:1 results in S immobilization and at ratios of less than 200:1, S is generally mineralized in the soil environment (Plante, 2007).

2.3.2.2 Oxidation, reduction and volatilization

Reduced forms of S become oxidized in the presence of available electron acceptors: $HS^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_40_6^{2-} \rightarrow SO_4^{2-}$. A number of different organisms have the ability to oxidize S and are classified according to the metabolic pathway used (Plante, 2007).

Sulfate is reduced by microorganisms in one of two ways:

- S is incorporated into the cells and is known as assimilatory SO_4^{2-} reduction or immobilization and
- dissimilatory or respiratory SO_4^{2-} reduction where H_2S is formed as an end product (Plante, 2007).

Dissimilatory or respiratory SO_4^{2-} reduction is mediated by anaerobic, organotrophic organisms that may use hydrogen (H_2) as electron donors and oxidized S compounds as terminal electron acceptors (Plante, 2007), forming a process similar to denitrification. Sulfate reduction however, is not very important in aerated soils except in the anaerobic microsites that may develop during times of maximum rainfall. This is the process that releases H_2S in water-saturated soils. The bacteria responsible for SO_4^{2-} reduction are known to be found in soils with a wide range of pH and salt concentrations and are able to tolerate both heavy metals and dissolved S^{2-} concentrations up to 2.0% (Plante, 2007).

When soils are poorly drained and there is decomposition of organic S, volatile organic S compounds may be formed during a process known as volatilization (Plante, 2007). These compounds are known to inhibit plant growth and nitrification and some are listed as greenhouses gases that negatively affect climate.

2.3.2.3 Movement of sulfate in soil

Soil SO_4^{2-} concentration is partially dependent on topography and soil depth with total S known to be higher down-slope compared to up-slope and total SO_4^{2-} concentration generally increases with an increase in soil depth (Roberts & Bettany, 1985). The concentration of water soluble SO_4^{2-} in the soil solution is affected by S mineralization rate, leaching, the amount of SO_4^{2-} taken up by plants and the amount of acid deposition (Tabatabai, 1982).

The adsorption of SO_4^{2-} in soils, that acts to slow SO_4^{2-} leaching (McLaren & Cameron, 2004), is dependent on soil pH and becomes weaker at lower pH (Martini & Mutter, 1984). When the soil pH is above 6.5, SO_4^{2-} adsorption is insignificant and the majority of the soil SO_4^{2-} is present in solution (Curtin & Syers, 1990). When adsorption does occur, the length of time the SO_4^{2-} spends in contact with the soil is considered the most important factor that determines the amount of adsorption (Vance & David, 1992). The longer the contact time between the SO_4^{2-} and the soil, the less chance there is of SO_4^{2-} leaching. Sulfate leaching is mostly controlled by water movement and many systems show peak SO_4^{2-} concentrations in nearby surface water following the first rains of the season. Lynch & Corbett (1989) used a model to explain the large SO_4^{2-} export they recorded. They hypothesized that a buildup of SO_4^{2-} occurred during dry periods that was washed out of the soil during the early rains of each annual season (Lynch & Corbett, 1989). The amount of incoming S deposition is also a factor taken into

consideration when measuring the change in soil SO_4^{2-} throughout the year. Johnson & Cole (1977) recorded large increases in SO_4^{2-} leaching in the A horizon of the soil profile when acid deposition, in the form of H_2SO_4, was added. A simultaneous decrease in soil pH occurred to a low of 4.3, although the drop in pH was only recorded for as long as the acid deposition was added to the system (Johnson & Cole, 1977).

2.3.3 The Carbon and Phosphorus Cycles

The C cycle consists of the transfer of C between the atmosphere, oceans and land and comprises both the short-term and the long-term C cycles (Figure 2.4) (Horwath, 2007). The terrestrial C cycle is included in the short-term C cycle and includes plants, soil biota and soil organic matter. The short-term C cycle is principally dependant on CO_2 and CH_4, both well known greenhouse gases. Since the beginning of the Industrial Revolution, the atmospheric C content has risen from 260 ppm to 396 ppm currently (NOAA, 2012) as a result of increased fossil fuel burning, forest clearing and the conversion of land for agricultural use.

Photosynthesis is the process of converting inorganic C (CO_2) into organic C with the majority of the C converted to plant biomass (Horwath, 2007). The outcome of decomposition in the soil is dependent upon the amount and quality of the litter and the microbial turnover rate. The amount of N in the above-ground biomass and the amount of N in the soil microbial pool are very similar in most ecosystems, indicating the important role soil organisms play in influencing available N for plants to take up (Horwath, 2000). The capacity of the soil decomposers to consume the inputs into the system is also important in determining the availability of nutrients to plants. Elevated N (NO_3^- and NH_4^+) input into a system can result in a lower C:N

ratio that no longer limits N availability to decomposers, increasing the decomposition rate of the system (Horwath, 2007).

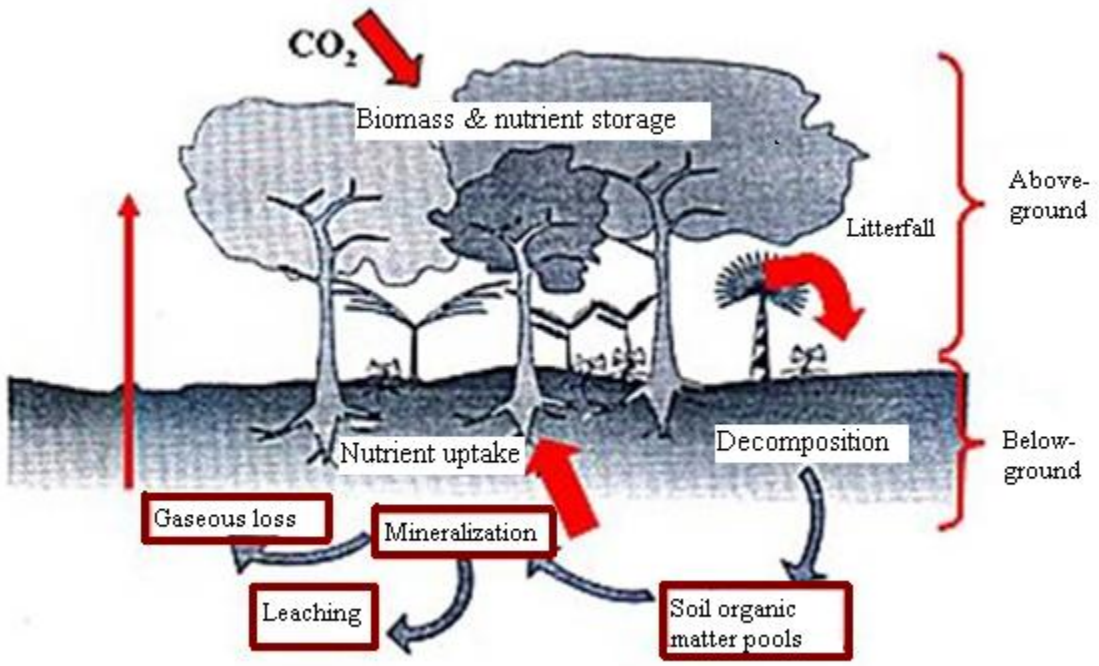

Figure 2.4: Representation of the terrestrial C cycle (Pendergast, 2004).

Nitrogen and P are the most common limiting nutrients to biological productivity and whereas N is most commonly atmospherically derived, P is mostly rock derived, making P depletable (Walker & Syers, 1976; Plante, 2007). Although P is not very mobile, it has been known to be lost from the soil in dissolved organic form in highly weathered soils, where dust deposition may then form the majority of the P input (Vitousek, 2004). Phosphorus does not show large fluxes between the atmosphere and terrestrial environments nor does it feature as a primary source of energy for microbial oxidations (Plante, 2007).

Phosphorus mainly occurs as inorganic phosphates (PO_4^{2-}) and in organic PO_4^{2-} derivatives in soil (Figure 2.5) and can range between 35.00-5 300.00 µg g^{-1} soil with an average of approximately 800.00 µg g^{-1} soil (Bowen, 1979). The concentration of P in soil solution is very rarely above 0.10-1.00 µg g^{-1} soil as soluble P quickly precipitates either as iron or aluminium phosphate ($FePO_4$ or $AlPO_4$) in acidic soils and as calcium phosphate ($CaPO_4$) in alkaline soils (Plante, 2007). Optimal P availability occurs in soil with a pH of approximately 6.5, an important consideration as there is ample evidence that P is the most important element controlling C and N immobilization in most biological systems (Plante, 2007). At a lower soil pH, when Al solubility increases, P fixation increases which reduces the amount of available P (Tainton, 1984).

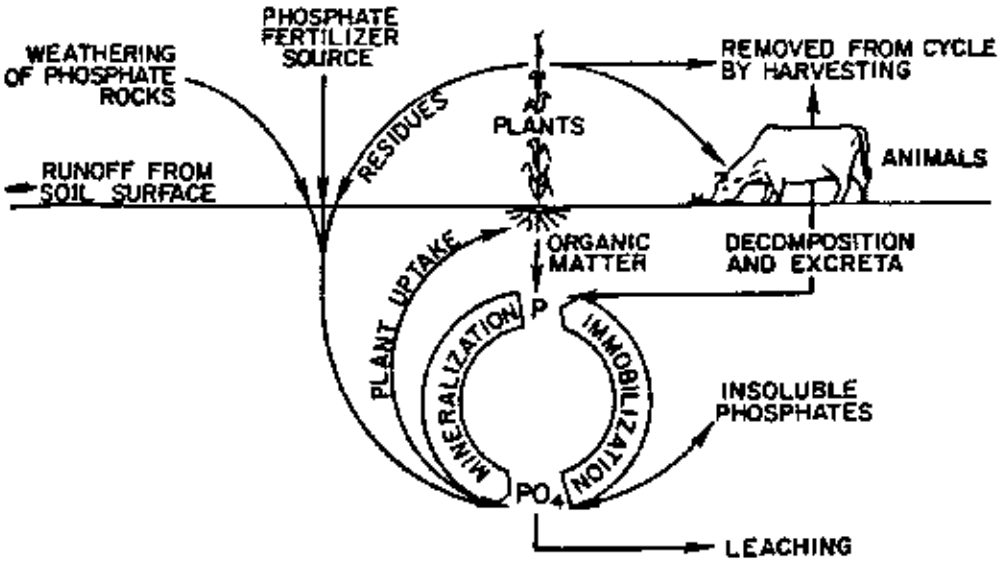

Figure 2.5: Simplified representation of the processes occurring in the P cycle (Marston, 1989).

2.3.3.1 Microbial transformations of phosphorus

Phosphorus mineralization needs to occur in the soil before mineral plant uptake can take place. Following P mineralization, P can be taken up by plants, immobilized by microbial biomass, changed into inorganic complexes and sorbed to available mineral surfaces (Plante, 2007). Microorganisms in the soil are responsible for P mineralization and immobilization where immobilization occurs through P fixation by calcium (Ca^{2+}), Al^{3+} or iron (Fe^{3+}) (Plante, 2007). The rate at which this immobilization occurs depends on the C:N ratio of organic materials decomposing in the soil and the amount of P already available in solution (Plants, 2007).

2.4 Nitrogen and Sulfur Deposition

2.4.1 Deposition Chemistry and Transformations

Gaseous SO_x and NO_x are oxidized to SO_4^{2-} and NO_3^- , undergo dissolution with water vapour and are deposited on the soil as sulfuric acid (H_2SO_4) and nitric acid (HNO_3), releasing H^+ ions into the soil when moving into solution (Cowling & Linthurst, 1981; Van Breemen *et al.*, 1983; Fey & Guy, 1993; Galloway, 1995; Mphepya, 2002). During the transformations of these gases in the atmosphere, the distance they are transported away from the source depends on the height they reach in the atmosphere, their particle size and various climate factors (Alloway & Ayres, 1997). The use of tall stacks in power stations means pollutants are sometimes released above the surface inversion layer, can be widely distributed by wind and turbulence and can undergo a large number of transformations before they are deposited (Cowling & Linthurst, 1981; Mphepya, 2002; Van Tienhoven & Scholes, 2003). During fairly stable conditions, such as during the night, emissions can be transported thousands of kilometers downwind of the source before reaching the ground, whereas during the day, when strong convection currents

occur, pollutants can be deposited on the ground close to the emission source (Turner *et al.*, 1996).

Atmospheric deposition can occur as either wet or dry deposition (Ulrich & Sumner, 1991). Wet deposition occurs when gaseous pollutants collect by dissolution into cloud droplets and refers specifically to the portion of the deposition that is carried down in precipitation (Ulrich & Sumner, 1991). Dry deposition is mostly dominated by gaseous components in the nearby vicinity of the pollutant source, decreasing with increasing distance from the emission source and refers to the portion of deposition that is deposited during dry weather via the processes of settling, impaction and adsorption (Ulrich & Sumner, 1991). Atmospheric deposition has been linked to a source of both nutrients for ecosystems as well as toxic substances, mostly deposited as acid deposition (Fenn *et al.*, 2003a). Acid deposition is mainly associated with N and S compounds that have reacted in the atmosphere to produce HNO_3 and H_2SO_4 respectively (Fowler *et al.*, 1989; Hornung *et al.*, 1995). Base cations that are present as common elements in the earth's crust are transferred to the atmosphere (as dust) and neutralize some of the acids present in precipitation (Ulrich & Sumner, 1991). The combination of an increase in the release of SO_x and NO_x and a decrease in neutralizing substances in the atmosphere can result in a significant decrease in precipitation pH and the formation of acid deposition (Kennedy, 1986; Ulrich & Sumner, 1991).

2.4.2 Deposition in Developed and Developing Countries

A summary of the measured and modelled N and S deposition rates across a number of developed and developing countries provides context for where South Africa and more specifically the Mpumalanga Highveld grasslands of South Africa fit into global deposition levels.

2.4.2.1 Nitrogen and sulfur deposition in developed countries

Nitrogen and S deposition is well studied and modelled in Europe and the US and has been monitored in a number of ecosystems although the majority of impact studies have taken place in forest and grassland ecosystems. The majority of the current research on deposition in developed countries focuses on N deposition since the implementation of environmental protection policies has been successful in drastically reducing S emissions, and thus S deposition (Berge *et al.*, 1999; Butler *et al.*, 2001; Driscoll *et al.*, 2001; Likens *et al.*, 2001; Reinds *et al.*, 2008).

Duprè *et al.* (2010) compiled data from various literature sources and databases on the rates of N and S deposition across a number of European grasslands over a period of approximately 70 years. Deposition rates were found to be highest in Denmark for N and in Great Britain for S with rates of between 256.5-298.0 kg N ha^{-1} yr^{-1} and 19.4-227.5 kg S ha^{-1} yr^{-1} respectively (Duprè *et al.*, 2010). The same study also provided N and S deposition data for Germany, the Netherlands and Sweden. Nitrogen and S deposition in Germany ranged between 7.1-27.2 kg N ha^{-1} yr^{-1} and 3.6-9.1 kg S ha^{-1} yr^{-1} respectively (Duprè *et al.*, 2010). Nitrogen and sulfur deposition in the Netherlands ranged between 1.0-3.4 kg N ha^{-1} yr^{-1} and 0.7-1.3 kg S ha^{-1} yr^{-1} respectively (Duprè *et al.*, 2010). Nitrogen and sulfur deposition in Sweden was relatively high and ranged between 139.5-140.8 kg N ha^{-1} yr^{-1} and 79.8-81.1 kg S ha^{-1} yr^{-1} respectively (Duprè *et al.*, 2010).

When comparing the deposition rates complied from literature sources and data bases to modelled deposition rates, a large variance exists. In two deposition modeling studies of 68 and 153 grasslands across Europe, the N and S deposition rates ranged between 2.4-43.5 kg N ha^{-1} yr^{-1} and 6.6-28.7 kg S ha^{-1} yr^{-1}

respectively (Stevens *et al.*, 2009a; Stevens *et al.*, 2010a), far less than the deposition rates compiled using literature and database sources. Similarly, modelled N and S deposition from 56 grasslands across the UK predicted deposition rates of between 7.3- 40.7 kg N ha^{-1} yr^{-1} and 8.0-27.6 kg S ha^{-1} yr^{-1} (van den Berg *et al.*, 2011). A continental-scale study performed by Holland *et al.* (2005) compiled N budgets that used site observations across the US and estimated the average N deposition rate to be approximately 4.6 kg N ha^{-1} yr^{-1}.

2.4.2.2 Nitrogen and sulfur deposition in developing countries

A number of developing countries including China, Brazil, Slovakia, Argentina and South Africa have researched the rates of N and S deposition in agricultural, forested and grassland ecosystems.

A network of 40 monitoring stations located across China measured the N deposition on croplands, grasslands, forests and urban ecosystems to range between 27.0 and 39.0 kg N ha^{-1} yr^{-1} (Liu *et al.*, 2006; Liu *et al.*, 2011). A campaign in 2002 in the Brazilian Amazon basin named the Large-Scale Biosphere-Atmosphere Experiment in Amazon-Smoke, Aerosols, Clouds, Rainfall and Climate (LBA-SMOCC) estimated the average N deposition to be 11.0 kg N ha^{-1} yr^{-1} (Trebs *et al.*, 2006). Similarly, Krusche *et al.* (2003) measured N deposition of approximately 9.0 kg N ha^{-1} yr^{-1} in forests, pastures and sugarcane in a river basin in South Eastern Brazil. Periodic bulk deposition measurements made in Slovakia measured N and S deposition rates of approximately 12.0 kg N ha^{-1} yr^{-1} and 11.0 kg S ha^{-1} yr^{-1} respectively (Bowman *et al.*, 2008). A CALPUFF modeling study performed by Rojas & Venegas (2008) in Argentina modelled N deposition in and around the city of Buenos Aires and found an average deposition rate of

20.2 kg N ha^{-1} yr^{-1}, that consisted of 89.0% dry deposition and 11.0% wet deposition (Rojas & Venegas, 2008).

Nitrogen and S deposition are fairly well studied on the Mpumalanga Highveld of South Africa and have been recorded using field measurements, inferential modeling and passive monitoring stations. Field measurements at Eskom's Elandsfontein ambient air quality monitoring site in central Mpumalanga were used to estimate N deposition in the range of 6.7 and 13.1 kg N ha^{-1} yr^{-1} (Collett *et al.*, 2010). Nitrogen deposition data from a local monitoring site in a commercial forest plantation and adjacent natural grassland on the Highveld were used in inferential modeling of the N deposition expected on the plantation and grassland that included deposition from fog. Nitrogen deposition on the plantation and neighbouring grassland were estimated to be 70.0 kg N ha^{-1} yr^{-1} and 25.0 kg N ha^{-1} yr^{-1} respectively (Lowman, 2003). A passive monitoring network of 37 stations located in the northern and eastern regions of South Africa measured ambient trace gas concentrations and long-term acid rainfall from which N and S deposition rates were calculated (Josipovic, 2009). Nitrogen and S deposition rates were calculated as an average of the combined wet and dry deposition measurements across the 37 sampling sites to be approximately 3.5 kg N ha^{-1} yr^{-1} and 7.7 kg S ha^{-1} yr^{-1} respectively (Josipovic, 2009). A very large difference exists between the estimated and calculated N deposition rates reported by Lowman (2003) and Josipovic (2009) and may be a result of the chosen location for the plantation and grassland sites and the large spatial scale of the ambient gas concentration measurements made by Josipovic (2009).

Blight *et al*. (2009) used CALPUFF to model the rates of N and S deposition on the Mpumalanga Highveld under different rainfall conditions. Sampling took place at Amersfoort air quality monitoring station, located near Amersfoort, 19.0 km north of

the Sandspruit system. Peak N deposition was calculated to be 8.5 kg N ha^{-1} yr^{-1}, comprising 63.0% wet deposition and 37.0% dry deposition (Blight *et al.*, 2009). The total maximum N deposition was estimated to occur in an easterly direction compared to the peak S deposition that occurred near the towns of Bethal and Ermelo. Total peak S deposition was modelled to be 35.0 kg S ha^{-1} yr^{-1} over the central Highveld region including Witbank, Secunda and Bethal (Blight *et al.*, 2009). The peak annual wet S deposition on the central Highveld was modelled to be approximately 20.0 kg S ha^{-1} yr^{-1} with wet deposition in the greater Highveld region modelled to be less than half that at between 3.0 and 7.0 kg S ha^{-1} yr^{-1} (Blight *et al.*, 2009). The peak annual dry S deposition on the central Highveld was modelled to be approximately half that of the wet deposition at >10.0 kg S ha^{-1} yr^{-1} with dry deposition in the greater Highveld region modelled to be between 3.0 and 13.0 kg S ha^{-1} yr^{-1} (Blight *et al.*, 2009).

During the study by Blight *et al.* (2009), it was determined that the total deposition loads decreased substantially with a decrease in rainfall as the deposition rates modelled for years with above average rainfall were significantly higher than those for an average rainfall year. Any increase in modelled S deposition was found to be a result of a much higher wet S deposition component and slightly higher dry S deposition component (Blight *et al.*, 2009). The dry S deposition rate however, increased during wet years as a result of ground level removal of S increased by wet surfaces. The modelled N and S deposition rates, the spatial variation in these rates and the wet and dry ratios of deposition during the study were found to be significantly affected by changes in rainfall intensity and spatial rainfall patterns (Blight *et al.*, 2009).

2.5 Impacts of Deposition on Key Components of a Grassland Ecosystem

Nitrogen oxides and SO_x released as products of coal combustion are both equally important in their ecosystem impacts (Vitousek, 1994; Bobbink & Roelofs, 1995; Vitousek *et al.*, 1997; Bobbink *et al.*, 1998; Galloway, 1998; Goulding *et al.*, 1998; Galloway *et al.*, 2004; Baisre, 2006), the greatest being within 10.0-15.0 km of the release source (Freedman & Hutchinson, 1980; Annegarn *et al.*, 1996; E'k *et al.*, 2001). These precursors to acidic compounds have been investigated as potential threats to ecosystems, their functioning and their health (Kennedy, 1986) (Figure 2.6) and will further be discussed with regards to their impacts on key soil properties and processes, plant species diversity, plant growth and productivity, and surface water quality.

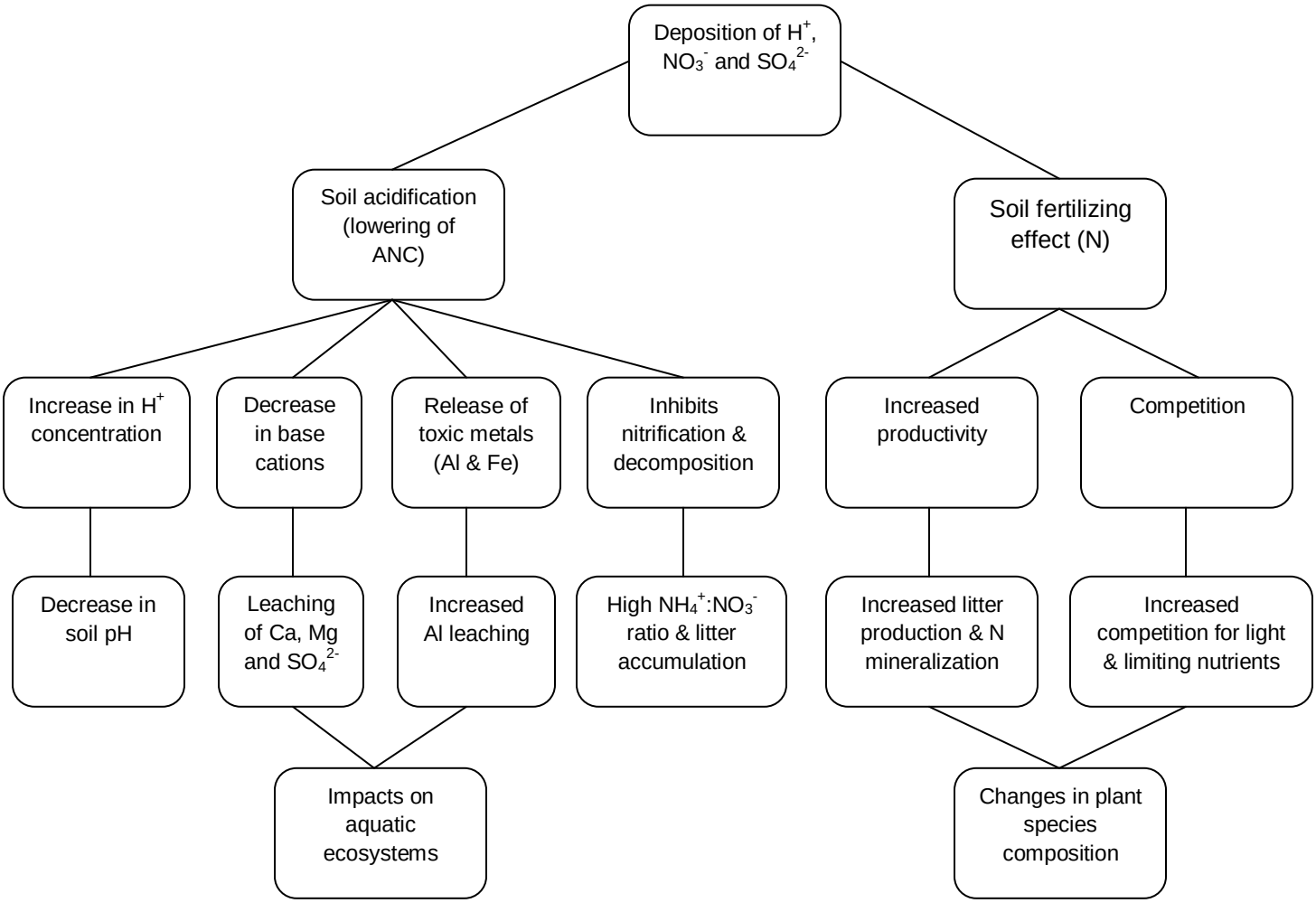

Figure 2.6: Diagrammatic representation of the main impacts of N and S deposition on vegetation and soil of an ecosystem. (Modified from Aerts & Bobbink, 1999; Bobbink & Lamers, 2002 & Bird, 2011).

2.5.1 Key Soil Properties and Processes

Acidic compounds, such as HNO_3, reaching the surface of the earth in precipitation have the ability to cause N saturation of a system. This is defined as a system where the primary production will not be further increased by any increase in N supply (Nilsson, 1986) and was first thoroughly studied by Aber *et al.* (1989). This phenomenon is thought to have occurred in forested areas in the San Bernardino Mountains in southern California. Fenn *et al*. (1996) recorded NO_3^- concentrations

of up to 44 times greater in the soils of areas that received elevated N deposition over the last 40 years. Gaseous losses in the forms of NO_2 and N_2O, nitrification, N mineralization and NO_3^- leaching were found to be larger in the soil in areas most impacted by N deposition (Fenn *et al.*, 1996; Fenn *et al.*, 1998). In a system that did not receive anthropogenic N and S deposition, the mineralization of organic N in the litter to NH_4^+ would result in nitrification and plant NH_4^+ and NO_3^- uptake. However when additional inputs of NH_3 from deposition occur, nitrification could also result in soil acidification (Horswill *et al.*, 2008). If the excess NO_3^- produced by nitrification is leached, there is an addition of $2H^+$ ions to the soil whereas if the NO_3^- is taken up by plants, there is addition of $1H^+$ ion to the soil (Horswill *et al.*, 2008). High levels of N and S inputs from deposition create further changes in the chemistry and chemical processes of the soil. Nitrogen saturation and N and S deposition are known to affect the acid status of soil, the amount of base cations leached from the soil and toxic ion release from the soil (Grennfelt & Hultberg, 1986).

An increase in the acid status of soils is known to affect the soil's acid neutralizing capacity (ANC). The ANC measures the buffering capacity of the soil against acidic inputs and measures the ability of the soil to resist a change in pH (Singer & Munns, 1987). Large inputs of strong acids to soils decrease the ANC of the soil. This occurs by addition of H^+ ions from the input of HNO_3 and H_2SO_4 that directly decreases the ANC of the soil and by displacing base cations such as sodium (Na^+), potassium (K^+), Ca^{2+} and magnesium (Mg^{2+}) or causing the leaching of base cations into solution (Kennedy, 1986; Ulrich & Sumner, 1991). The loss of base cations from the top soil is thought to be of concern in the United Kingdom as various models have predicted that it is unlikely that base saturation will naturally return to levels before large-scale deposition became an issue (Coyle & Vipond, 2001).

This displacement of base cations further increases the acid status of the soil and the leaching of base cations from the upper to the lower soil horizons is known as podzolisation. The combined increase of the acid status of the soil and base cation leaching can result in increased mobility of Al^{3+} and H^{+} ions (Singer & Munns, 1987; Ulrich & Sumner, 1991). Aluminium becomes more soluble in soils with a pH < 5 and the positive relationship between Al^{3+} and N deposition is well studied (Blake *et al.*, 1999; Dise *et al.*, 2001, Stevens *et al.*, 2011). As soils become more acidic, certain base cations such as Mg^{2+} and Ca^{2+} are released and neutralize the acidity, although once the base cations are depleted, the release of Al^{3+} increases (Stevens *et al.*, 2006; Bowman *et al.*, 2008). This was found to be the case by Jacobson *et al.* (2011) where the soil Al^{3+} concentration increased in the top 30 cm of soil under N and N+P fertilization experiments. An increase in Al^{3+} ions can be harmful to ecosystem functioning and affect the system directly and indirectly. These ions directly affect the system as they are toxic to most plants and affect the system indirectly as they affect the nutrient uptake ability of plants (Cowling & Linthurst, 1981; Singer & Munns, 1987; Van Miegroet *et al.*, 1992b; Scholes *et al.*, 1996).

2.5.2 Plant Species Diversity

The numerous changes that have been recorded in soil chemistry as a result of N and S deposition can be considered important drivers of biodiversity changes in a number of ecosystems, including grasslands (Smart *et al.*, 2003; Stevens *et al.*, 2006). Nitrogen and S deposition have been responsible for the decline in plant species richness and diversity in a number of sites studied across the US and Europe (Al-Mufti *et al.*, 1977; Bobbink *et al.*, 2010; van den Berg *et al.*, 2011; Jacobson *et al.*, 2011; Stevens *et al.*, 2011) with a number of suggested reasons for this occurrence.

The availability of nutrients is a key determining factor in the plant species composition of ecosystems and by changing nutrient availability, a change in plant species composition often follows soon after. It is thought that the addition of N through deposition changes the competitive relationship between plant species in an ecosystem (Lawes & Gilbert, 1880; Al-Mufti *et al.*, 1977; Bobbink *et al.*, 1998). Many plant species that occur in N limited systems are adapted to those nutrient conditions and with the addition of N become outcompeted by species that perform better when supplied with larger amounts of N (Chapin, 1980; Bobbink *et al.*, 1998). As well as being outcompeted by the N adapted plant species in the system, the addition of N and S may also change the limiting factor for more sensitive plant species. In systems where N was the limiting factor prior to atmospheric deposition, light and P have often emerged as the new limiting factors following years of N and S deposition (Bobbink, 1991; Phoenix *et al.*, 2006; Huang *et al.*, 2010). A change in the plant species composition of a system may also occur following a decrease in the pH of the soil as a result of acid deposition. Plants that are more tolerant of acid soils become more dominant and may outcompete plants that are adapted to soil with a more intermediate pH (Bobbink *et al.*, 1998). van den Berg *et al.* (2011) modelled the impact of N and S deposition on plant species diversity in UK grasslands from 1993 to 2009 and the model revealed a significant indirect effect of the deposition as a result of changes in soil pH.

Research has been conducted that includes field experiments to quantify the rate of forb species loss in grasslands across N and S deposition gradients. Carroll *et al.* (2003) simulated N deposition using additions of between 35.0-140.0 kg $N\text{-}NH_4NO_3$ ha^{-1} yr^{-1} and 140.0 kg $N\text{-}(NH_4)_2SO_4$ ha^{-1} yr^{-1} to UK grasslands over a six year period. These treatments resulted in drastic changes in the grass and forb species composition as well as the grass-forb ratio following the first four years of simulated deposition (Carroll *et al.*, 2003). It was postulated that these changes were a result of a combination of N accumulation in the tissue of impacted

plants and the changes to the soil properties and processes such as soil acidification (Carroll *et al.*, 2003). Stevens *et al.* (2010a) performed a study of 153 grasslands across Europe that revealed a decrease in forb and grass species richness and both decreases had a significant negative correlation with the rate of N deposition. In grasslands that received less than 20.0 kg N ha^{-1} yr^{-1}, a deposition rate of 2.3 kg N ha^{-1} yr^{-1} would reduce plant species richness by 1 species per 4 m^2 (Stevens *et al.,* 2010a). In grasslands that received more than 20.0 kg N ha^{-1} yr^{-1}, a deposition rate of 3.5 kg N ha^{-1} yr^{-1} would reduce plant species richness by the same amount (Stevens *et al.*, 2010a). As it only requires a small amount of additional N in high deposition areas to decrease the plant species richness by the same amount as in low deposition areas, it was suggested that there was already a decline in N sensitive species in high deposition areas leaving species that were more N tolerant at the time of sampling (Emmett, 2007; Stevens *et al.*, 2010a).

A number of studies have tested the duration of deposition before a consistent, significant reduction in the number of plant species can be expected in the system. Clark & Tilman (2008) and Haddad *et al.* (2000) determined this time lapse to be between 3 and 9 years and that the level of deposition during that time would be the most important factor determining when plant diversity changes would be detectable. Stevens *et al.* (2004) estimated that for every 2.5 kg N ha^{-1} yr^{-1} increase in deposition, a decrease of 1 plant species can be expected in a system. This would be a fair estimate for certain systems although the exact level of deposition that would cause such a decrease in plant species diversity differs for each system, depending on the systems buffering capacity and the number of plant species at the time of sampling. A report by Bobbink (2004), released in the same year may be a more widely accepted description of the impacts on deposition on plant species diversity across a deposition gradient. The report found a 40.0% plant species reduction across a deposition gradient of between 0.0 and 40.0 kg N ha^{-1} yr^{-1} in Europe (Bobbink, 2004).

2.5.3 Plant Growth and Productivity

The concept of increased productivity in an ecosystem with larger plant species diversity has been greatly debated. Tilman *et al.* (1997) suggested that if different plant species occupy the same habitat and have different temporal resource use, a multi-species coexistence can occur in one ecosystem that would lead to increased productivity. This was found to be the case by Tilman *et al.* (2001) as they recorded a significant increase in above-ground biomass and total biomass with an increase in the number of plant species.

The opposite to this debated concept of decreased productivity in a system with smaller plant species diversity is a concern for areas receiving N and S deposition. It has already been discussed that with nutrient inputs from deposition, a decrease in plant species diversity is expected however that would depend on the limiting nutrient in the system and the form of N and S deposition. Bobbink *et al.* (1988) and Bobbink (1991) found that N addition in the form of NH_4NO_3 to their study sites in European grasslands resulted in an increase in total biomass production. These studies appeared to show a fertilization effect and suggest the impact of N and S deposition on ecosystem productivity would depend on the forms of N and S deposited as well as the total amount of annual deposition. This fertilization effect would be expected in systems that are N limited, a deposition impact supported by the optimal partitioning theory that proposes plants will equalize their nutrient exchange ratios to gain the most from their available resources (Bloom *et al.*, 1985).

2.5.4 Surface Water Quality

Terrestrial effects of N and S deposition focused upon thus far have been those on soil and vegetation. Nitrogen and S deposition that impacts on soil chemistry and

plant diversity has the potential to impact on surface water quality. If that happens, terrestrial impacts would include those on the freshwater river and dam systems that receive water from impacted catchments. Although these impacts were not determined during this study as an initial investigation, river samples have been collected between October 2002 and October 2006 by collaborating colleagues (Blight *et al.*, 2009) and the potential exists to monitor surface water NO_3^- and SO_4^{2-} concentrations together with N and S deposition on the Mpumalanga Highveld.

Fresh waters are one of the most sensitive ecosystems to acid deposition and N and S deposition have been recorded to have impacted streams globally (Muniz, 1991). The nitrate concentration in the Mississippi River and rivers across the US has increased between three- and ten-fold since 1965 and shows a correlation with human population density in the watersheds of the rivers (Turner & Rabalais, 1991; Peierls *et al.*, 1991; Cole *et al.*, 1993). The degradation of water quality caused by an increase in the concentrations of limiting nutrients has the potential to cause acidification, affect the productivity and species composition of the aquatic ecosystems and impact on human health (Likens *et al.*, 1996).

A number of monitored catchments around the world that receive moderate to high levels of deposition appear to be becoming N saturated and show HNO_3 as the cause of acidification (Vitousek *et al.*, 1997). Nitrate and SO_4^{2-} can leach through the soil profile into surface and ground water creating an acidifying effect and changing the ecosystem downstream (Likens *et al.*, 1996). This occurs as NO_3^- and SO_4^{2-} are relatively mobile in the soil and travel through the soil profile, often pulling base cations with them (e.g. magnesium sulfate ($MgSO_4$)) into surface waters (Likens *et al.*, 1970). Nitrate leaching is high in regions receiving high levels of N deposition (35.0 kg N ha^{-1} yr^{-1}) in China (Chen *et al*., 2004). Nitrate and SO_4^{2-}

levels at a number of water quality monitoring sites in the Vaal Dam catchment ranged between 0.15-3.23mg NO_3^- L^{-1} and 12.01-82.70 mg SO_4^{2-} L^{-1} (Fey & Guy, 1993; Bird, 2011). Freshwater NO_3^- and SO_4^{2-} levels in the Vaal Dam catchment are broader than the levels in Japan (Shrestha & Kazama, 2007) and India (Singh *et al.*, 2004) but are similar to a number of streams in the UK (Evans *et al.*, 2001).

Nitric acid contributes directly to acidification and NH_4^+ contributes during its formation as H^+ ions are produced during nitrification (Schindler *et al.*, 1985). The resultant acidification can also affect the N cycle as nitrification of NH_4^+ slows at soil pH levels < 5.6 (Rudd *et al.*, 1988). These changes in the chemistry of river and dam waters have affected species composition and plant productivity in the impacted water systems. The acidification of surface water enhances mercury (Hg) accumulation in fish (Driscoll *et al.*, 1994) while a decrease in pH and increase in Al^{3+} ion concentration lead to a decline in species richness and abundance of zooplankton, microinvertebrates and fish (Schindler *et al.*, 1985). The increase in N concentration and flux in large rivers around the world correlate with high human population densities and the rate of deposition in the catchments of these rivers (Peierls *et al.*, 1991; Cole *et al.*, 1993).

CHAPTER 3: MATERIALS AND METHODS

3.1 Research Site Description

The research site is located in the south of the Mpumalanga province of South Africa (Figure 3.1). This site was identified and selected as it is in the vicinity of three coal-fired power stations, the closest being the Majuba power station, and it receives elevated N and S deposition (Lorentz *et al*., 2007).

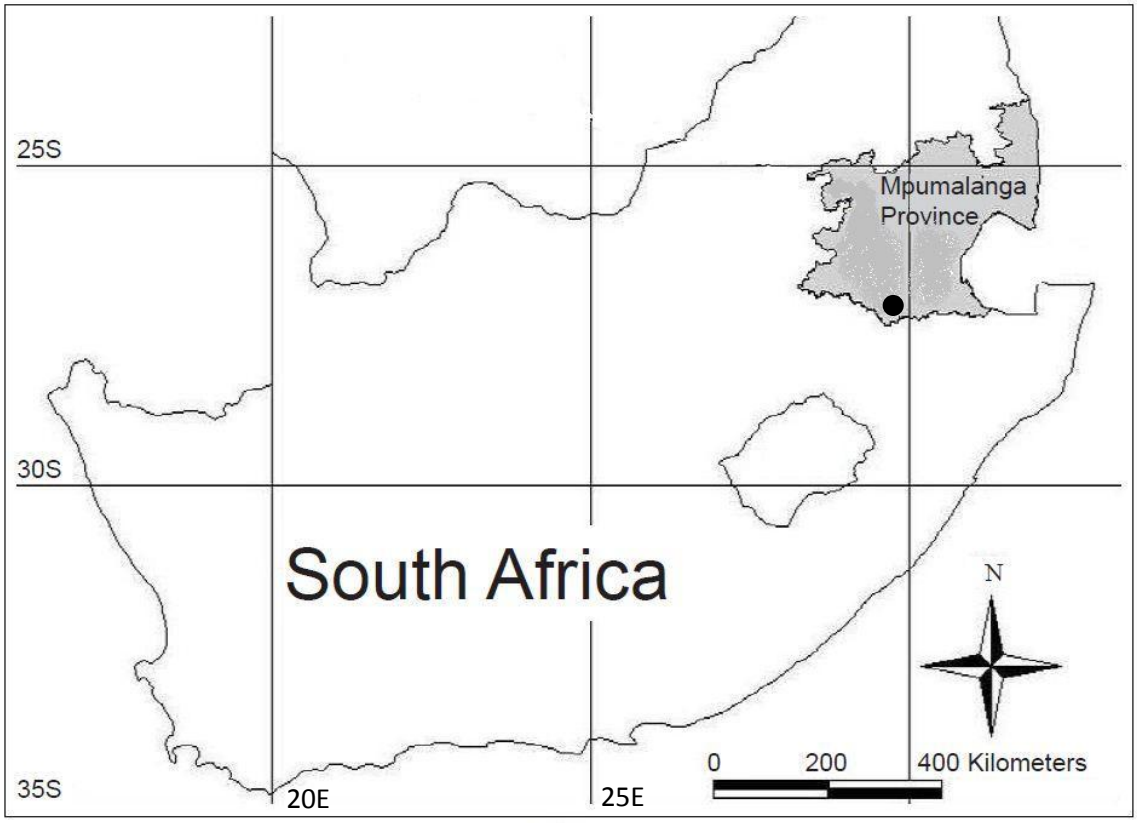

Figure 3.1: The position of the Sandspruit catchment in the southern part of the Mpumalanga province of South Africa (●). Vertical y axis in degrees (°) of latitude (south) and horizontal x axis in degrees (°) of longitude (east).

Blight *et al.* (2009) modelled N and S deposition on the Mpumalanga Highveld. The source of the emissions and the emissions data for the Highveld, including current, historical and projected future emissions were used in hourly CALPUFF modeling of NO_x and SO_x emissions that were used to model wet, dry and total N and S deposition during an average rainfall year (Figure 3.2 & 3.3). The peak total N deposition rate was modelled at 8.5 kg N ha^{-1} yr^{-1} (Figure 3.2) and consisted of 63.0% wet deposition (5.4 kg N ha^{-1} yr^{-1}) and 37.0% dry deposition (3.1 kg N ha^{-1} yr^{-1}) (Blight *et al.*, 2009). The peak total S deposition rate was estimated historically to be in the range of 50.0-80.0 kg S ha^{-1} yr^{-1} and was modelled by Blight *et al.* (2009) to be 35.0 kg S ha^{-1} yr^{-1} (Figure 3.3). This was divided into wet and dry S deposition with wet S deposition on the Highveld modelled at 20.0 kg S ha^{-1} yr^{-1} and dry S deposition between 3.0-6.0 kg S ha^{-1} yr^{-1} (Blight *et al.*, 2009).

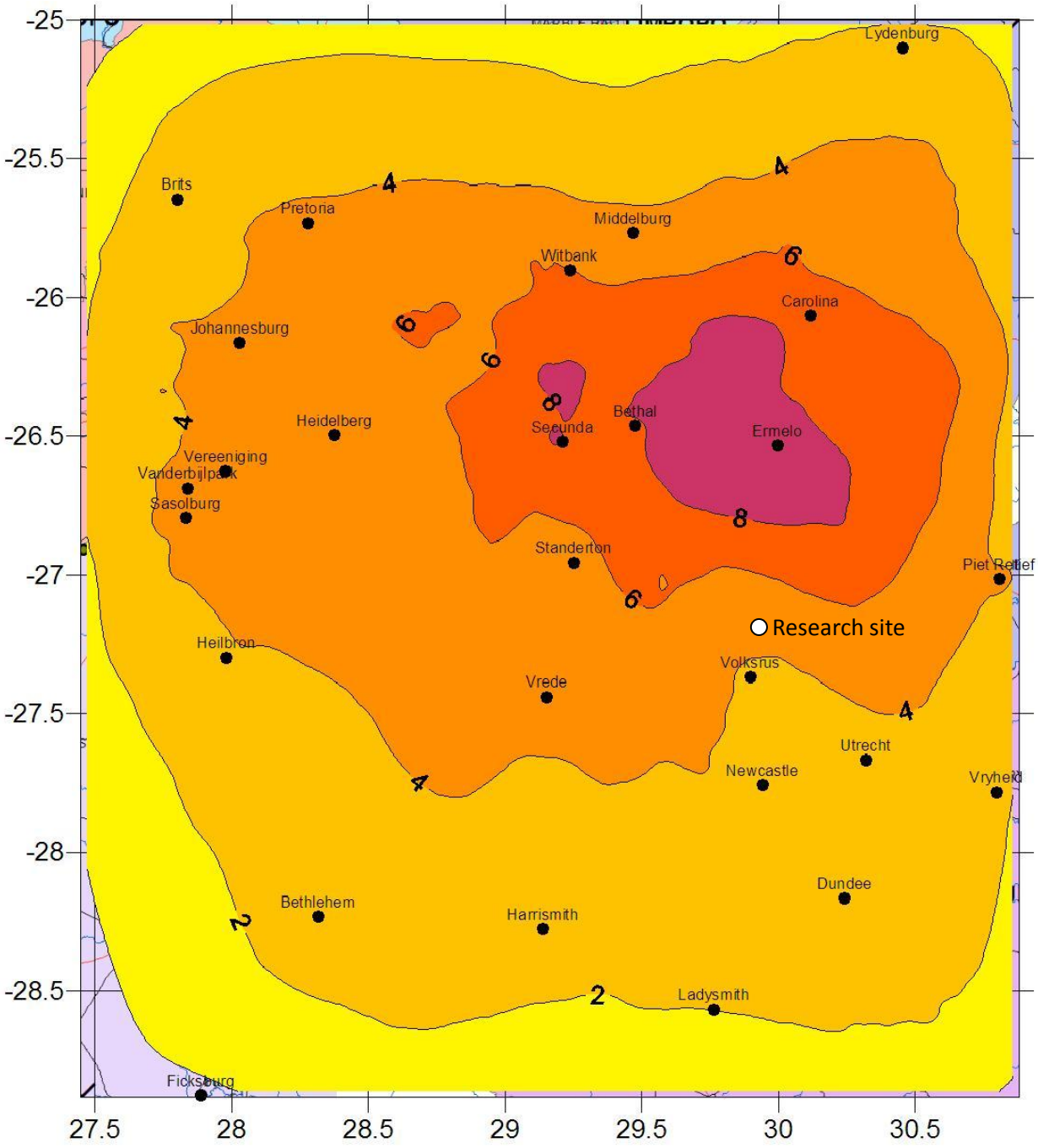

Figure 3.2: Modelled total N deposition (kg N ha^{-1} yr^{-1}) for an average rainfall year (Blight *et al.*, 2009). The location of the research site is indicated by the white dot, 14.0 km north of Volksrust. Axes in degrees (°) of latitude (south) and longitude (east).

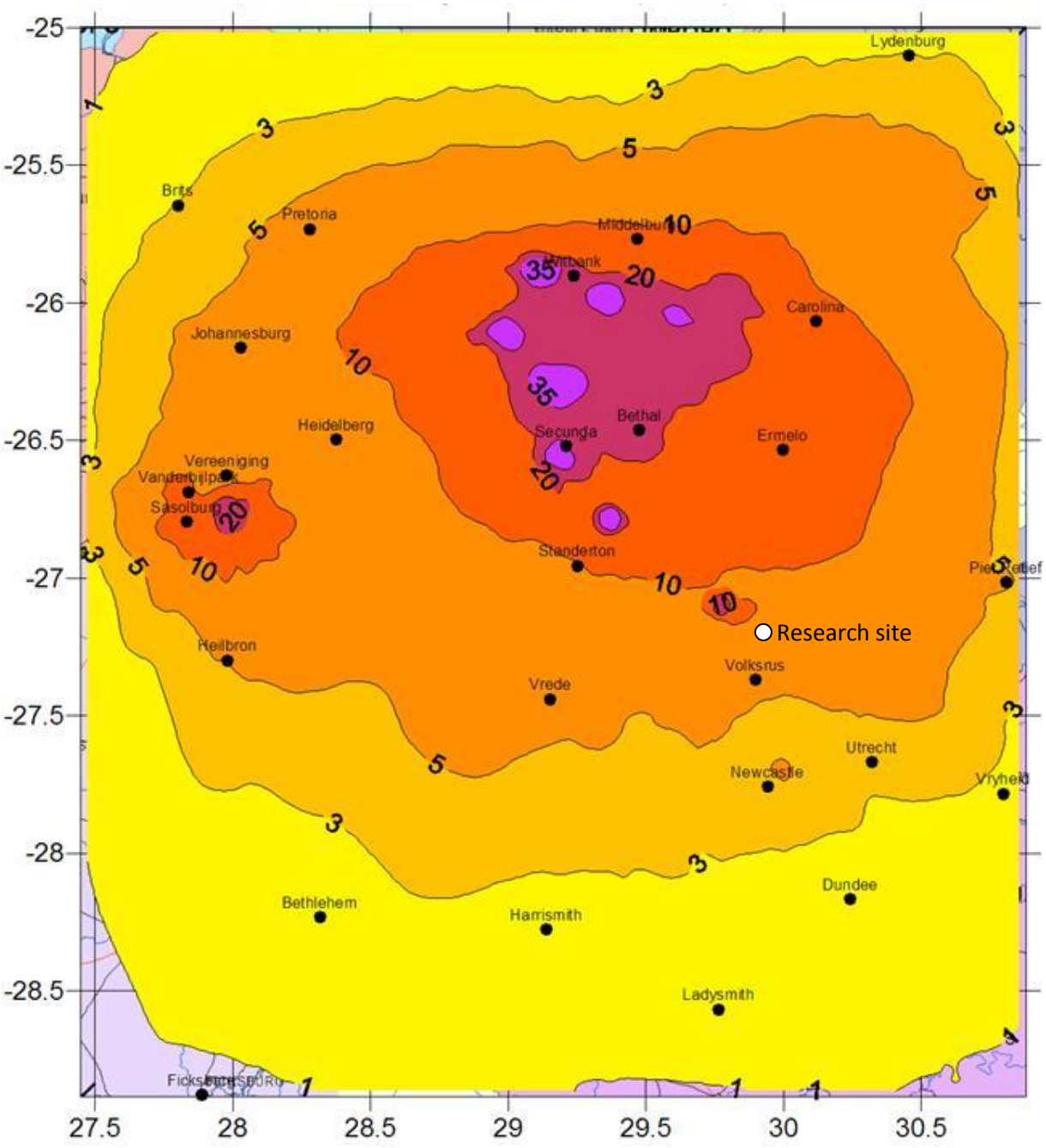

Figure 3.3: Modelled total S deposition (kg S ha^{-1} yr^{-1}) for an average rainfall year (Blight *et al.*, 2009). The location of the research site is indicated by the white dot, 14.0 km north of Volksrust. Axes in degrees (°) of latitude (south) and longitude (east).

The research site is located in the Sandspruit catchment in the upper reaches of the Klip River, with the Vaal River as its primary river system. The Sandspruit catchment lies at an approximate latitude of -27.2323 S and approximate longitude of 29.9177 E and comprises a 207.0 km^2 section of the quaternary catchment C13A (Figure 3.4). The catchment receives a mean annual rainfall of 778.0 mm and has a mean annual evaporation of between 1300.0 and 1400.0 mm (Midgley *et al*., 1994). The research site lies approximately 14.0 km north of the town of Volksrust and is located 21.0 km south east of the Majuba power station (Figure 3.5). The first order stream in the Sandspruit catchment is approximately 3.0 km long and comprises a channel with a spring that surfaces in the lower reaches. The catchment is approximately 0.8 km wide and drains towards the west.

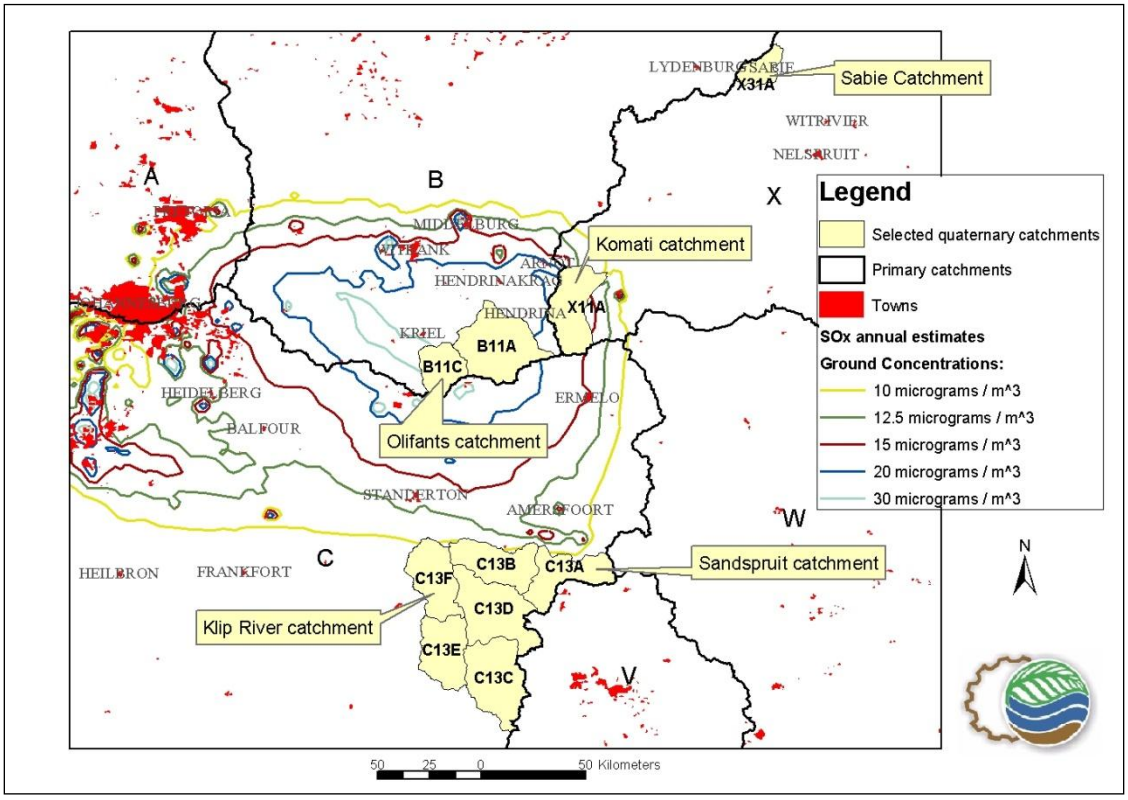

Figure 3.4: Position of the Sandspruit catchment at C13A relative to the major SO_x pollution sources (Lorentz *et al.*, 2010).

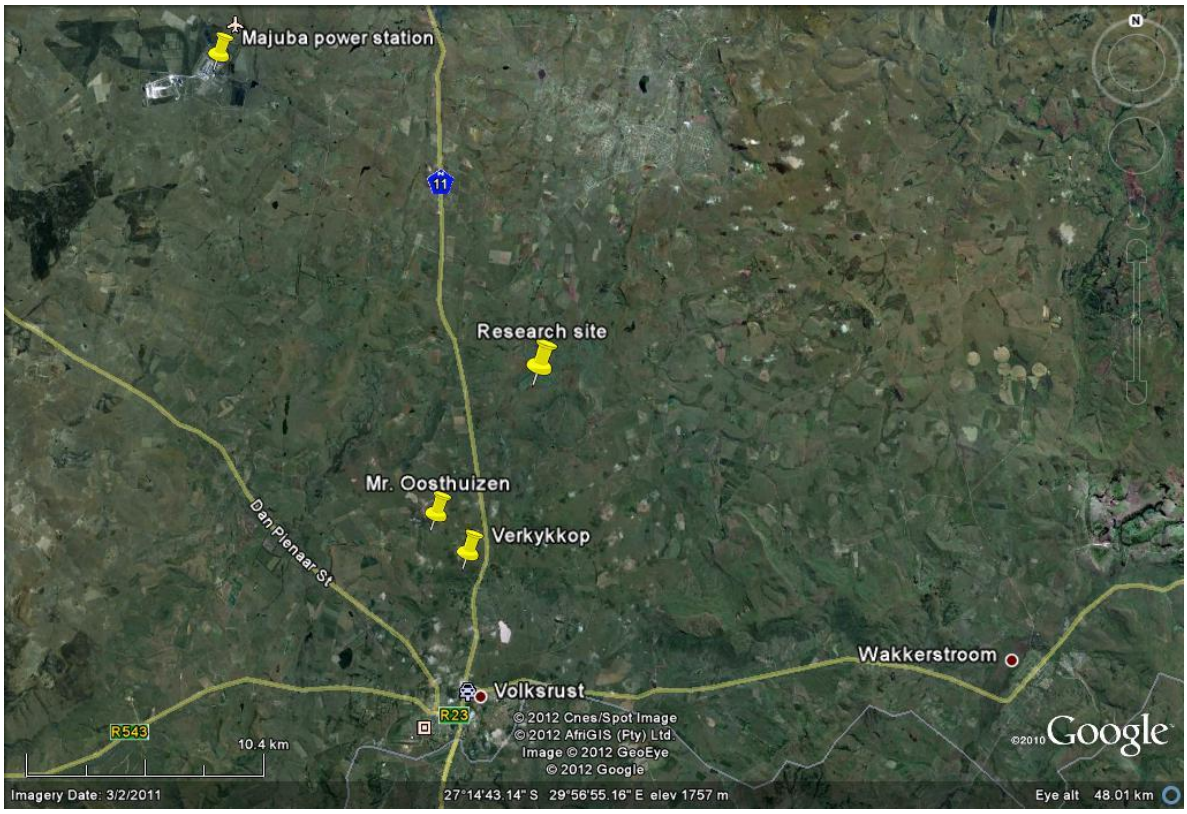

Figure 3.5: Position of the research site relative to the town of Volksrust, Verkykkop (weather station location), Mr. Oosthuizen's farm (rainfall measurements location) and Majuba power station (Google Earth© 2010 image).

Daily rainfall measurements were recorded by Mr. Oosthuizen from 1 October 2010 to 31 December 2011 at his house (-27° 17' 39" S; 29° 52' 22" E), located 8.0 km SSW of the research site (Figure 3.5). Long term (1961 to 2010) rainfall data for Volksrust was accessed from the South African Weather Service. Half hourly ambient temperatures were accessed from Eskom's air quality monitoring station located at Verkykkop (latitude -27° 18' 35.92" S; longitude 29°53' 14.44" E), 8.8km SSW of the research site (Figure 3.5) for the period from 1 October 2010 to 31 May 2011. These sites were the nearest to the research site except for the weather station located within the site that was operational from mid-March 2011 and failed from mid-June to mid-September 2011. The minimum and maximum

temperatures measured from March-May 2011 on Verkykkop and within the research site did not differ significantly and so it was assumed that temperatures from Verkykkop were representative of the research site.

The sampling site comprised mixed grassland with mostly sourveld species and was dominated by large stands of *Hyparrhenia hirta* (L.) Stapf and a stocking density of one animal per four hectares. The hill on which the research site was established became steeper towards the crest of the hillslope, on which a windmill draws groundwater in the upper reaches of the catchment from 20-30 m below ground (Figure 3.6). The exact position of the 30 m x 30 m research site within the Sandspruit catchment (latitude -27.236427 S, longitude 29.920112 E, altitude 1814 m) was chosen to be on a mid- to upper west facing slope as the east facing slopes were shorter, steeper and the soils were deemed to be shallow and stony. The soils collected in an initial site visit in September 2010 were dark grey, fairly moist immediately below the surface and appeared clayey.

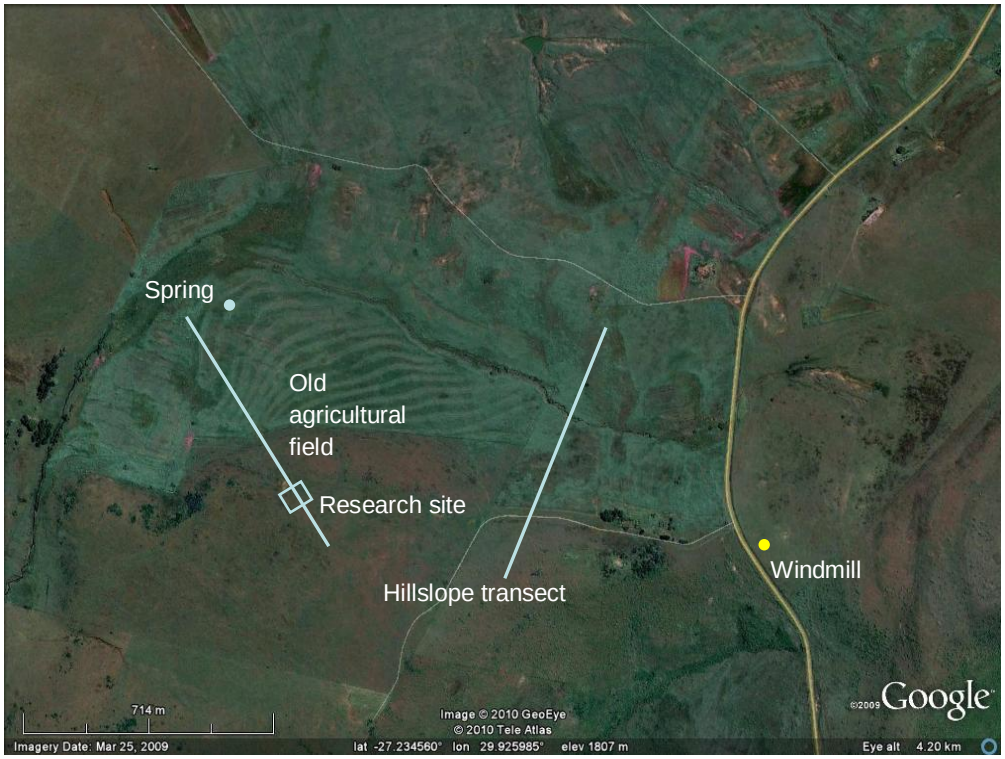

Figure 3.6: Position of the research site in relation to the spring, the windmill and an old agricultural field on Wessel Oosthuizen's farm in the Sandspruit catchment (Google Earth$_{© 2010}$ image).

3.1.1 Research Site's Historical and Current Land Use

The farm on which the research site was located was used for farming agricultural crops more than 60 years ago and has been in the Oosthuizen family for the past 150 years. A large proportion of the mid and valley slopes of the catchment were previously tilled for crop production and still show elevated ridges and depressions, including the area within the research site (Figure 3.7). The land was used for beef cattle grazing following the agricultural farming, although the stocking density has never been particularly high (Figure 3.8). No specific burning regime has been followed by Mr. Oosthuizen in the past and the decision to burn is made by visually assessing the grass fuel load at the end of each dry season. These controlled

burns are performed on a day with minimum wind speed so they can be kept within the confines of the farmer's land and do not spread to adjacent fields.

Mr. Oosthuizen was subcontracted to buy materials for and erect the experimental research site (30 m x 30 m) in the Sandspruit catchment (Figure 3.7). This research site was erected (in November 2010) to exclude the effects of cattle dung, trampling and grazing. This was necessary as cattle move nutrients directly by ingestion, retention and excretion and indirectly by modifying mineralization rates, dry deposition, leaching and soil erosion (Bokdam & Gleichman, 2000).

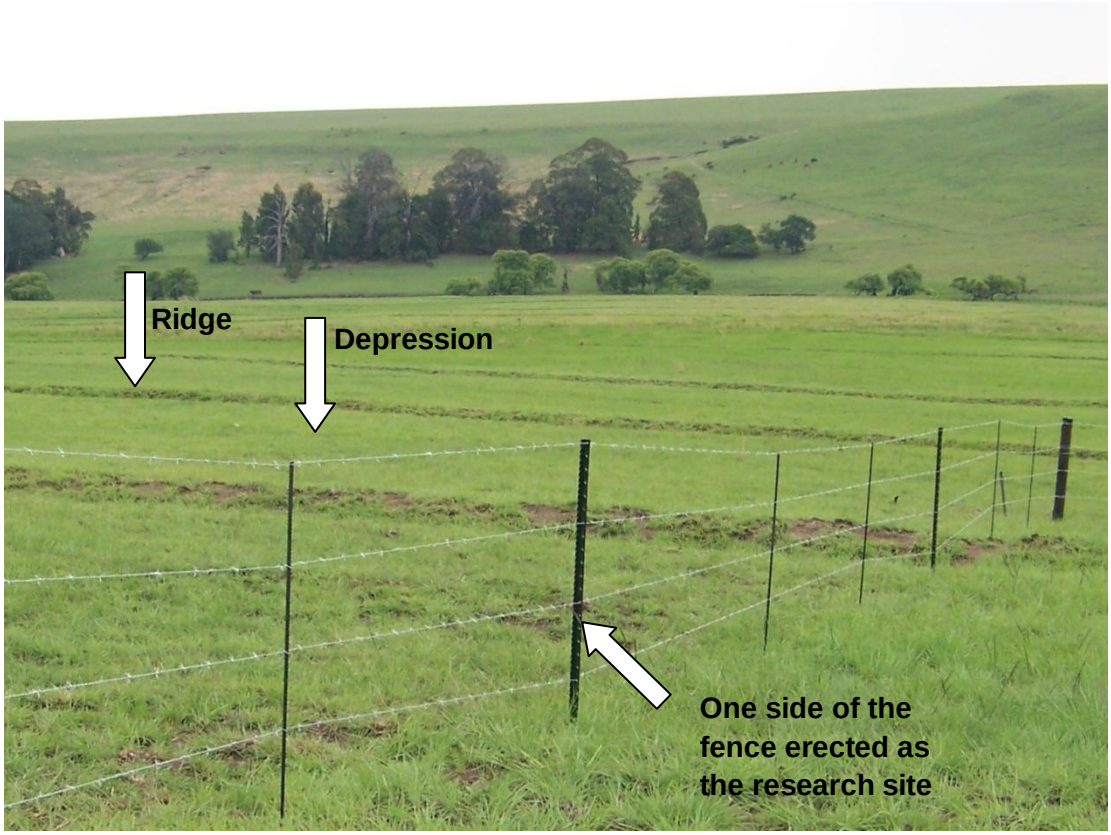

Figure 3.7: Ridges and depressions from tillage approximately 60 years ago running across the field, through the fenced-off research site and parallel to the contours of the land (Photo: L. Hutchinson).

Figure 3.8: Sourveld grassland during the initial site visit (September 2010) before the research site was erected, showing current land-use for free range beef cattle (Photo: Prof. S. Lorentz).

3.2 Sampling Timeline

Vegetation, litter, soil and insect sampling were performed during the eight month sampling period from October 2010 to May 2011. The change in the landscape can be seen from the beginning of the growing season in October 2010 (Figure 3.9), during the growing season from December 2010 to April 2011 (Figures 3.10 to 3.14) to the end of the growing season in May 2011 (Figure 3.15). Figures 3.9 and 3.13 show the research site during the months when vegetation and litter sampling was performed and Figures 3.12 and 3.15 show the research site during the months when insect sampling was performed. No photos were taken during the November 2010 sampling and the research site had burnt earlier on the day of sampling.

Figure 3.9: Research site on the day of sampling in October 2010 (Photo: L. Hutchinson).

Figure 3.10: Research site on the day of sampling in December 2010, one month after a planned burn on the farm (Photo: L. Hutchinson).

Figure 3.11: Research site on the day of sampling in January 2011 (Photo: L. Hutchinson).

Figure 3.12: Research site on the day of sampling in February 2011 (Photo: L. Hutchinson).

Figure 3.13: Research site on the day of sampling in March 2011 (Photo: L. Hutchinson).

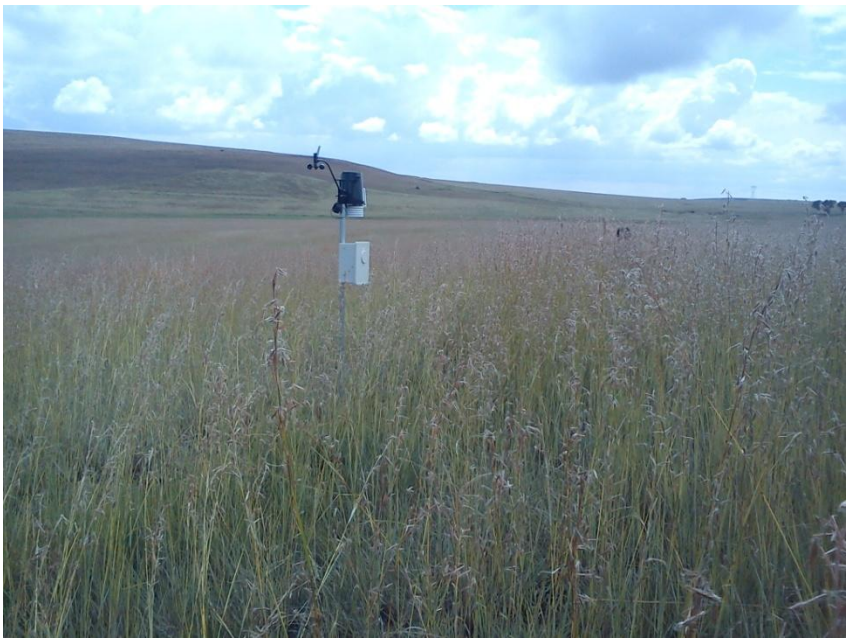

Figure 3.14: Research site on the day of sampling in April 2011 (Photo: L. Hutchinson).

Figure 3.15: Research site on the day of sampling in May 2011 (Photo: L. Hutchinson).

3.3 Species Lists

Two species lists were compiled which document the grass and insect species found within the research site. The grass species were recorded during sampling in October 2010 and March 2011 when vegetation and litter sampling was performed (Table 3.1). The insect species were recorded using sweep-net sampling in February and May 2011 at the beginning and end of their growing season (Table 3.2).

Table 3.1: Grass species recorded during the sampling in October 2010 and March 2011 including species name, common name and authority.

Sampling Month	Species Name	Common Name	Authority
October 2010 and March 2011	*Hyparrhenia hirta*	Common thatching grass	(L.) Stapf
	Sporobolus africanus	African dropseed	Poir.

Table 3.2: Insect species recorded during the sampling in February and May 2011 including species name, common name and authority.

Sampling Month (in 2011)		Species Name	Common Name	Authority
February	May			
✓		*Musca domestica*	House fly	Linnaeus, 1758
✓		*Chilocorus stigma*	Black two-spot ladybug	Say, 1835
✓		*Eyprepocnemis*	Locust	Fieber, 1853
✓		*Nephrotoma*	Cranefly	Meigen, 1828
✓		*Palpita unionalis*	White pearl moth	Hubner, 1796
✓		*Hermyia diabolus*	Fly	Wiedemann, 1830
✓	✓	*Cheilomenes lunata*	Lunate ladybug	Fabricius, 1775
✓		*Conocephalus caudalis*	Meadow katydid	Walker F., 1869
✓		*Leucochitonea levubu*	White cloaked skipper	Wallengren, 1857
✓		*Acrida acuminata*	Common stick grasshopper	Stål, 1873
✓		*Stenocoris apicalis*	Broad-headed bug	Westwood
✓		*Lucilia sericata*	European green blowfly	Meigen, 1826
✓		*Drosophila*	Vinegar fly	Fallén, 1823
✓		*Lycus melanurus*	Hook-winged net-winged beetle	Blanchard, 1843
✓		*Spilostethus pandurus*	Milkweed bug	Scopoli, 1763
✓		*Elassogaster*	Black scavenger fly	Bigot, 1860
✓		*Lagria vulnerata*	Hairy darkling beetle	Fåhraeus, 1870
✓		*Alcimus tristrigatus*	Robber fly	Loew, 1858
	✓	*Allograpta fuscotibialis*	Hover fly	Macquart, 1842
	✓	*Homoeocerus*	Bug species	Bermeister, 1835
	✓	*Polyrhachis gagates*	Ant species	Smith, 1858
	✓	*Catantops humeralis*	Grasshopper species	Thunberg, 1815
	✓	Coccinellidae	Ladybird / Ladybug family	Latreille, 1807
	✓	*Mirperus jaculus*	Broad-headed bug species	Thunberg, 1783
	✓	Thericleidae	Grasshopper family	Burr, 1899
	✓	*Conocephalus conocephalus*	Meadow katydid	Linnaeus, 1767
	✓	*Bittacus sp.*	Hanging fly	Latreille, 1805
	✓	*Truxalis*	Grasshopper genus	Fabricius, 1775
	✓	Chrysomelidae	Leaf beetle	Latreille, 1802
	✓	*Daspletis placodes*	Robber fly	Londt., 1983

3.4 Sampling Methodology

3.4.1 Vegetation and Litter

Vegetation and litter sampling were performed during October 2010 as an estimate of standing biomass at the end of the 2010 growing season and during March 2011 as an estimate of the peak biomass for 2011. The vegetation sampling in March 2011 supplied an estimate of the total biomass produced over the 2010-2011 growing season as the research site burnt in November 2010. Sampling consisted of randomly selecting 50 0.5 m x 0.5 m quadrats. The vegetation in each quadrat was ranked between 1 and 5 for basal cover (1 being the least amount of basal cover and 5 the most). The three most abundant grass species in the first 30 quadrats were identified and ranked using the dry-weight rank method (DWR) (t'Mannetjie & Haydock, 1963). The proportion of each species in each rank in the quadrats was multiplied by 70.2, 21.1 and 8.7 for rank 1, 2 and 3 respectively (t'Mannetjie & Haydock, 1963). This provided the percent contribution, separated by rank, which were summed to provide the percent dry mass contribution of each grass species in October 2010 and March 2011. The DWR method was used to calculate above-ground biomass (kg ha^{-1}) within a single growing season (Tilman *et al.*, 2001) and estimate species composition in terms of contribution to the total mass (Dekker *et al.*, 2001). To quantify the mass given to each rank and the total biomass over the 2010-2011 growing season, all the above ground vegetation of these quadrats was cut, bagged and all the litter in each quadrat was collected and bagged. For the purpose of this study, vegetation was defined as above-ground living plant parts or parts still attached to the plant and litter was defined as leaf and stem material that was no longer attached to the plant (Woods & Raison, 1983). Each vegetation and litter sample was weighed, dried for three days at 50.0 °C, re-weighed and milled to a fine powder. Sub-samples of each vegetation and litter sample were analyzed according to Table 3.3.

Twenty randomly selected grass tufts were collected in August 2011 and each one was divided in half. One half was rinsed in de-ionized water and the other half was not rinsed. All 40 samples were dried for three days at 50.0 °C and analyzed for total N and S (%). Comparing the samples when rinsed and when un-rinsed provided an indication of whether deposition of N and S on the surface of the grass stems and leaves was contributing significantly to the total plant N and S (%) at the end of the growing season in March 2011.

3.4.2 Soil

The methodology for mineralization studies (Raison *et al.*, 1987) involves placing 25 cm long stainless steel tubes into the soil to a depth of 15 cm. This was done using 10 tubes inserted diagonally across the research site (Figure 3.16). All ten tubes were immediately removed for an initial estimate of inorganic N and S (Time 0) (Figure 3.17 & 3.18). The tubes were knocked back into the ground, securely covered with plastic and left to incubate in the field for the month (Time 1), until the time of the following sampling. This method allowed calculation of the amount of NH_4^+, NO_3^- and SO_4^{2-} in the soil before and after incubation without removal by plant uptake, evapotranspiration and volatilization but with the continuation of other microbial processes. The first soil samples were collected before the 2010-2011 rainy season began and all subsequent soil sampling was completed during the first week of every month following October 2010. All tubes were inserted in the elevated ridges of the field to keep variables such as soil compaction constant and all tubes were secured to prevent leaching of inorganic N and S by percolation of precipitation. Soil samples were stored at 4.0 °C and analyzed within 48 hours. All tubes were inserted at least 10 cm away from previous tube holes at each sampling point to remove any effects of standing water in holes left from previously collected soil samples.

Gravel and large roots were removed from all soil samples. Each soil sample was manually mixed as their high clay content prevented them from being sieved. Sub-samples were taken from soil samples for all analyses. Complete soil analyses are presented in Table 3.3.

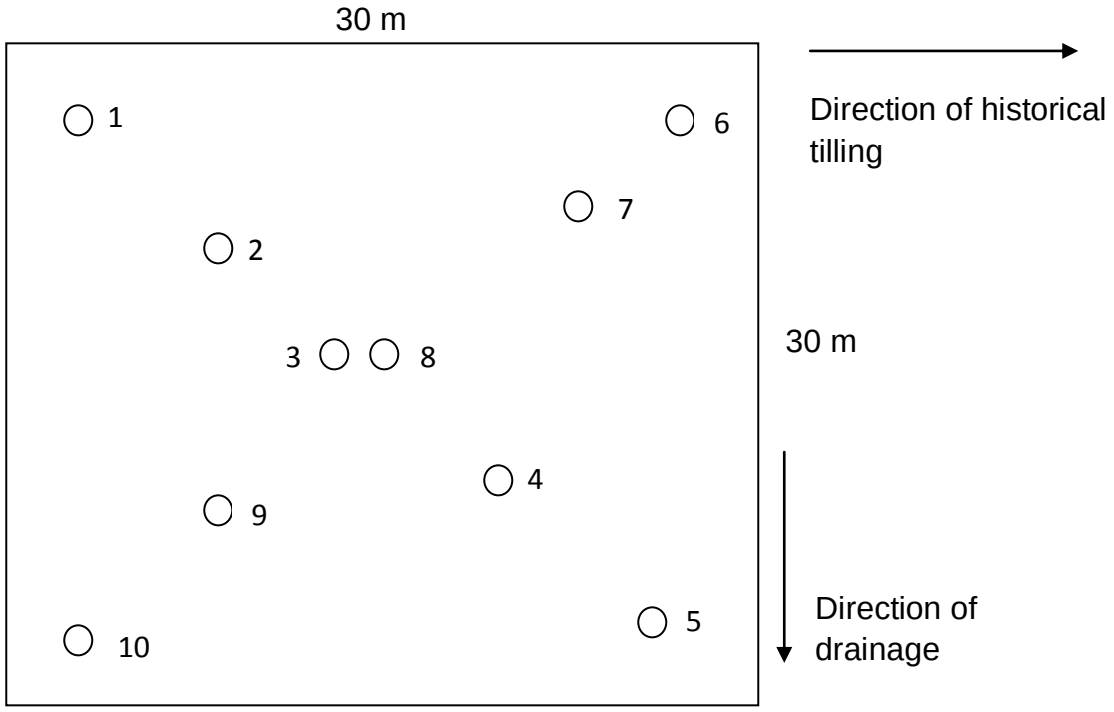

Figure 3.16: Representation of the 30 m x 30 m research site indicating the positioning of the ten sampling points in relation to the tillage and drainage directions of the field.

Figure 3.17: Elevated ridge in the research site where tubes were inserted and removed for Time 0 and Time 1 sampling in December 2010 (Photo: L. Hutchinson).

Figure 3.18: Removal of the Time 0 soil sample from the tube during sampling in December 2010 (Photo: L. Hutchinson).

3.5 Laboratory Analyses

Table 3.3: Ecosystem component, variable measured, reagents used, procedure conducted and the months during which the variables were analyzed are presented. The method reference and the institution at which the analysis was performed are provided.

Ecosystem Component	Variable	Reagent/s	Procedure	Months Conducted	Reference	Institution / Laboratory
Soil	Total N (%)	-	Leco combustion	Oct & Nov 2010	Horneck & Miller (1998)	BEM Laboratory
	Total S (%)	Potassium sulfate (99% min.) Phosphoric acid (concentrated) Calcium carbonate (98% min.)	ICP-OES	Oct & Nov 2010	Soil and Plant Analysis Council (1999b)	BEM Laboratory
	Total C (%)	Potassium dichromate (0.167M) Iron (II) ammonium sulfate (0.5M) N-phenylanthranilic acid indicator	Walkley-Black method	Oct & Nov 2010	Soil and Plant Analysis Council (1999b)	BEM Laboratory
	Total P (%)	Ammonium fluoride Bray (II) extraction solution	ICP-OES	Oct & Nov 2010	Soil and Plant Analysis Council (1999b) Soil Science Society of South Africa	BEM Laboratory
	Exchangeable base cations (c mol (+) kg^{-1})	Ammonium acetate (1M, pH7)	ICP-OES	Oct & Nov 2010	Soil and Plant Analysis Council (1999b) Soil Science Society of South Africa (1998)	BEM Laboratory
	Exchangeable Al (c mol (+) kg^{-1})	Potassium hydroxide (0.1M) Sodium hydroxide (0.05M)	Titration with NaOH	Oct & Nov 2010	Soil and Plant Analysis Council (1999b) Eksteen (1969)	BEM Laboratory

Table 3.3: Continued.

Soil	Texture (% clay, silt & sand)	Sodium hexametaphosphate solution (4%) buffered with sodium carbonate	Dispersion and hydrometer method	Nov 2010	Gee & Bauder (1986)	Phytotron, University of the Wiwatersrand
	Ammonification (µg g^{-1} soil day^{-1})	N1 (sodium salicylate, sodium citrate, sodium tartrate, sodium nitroprusside, water) N2 (sodium hydroxide, sodium hypochlorite, water)	Colorimetric determination of ammonium	Oct 2010 - Jun 2011	Anderson & Ingram (1996)	Phytotron, University of the Wiwatersrand
	Nitrification (µg g^{-1} soil day^{-1})	Sodium hydroxide (4M) Salicylic acid and sulfuric acid (concentrated)	Colorimetric determination of nitrate	Oct 2010 - Jun 2011	Anderson & Ingram (1996)	Phytotron, University of the Wiwatersrand
	N mineralization (µg g^{-1} soil day^{-1})	-	Field incubation method	Oct 2010 - Jun 2011	Anderson & Ingram (1996)	Phytotron, University of the Wiwatersrand
	S mineralization (µg g^{-1} soil day^{-1})	Potassium sulfate (99% min.) Phosphoric acid (concentrated) Calcium carbonate (98% min.)	Field incubation method	Oct 2010 - Jun 2011	Soil and Plant Analysis Council (1999b)	BEM Laboratory
	Anaerobic N mineralization (µg g^{-1} soil day^{-1})	Potassium chloride (1M) Potassium chloride (2M)	Anaerobic nitrogen mineralization index	Oct 2010, Feb & Mar 2011	Anderson & Ingram (1996)	Phytotron, University of the Wiwatersrand
	Soil moisture (%)	-	Water correction factor	Oct 2010 - Jun 2011	Anderson & Ingram (1996)	Phytotron, University of the Wiwatersrand
	pH	Potassium chloride (1M)	Suspension in potassium chloride solution	Oct & Nov 2010	Soil and Plant Analysis Council (1999b)	BEM Laboratory
Vegetation and litter	Dry weight contribution	-	Dry weight rank method	Oct 2010 & Mar 2011	t'Mannetjie & Haydock (1963)	Phytotron, University of the Wiwatersrand
	Total N (%)	-	Leco combustion	Oct 2010 & Mar 2011	Horneck & Miller (1998)	BEM Laboratory

Table 3.3: Continued.

Vegetation and litter	Total S (%)	Nitric acid (1M)	ICP-OES	Oct 2010 & Mar 2011	Kowalenko & van Laerhoven (1998)	BEM Laboratory
	Total C (%)	-	Leco combustion	Oct 2010 & Mar 2011	Horneck & Miller (1998)	BEM Laboratory
	Total P (%)	Hydrochloric acid (32%) Hydrochloric acid (1:1 by volume)	ICP-OES dry ashing extraction	Oct 2010 & Mar 2011	Campbell & Plank (1998) Miller (1998)	BEM Laboratory

3.6 Statistical Analyses

The data were tested for normality using the Shapiro-Wilks test for normality. Data found to be non-normally distributed were log transformed prior to statistical analyses. Repeated measure Analysis of Variance (ANOVA) (Klugh, 1986) were used to test for significant differences between the long-term annual rainfall and the mean minimum and maximum temperatures for the eight month sampling period which allowed for comparisons within and between the data sets. Repeated measure ANOVA's were used to test for significant differences within and between variables (ammonification, nitrification, N mineralization, S mineralization, mean % soil moisture and total soil N, S, C and P). Repeated measure ANOVAs were used to test for significant differences between the values calculated for each month for the vegetation and litter total N, S, C and P for each quadrat. All results from the ANOVA tests were presented as the F statistic and the p value. Correlations were performed between N mineralization and nitrification, between N mineralization and soil moisture, as well as between S mineralization and soil moisture. Bulk density, measured in the research site (S. Lorenz, 201x, pers. comm.) was used to calculate total N, S, C and P pool sizes (kg ha^{-1}) and annual N and S mineralization rates (kg ha^{-1} yr^{-1}) to a depth of 0.15 m. All normality testing was performed using XLSTAT statistical analysis software (www.xlstat.com) and all other statistical analyses were performed using Statistica® 8.0 (www.statsoft.com).

Three N and three S input-output budgets were created using all data from the soil, vegetation and litter analyses during the eight month sampling period. Values not provided by the analyses in this study were used from relevant literature sources for use in the N and S input-output budgets. The minimum, average and maximum pool sizes and fluxes were calculated using data from vegetation, litter and soil in the study site and used to construct the N and S input-output budgets that could occur under minimum, average and maximum levels of deposition (Figures 4.14-4.19). The minimum, average and maximum budgets for the Sandspruit system were created under the assumption that the amount of N gained through N fixation and the amount of S lost from the system as H_2S gas were negligible. The values for the below-ground living component of N and S, the microbial N and S in the soil and the N and S deposition were used from the presented literature sources (Table 4.7 & 4.8). Nitrogen may also have been lost through denitrification as nitrogen dioxide (NO_2) and nitrous oxide (N_2O) gases, especially under any anaerobic soil conditions when the tubes were uncovered in October and November 2010. This was estimated as 5.0% of the nitrification rate (Scholes & Walker, 1993). Nitrogen lost to the atmosphere when the vegetation and litter burnt in this system was estimated at 75.0% of the N stored in the above-ground pools. This calculation was based on the loss of N through fires in savanna ecosystems where the fuel loads are made up of approximately 80.0% grass (Scholes *et al.*, 2003).

CHAPTER 4: RESULTS

The results are presented in an order that provides a logical flow of information on the inputs, cycling and outputs of key nutrients in the Sandspruit system. Firstly, the long-term, eight-month and annual rainfall, mean monthly minimum and maximum temperatures and land type description are presented for the research site. Key properties and processes of the above-ground (vegetation and litter) and below-ground (soils) components are then presented to provide an overall idea of the N and S cycling in this system. Vegetation and litter data include a suite of data provided by quadrat sampling and the dry weight rank method and the concentrations (%) and ratios of N, S, C and P at the beginning and end of the growing season. The key soil properties and processes associated with N and S cycling that influence system outputs are presented. The results section on soil includes data on soil nutrient pools and properties, N and S mineralization rates, with the mineralization rates plotted alongside soil moisture. The soil pools and properties are presented in tables and include certain data that are not used in the preparation of the N and S input-output budgets for the system although they are important in the discussion of the functioning of the system as a whole. The majority of the results are depicted either in tables or graphs with associated statistical analyses to highlight trends, key findings and interesting relationships between the variables measured.

4.1 Meteorological Records and Land Type Description

Inorganic N and S mineralization rates are often dependant on the amount of soil moisture and soil temperature (Singer & Munns, 1987). Hourly temperatures were accessed from Eskom's air quality monitoring station at Verkykkop (October 2010 to May 2011) and daily rainfall was measured at Mr. Oosthuizen's house (October 2010 to May 2011) (Figure 3.5). Long-term annual rainfall, from 1961 to 2010 for Volksrust, was accessed from the South African Weather Service.

The total rainfall from October to May each year from 1961 to 2010 was graphed to compare rainfall during the eight months of sampling (October 2010-May 2011) with long-term rainfall for the same months of each year (October-May) (Figure 4.1). The average long-term rainfall for the period from October to May was 688.7 mm. The total rainfall for the eight month sampling period (beginning October 2010) was 587.5 mm which was significantly less than the eight month rainfall in 1974, 1975, 1976, 1977, 1983, 1989, 1995 and 2005 ($p < 0.05$ in all cases). The total rainfall for the eight month sampling period (beginning October 2010) did not differ significantly from any other remaining years and was considered an average rainfall period.

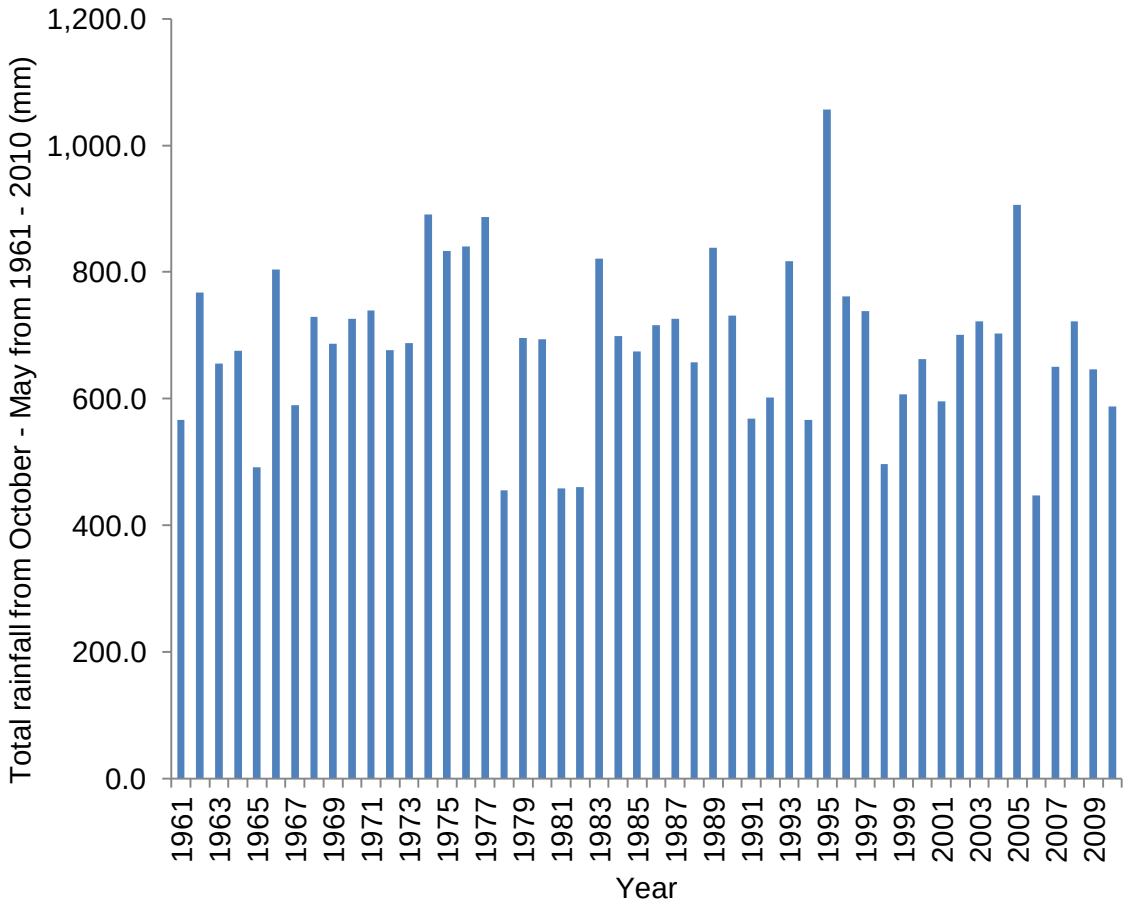

Figure 4.1: Total rainfall (mm) for Volksrust measured from October to May each year from 1961 to 2010.

The average long-term annual rainfall (January-December) from 1961 to 2011 for Volksrust was 760.0 mm. The total annual rainfall for 2010 and 2011, the years in which sampling months fell for this study, was 638.2 mm and 564.5 mm respectively (Figure 4.2). The average annual rainfall in 2010 was significantly lower than the average annual rainfall in 1963, 1967, 1974, 1975, 1987, 1996, 1997, 2000, 2006 and 2009 ($p < 0.05$ in all cases) and significantly higher than 1999 ($p < 0.05$). The average annual rainfall in 2011 was significantly lower than the average annual rainfall in 1961, 1963, 1967, 1969, 1971, 1973, 1974, 1975, 1976, 1977, 1978, 1985, 1987, 1991, 1996, 1997, 2000, 2004, 2006 and 2009

($p < 0.05$ in all cases). Rainfall during 1982, 1999 and 2007 was particularly low and these years were classified as either strong El Niño (1982) or moderate La Niña (1999 & 2007) events (Wolter & Timlin, 2011).

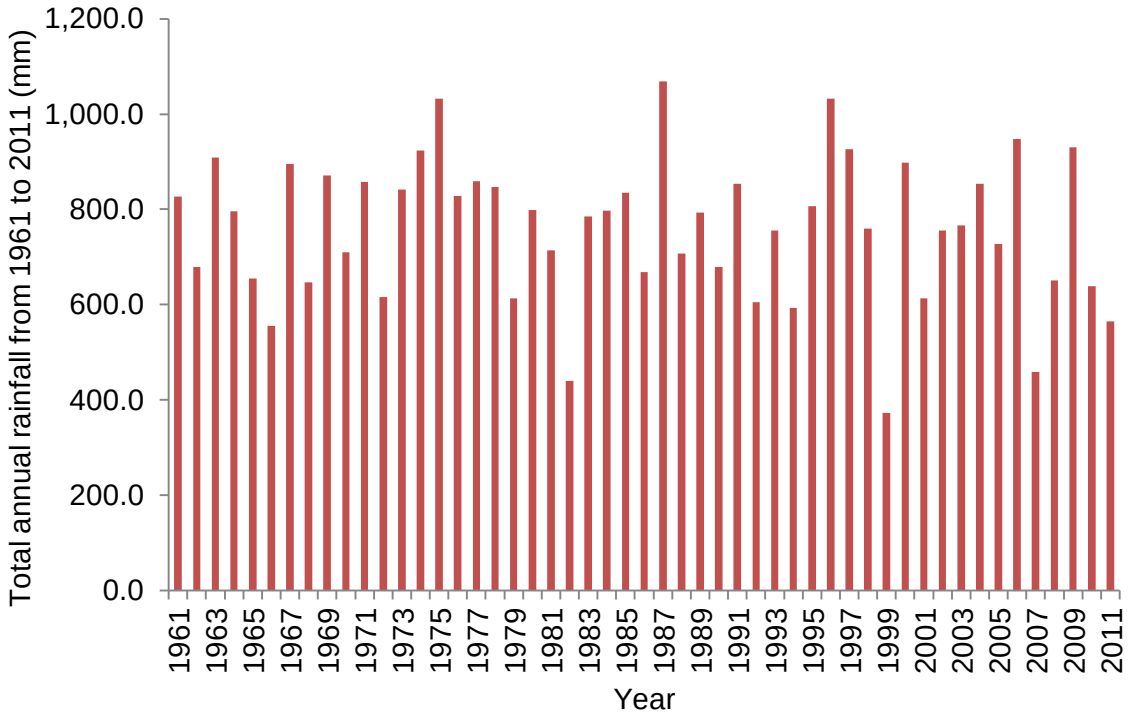

Figure 4.2: Total annual rainfall (mm) for Volksrust from 1961 to 2011.

Three rainfall scenarios (above-average, average and below-average rainfall years) were used for CALPUFF modeling of N and S deposition on the Highveld grasslands (Blight *et al.*, 2009). The years chosen to be used in the models ran from October 1995 to September 1996 (above-average rainfall), October 2000 to September 2001 (average rainfall) and October 2006 to September 2007 (below-average rainfall). These years were chosen so the model could provide the expected ratios of wet and dry deposition for all three rainfall scenarios. Years with above-average rainfall lead to an increase in the wet component of total N and S

deposition and years with below-average rainfall lead to an increase in the dry component (Hicks *et al.*, 1989; Blight *et al.*, 2009). The years chosen to represent above-average, average and below-average rainfall years correlate well with long-term rainfall measured in Volksrust. Rainfall in 1996 was measured as third highest between 1961 and 2011, rainfall in 2001 was measured as average for the region (612.9 mm) and rainfall in 2007, under La Niña conditions, was measured as third lowest between 1961 and 2011 (Figure 4.2). The N and S deposition modeling study was conducted by colleagues (Blight *et al.*, 2009) but the data are presented here as they provide the context and rationale for this study and were used in the preparation of the N and S input-output budgets.

Daily minimum and maximum temperatures were averaged for each month to provide the mean monthly minimum and maximum temperatures (Figure 4.3). Daily rainfall data were summed for each month to provide the total monthly rainfall for each month during the eight month sampling period (Figure 4.3). The total monthly rainfall ranged from 2.5 mm in May 2011 to 176.5 mm in December 2010 with an average of 73.4 ± 19.9 mm per month over the sampling period. The minimum temperature ranged from 6.2 °C in May 2011 to 12.6 °C in March 2011 and the maximum temperature from 19.4 °C in May 2011 to 28.2 °C in March 2011 (Figure 4.3). Significant month and minimum-maximum effects were calculated ($F = 20.4350$, $p = 0.0000$; $F = 2296.0080$, $p = 0.0000$) with April and May being significantly colder than all other months ($p < 0.001$ in all cases). A significant month-minimum-maximum interaction was calculated ($F = 3.2830$, $p = 0.0024$) with the minimum temperature in May 2011 being significantly lower than all other variable combinations except for April 2011 ($p < 0.05$ in all cases). The maximum temperature in April 2011 was significantly lower than all other variable combinations except for the maximum temperature in May 2011 ($p < 0.01$ in all cases).

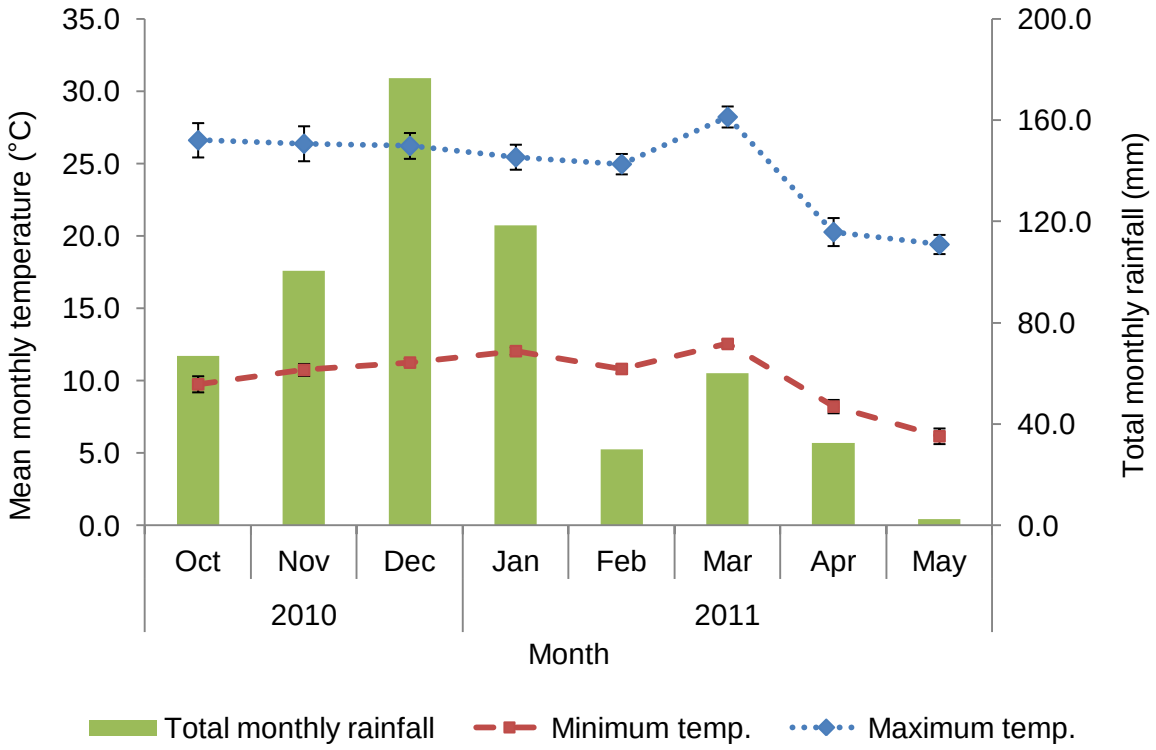

Figure 4.3: Mean (± standard error) monthly minimum and maximum temperatures (°C) and total monthly rainfall (mm) from October 2010 to May 2011.

According to Land Type Survey Staff (2002), the sampling site was located on land type Fa24, a landtype covering an area of approximately 32 698 ha in South Africa. This land type generally occurs on young landscapes in which the dominant soil forming processes were rock weathering and the formation of orthic topsoil horizons with clay illuviation (Land Type Survey Staff, 2002). The weathering of mudstone and sandstone parent material contribiute to Ca^{2+} and Mg^{2+} base cations that may be available in the soil or attached to exchange sites on the cation exchange complex (Singer & Munns, 1987).

4.2 Vegetation and Litter

4.2.1 Dry Mass Contribution of the Dominant Grass Species

The dry weight rank method provided a way to quantify the dry mass contribution of the dominant grass species in the research site (*H. hirta* and *S. africanus*). The mass (in g per 0.25 m^{-2}) of vegetation of 30 0.25 m^2 quadrats were measured directly and the dominant grass species were ranked according to visual dry mass contribution in each quadrat. A contribution of approximately 88.0% to the total dry mass at the beginning of the growing season was made by *H. hirta* whereas *S. africanus*, the only other grass species sampled during October 2010 contributed only 12.1% to the total dry mass (Table 4.1). The greatest contribution to total dry mass at the end of the growing season in March 2011 was made by *H. hirta* (84.2%) (Table 4.1). Similar contributions to total dry mass were made by *S. africanus* at the beginning and end of the growing season in October 2010 (12.1%) and March 2011 (14.5%) respectively (Table 4.1). Several forbs sampled in March 2011 contributed 1.4 % to total dry mass contribution.

Table 4.1: Percent (%) contribution made by the dominant grass species to total dry mass of the vegetation sampled in October 2010 and March 2011. Percent (%) contribution presented separated by rank and as a total contribution for each grass species.

	Rank 1 (%)	Rank 2 (%)	Rank 3 (%)	% dry mass contribution
October 2010				
H. hirta	63.2	19.0	5.7	87.9
S. africanus	7.0	2.1	3.0	12.1
March 2011				
H. hirta	63.2	16.5	4.5	84.2
S. africanus	7.0	4.6	2.8	14.5

The percent dry mass contribution made by each grass species in October 2010 and March 2011 (Table 4.1) was multiplied by total vegetation biomass and by litter biomass (Table 4.2) that provided the total dry mass (kg ha^{-1}) of vegetation and litter of each grass species in October 2010 and March 2011 (Table 4.2). It was assumed that the different grass species provided litter in the same proportions in which vegetation occurred in the quadrats when ranked. This was done to calculate dry mass (kg ha^{-1}) for vegetation and litter separately at the beginning and end of the growing season for each grass species.

The greatest dry mass contribution made by *H. hirta* indicated it was the dominant grass species at the beginning (October 2010) and end (March 2011) of the growing season with 1 792.6 kg ha^{-1} and 2 145.9 kg ha^{-1} respectively (Table 4.2). The dry vegetation biomass of *S. africanus* increased from 246.5 kg ha^{-1} in October 2010 to 368.3 kg ha^{-1} in March 2011 but remained less dominant in both sampling months compared to *H. hirta* (Table 4.2). Significant month and vegetation-litter effects were calculated to compare the dry masses of the vegetation and litter in October 2010 and March 2011 ($F = 8.2970$, $p = 0.0044$; $F = 91.2680$, $p = 0.0000$ respectively). The overall dry mass of vegetation was significantly higher than the dry mass of the litter, irrespective of month ($p = 0.0001$). A significant month-vegetation-litter interaction was calculated ($F = 44.9870$, $p = 0.0000$) with overall dry mass of litter in March 2011 being significantly less than all other variable combinations ($p < 0.001$ in all cases). The overall dry masses of vegetation and litter in October 2010 were significantly higher than the overall dry masses in March 2011 ($p = 0.0054$).

Table 4.2: Total vegetation and litter biomass (kg ha^{-1}), separated by month and further by grass species for October 2010 and March 2011.

	Total vegetation biomass (kg ha^{-1})	Vegetation biomass per grass species (kg ha^{-1})	Total litter biomass (kg ha^{-1})	Litter biomass per grass species (kg ha^{-1})
	October 2010			
H. hirta	2 039.1	1 792.6	1 491.2	1 310.9
S. africanus		246.5		180.3
	March 2011			
H. hirta	2 514.2	2 145.9	675.6	576.6
S. africanus		368.3		99.0

The difference between the amounts of vegetation and litter that contributed to dry mass is indicative of the season in which sampling was performed and the degree to which plants in the research site had senesced at the time of sampling (Figure 4.4 & 4.5). Each of the dominant grass species showed a visible difference between the amounts of vegetation and litter that contributed to the overall dry mass in October 2010, at the beginning of the growing season (Figure 4.4). Vegetation contributed 1.4 times more dry mass than litter in the October 2010 sampling (Table 4.2). Litter was expected to contribute more towards the overall dry mass in October 2010 than in March 2011 as the plants had senesced during the winter months (April-August) providing litter that contributed to overall dry mass. Each of the dominant grass species showed a visible difference between the amount of vegetation and litter that contributed to the overall dry mass in March, at the end of the growing season (Figure 4.5). Vegetation contributed 3.7 times more to overall dry mass than litter in the March 2011 sampling (Table 4.2). Vegetation was expected to contribute more towards the overall dry mass in March 2011 than in October 2010 as plants had not begun to senesce, providing very little litter that contributed to the overall dry mass.

Figure 4.4: View of the study site during the October 2010 site visit showing standing vegetation and litter at the time of sampling.

Figure 4.5: View of the study site during the March 2011 site visit showing standing vegetation and litter at the time of sampling.

4.2.2 Nitrogen, Sulfur, Carbon and Phosphorus Concentrations in the Vegetation and Litter at the Beginning and End of the Growing Season

Analyses of the concentrations (%) of N, S, C and P in the vegetation and litter included nutrients taken up and metabolically incorporated into plant tissues and nutrients deposited on the surfaces of the leaves and stems. Initially, no distinction was made in the analyses between internal (metabolically taken up) and external (from atmospheric deposition) N, S, C and P. Following interesting N and S results in the vegetation and litter, vegetation samples were collected and washed to analyze the difference between the N and S concentrations internally and externally.

The average concentration of N in the vegetation increased from 0.58 ± 0.02% in October 2010 to 1.67 ± 0.03% in March 2011 (Figure 4.6), an almost three fold increase over the 2010-2011 growing season. The average concentration of N in litter increased from 0.45 ± 0.02% in October 2010 to 1.46 ± 0.03% in March 2011 (Figure 4.6). Significant month and vegetation-litter effects were calculated ($F = 1755.7830$, $p = 0.0000$; $F = 46.1520$, $p = 0.0000$ respectively) with N in March 2011 samples being significantly higher than N in October 2010 samples ($p = 0.0001$) and the concentration of N (%) in vegetation being significantly higher than in litter, irrespective of month ($p = 0.0001$). The concentration of N (%) in vegetation in October 2010 was significantly higher than the concentration of N in litter in the same month ($p = 0.0002$) with the same found for the samples in March 2011 ($p = 0.0000$).

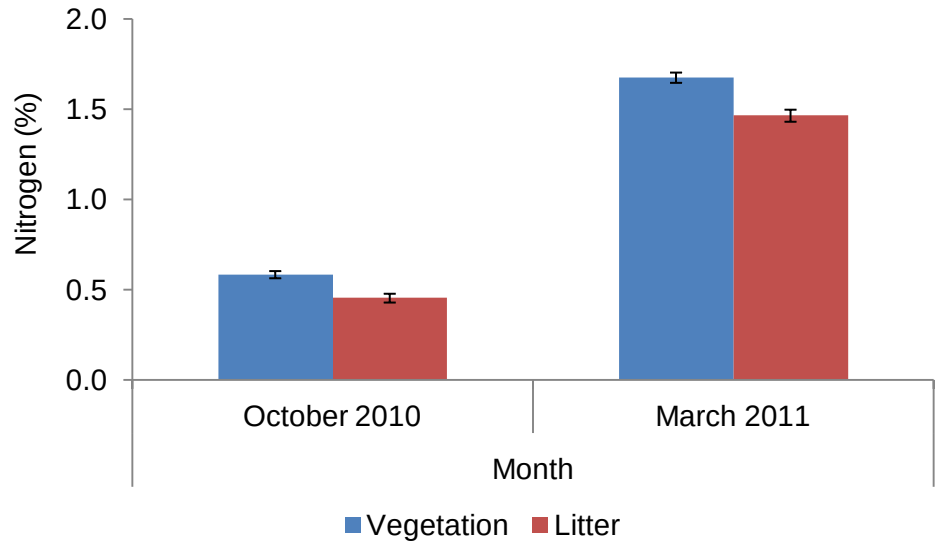

Figure 4.6: Mean concentration (% ± standard error) of N in vegetation and litter samples in October 2010 and March 2011.

The average concentration of S in the vegetation decreased from 0.16 ± 0.01% in October 2010 to 0.11 ± 0.00% in March 2011 (Figure 4.7), an almost 1½ fold decrease over the 2010-2011 growing season. The average concentration of S in the litter decreased from 0.13 ± 0.00% in October 2010 to 0.07 ± 0.00% in March 2011 (Figure 4.7). Significant month and vegetation-litter effects were calculated ($F = 170.4210$, $p = 0.0000$; $F = 61.8770$, $p = 0.0000$ respectively) with S in March 2011 samples being significantly lower than in October 2010 samples ($p = 0.0001$) and the concentration of S (%) in vegetation being significantly higher than in litter, irrespective of month ($p = 0.0001$). The concentration of S (%) in vegetation in October 2010 was significantly higher than the concentration of S (%) in litter in the same month ($p = 0.0002$) with the same result found in March 2011 ($p = 0.0000$).

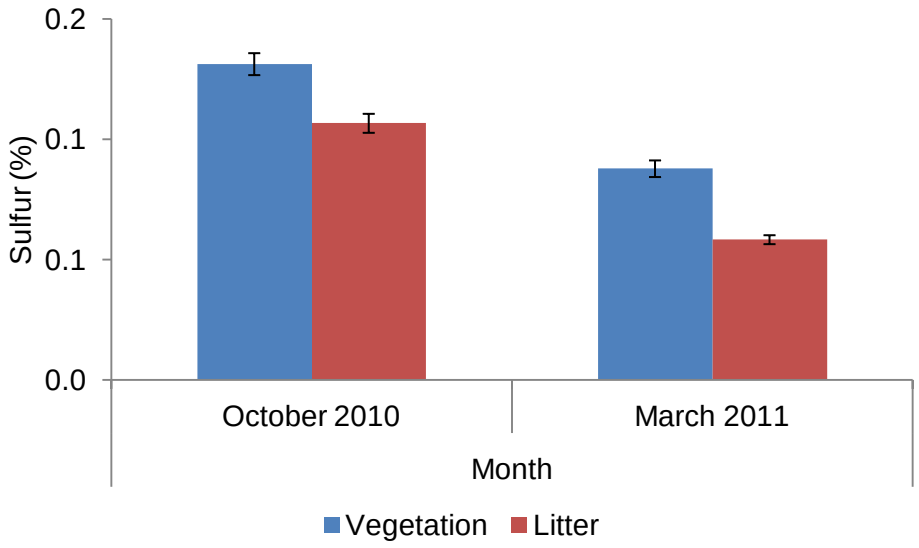

Figure 4.7: Mean concentration (% ± standard error) of S in vegetation and litter samples in October 2010 and March 2011.

The concentrations of N and S in the vegetation and litter produced interesting results. It was suggested that the high concentration of S in the vegetation in October 2010 could have been a result of dry deposition on the leaves and stems of the grass samples. The N and S that was metabolically incorporated into the tissue of *H. hirta* and *S. africanus* and the N and S that was possibly deposited on the leaves and stems from dry deposition were analyzed by rinsing half of each grass tuft prior to analysis in August 2011. No significant difference was found between the concentrations of N and S in the vegetation that had been rinsed and that that had not been rinsed. However, the dry deposition that could have accumulated on the grass from August to October 2010 may have created a significant difference between the analyzed N and S in the vegetation in October 2010 and March 2011.

The average concentration of C in the vegetation remained fairly stable (± 42.00%) throughout the 2010-2011 growing season with a slight increase from 41.76 ± 0.60% in October 2010 to 42.19 ± 0.63% in March 2011 (Figure 4.8). The average concentration of C in litter decreased from 41.79 ± 0.47% in October 2010 to 36.60 ± 0.87% in March 2011 (Figure 4.8). Significant month and vegetation-litter effects were calculated ($F = 15.3200$, $p = 0.0002$; $F = 20.9000$, $p = 0.0000$ respectively) with C in October 2010 samples being significantly higher than in March 2011 samples ($p = 0.0003$). A significant month-vegetation-litter interaction was also calculated ($F = 21.2400$, $p = 0.0000$) with litter samples in March 2011 having significantly less C than all other variable combinations ($p < 0.001$ in all cases). The concentration of C in the vegetation was positively correlated with the concentration of C in the litter in the October 2010 and March 2011 samples ($R = 0.5748$, $p < 0.05$).

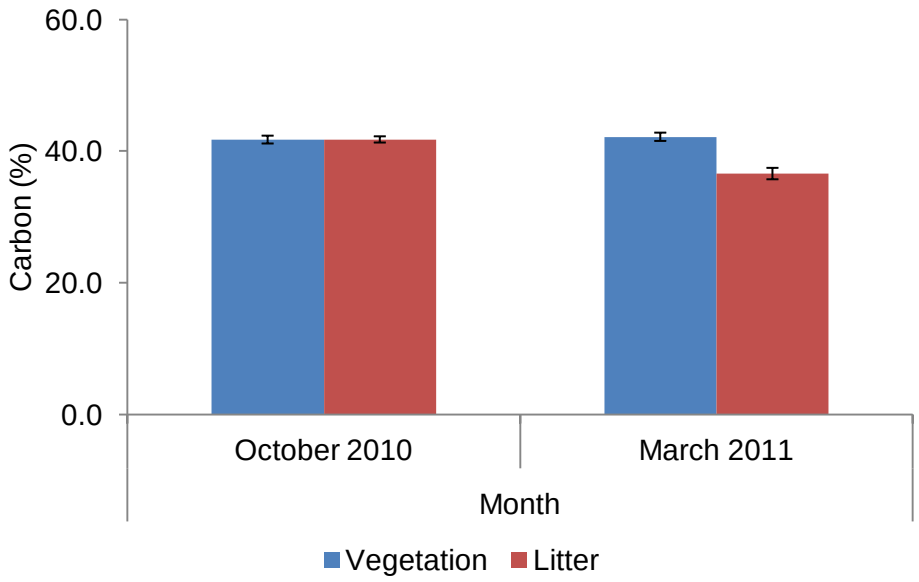

Figure 4.8: Mean concentration (% ± standard error) of C in vegetation and litter samples in October 2010 and March 2011.

The average concentration of P in the vegetation increased from 0.06 ± 0.00% in October 2010 to 0.08 ± 0.00% in March 2011 (Figure 4.9), an almost 1½ fold increase over the 2010-2011 growing season. The average concentration of P in the litter did not change from October 2010 to March 2011 (Figure 4.9). Significant month and vegetation-litter effects were calculated ($F = 20.5690$, $p = 0.0000$; $F = 48.5270$, $p = 0.0000$ respectively) with the concentration (%) of P in vegetation being significantly higher than in litter, irrespective of month ($p = 0.0001$). A significant month-vegetation-litter interaction was also calculated ($F = 12.5330$, $p = 0.0007$) with vegetation samples in March 2011 having significantly more P than all other variable combinations ($p < 0.001$ in all cases). The concentration of P (%) in the vegetation was significantly higher than the concentration of P (%) in litter in March 2011 ($p = 0.0000$). The concentration of P in the vegetation was positively correlated with the concentration of P in the litter in the October 2010 and March 2011 samples ($R = 0.8409$, $p < 0.05$).

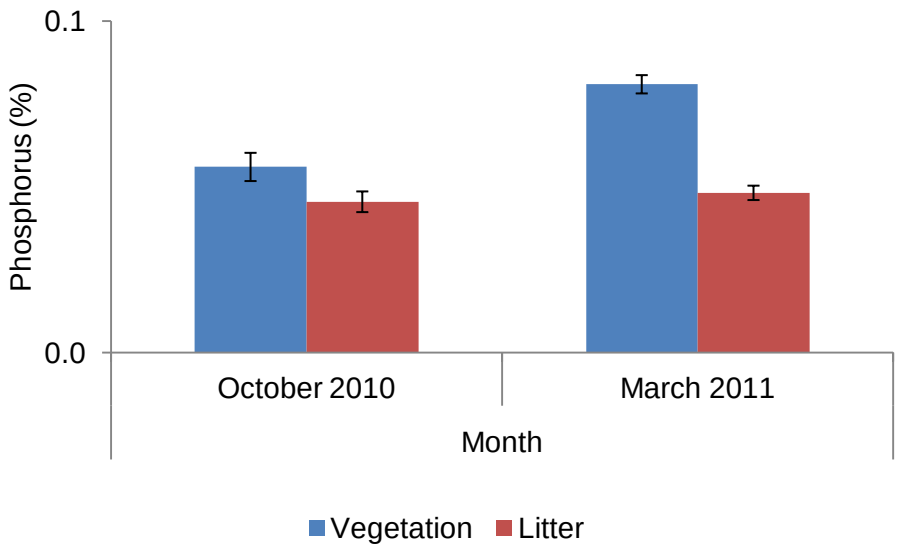

Figure 4.9: Mean concentration (% ± standard error) of P in vegetation and litter samples in October 2010 and March 2011.

The ratios of N, S, C and P calculated in the vegetation and litter at the beginning and end of the growing season provided a means to determine which nutrients may be limited in the system. The literature sources of Kathawaroo (2005) and Scholes & Walker (1993) were selected as they provided the necessary information for comparison whilst their chosen locations were similar to the current study site. The grassland study site that was used by Kathawaroo (2005) was located on the eastern escarpment of South Africa, received between 850-1000 mm of rain annually and was located on silty, clay-loam soil. The site used by Scholes & Walker (1993) was located in the Nylsvley broad-leaf savanna, 200.0 km north of Johannesburg and received an annual rainfall of 623.0 ± 134.0 mm.

The C:N ratio of 70:1 in the vegetation in October 2010 was slightly lower than the ratio of 84:1 in March 2011 and both ratios were lower than the literature ratio of 113:1 (Table 4.3). There was a greater difference between the C:N ratio in the vegetation and the C:N ratio in the litter at the end of the growing season (October 2010) compared to the beginning of the growing season (March 2011) (Table 4.3). The amount of C relative to the amount of P remained unchanged in the vegetation and litter sampled in October 2010 but was less in the litter than in the vegetation in March 2011 (Table 4.3). Kathawaroo (2005) found a higher C:P ratio in October (1 036:1) and a lower C:P ratio in March (288:1) (Table 4.3).

The C:S ratios in the vegetation and litter appeared to switch in October 2010 and March 2011 although they remained unchanged in the literature ratio at 427:1 (Scholes & Walker, 1993) (Table 4.3). The litter had the higher C:S ratio in October 2010 whereas the vegetation had the higher C:S ratio in March 2011 (Table 4.3). The N:S ratio for vegetation and litter increased from 3:1 and 5:1 respectively at

the beginning of the growing season in October 2010 to 17:1 and 15:1 respectively at the end of the growing season in March 2011 (Table 4.3).

Table 4.3: Ratio combinations of N, S, C and P in vegetation and litter samples from October 2010 and March 2011 compared with literature ratios from similar ecosystems (Kathawaroo, 2005 - highlighted green; Scholes & Walker, 1993 - highlighted pink).

	October 2010				March 2011			
	C:N	**C:P**	**C:S**	**N:S**	**C:N**	**C:P**	**C:S**	**N:S**
Vegetation	70:1	418:1	209:1	3:1	25:1	422:1	422:1	17:1
Litter	84:1	418:1	418:1	5:1	24:1	366:1	366:1	15:1
Literature	113:1	1 036:1	427:1	2:1	64:1	288:1	427:1	8:1

4.3 Soil: Key Properties and Processes

4.3.1 Nitrogen Mineralization

The net N mineralization rate, ammonification rate and nitrification rate were determined monthly from October 2010 to May 2011. Mineralization rates are positive slopes and immobilization rates are negatives slopes in Figure 4.10. The net N mineralization rate was the sum of the ammonification rate and the nitrification rate. The net N mineralization rate ranged between -1.03 ± 0.15 µg N g^{-1} soil day^{-1} in May 2011 and 1.05 ± 0.23 µg N g^{-1} soil day^{-1} in April 2011 (Figure 4.10). Nitrogen was immobilized between January and February 2011 and between April and May 2011 with mineralization occurring in the remaining months (Figure 4.10). The N mineralization rate was significantly affected by the month in which sampling was performed ($F = 5.3150$, $p = 0.0001$) with N mineralization in April 2011 significantly higher than N mineralization in all other months except December 2010 and January 2011 ($p < 0.05$ in all cases).

The ammonification rate ranged between -0.27 ± 0.13 µg $N\text{-}NH_4^+$ g^{-1} soil day^{-1} in October 2010, at the beginning of the growing season and 0.34 ± 0.19 µg $N\text{-}NH_4^+$ g^{-1} soil day^{-1} in January 2011 (Figure 4.10). The ammonification rate decreased steeply from January to February 2011 and remained fairly stable from February to March 2011 (Figure 4.10). The ammonification rate increased again towards the end of the growing season up to 0.15 ± 0.11 µg $N\text{-}NH_4^+$ g^{-1} soil day^{-1} in May 2011 (Figure 4.10). The ammonification rate was significantly affected by the month in which sampling was performed ($F = 2.9330$, $p = 0.0093$) with ammonification in October 2010 being significantly lower than ammonification in January 2011 ($p = 0.0499$).

Nitrification dominated the net N mineralization rate from October to November 2010 and from February to April 2011 and ranged between -1.18 ± 0.11 µg $N\text{-}NO_3^-$ g^{-1} soil day^{-1} in May 2011 and 0.94 ± 0.27 µg $N\text{-}NO_3^-$ g^{-1} soil day^{-1} in April 2011 (Figure 4.10). Nitrate loss from the soil from October to December 2010 may have been a result of water standing in the tubes during October and November 2010 when the plastic covering did not cover the tubes for the entire month. The high clay content of the soil prevented the water penetrating the soil and the N lost would have been as a result of denitrification. The nitrification rate was significantly affected by the month in which sampling was performed ($F = 7.5580$, $p = 0.0000$) with nitrification in April 2011 significantly higher than in all other months ($p < 0.05$ in all cases). The nitrification rate was slower than the ammonification rate only from December 2010 to January 2011 and in May 2011. A very strong positive correlation between nitrification rate and N mineralization rate ($R = 0.9306$) provided evidence that nitrification was the dominant process driving the net N mineralization in this grassland.

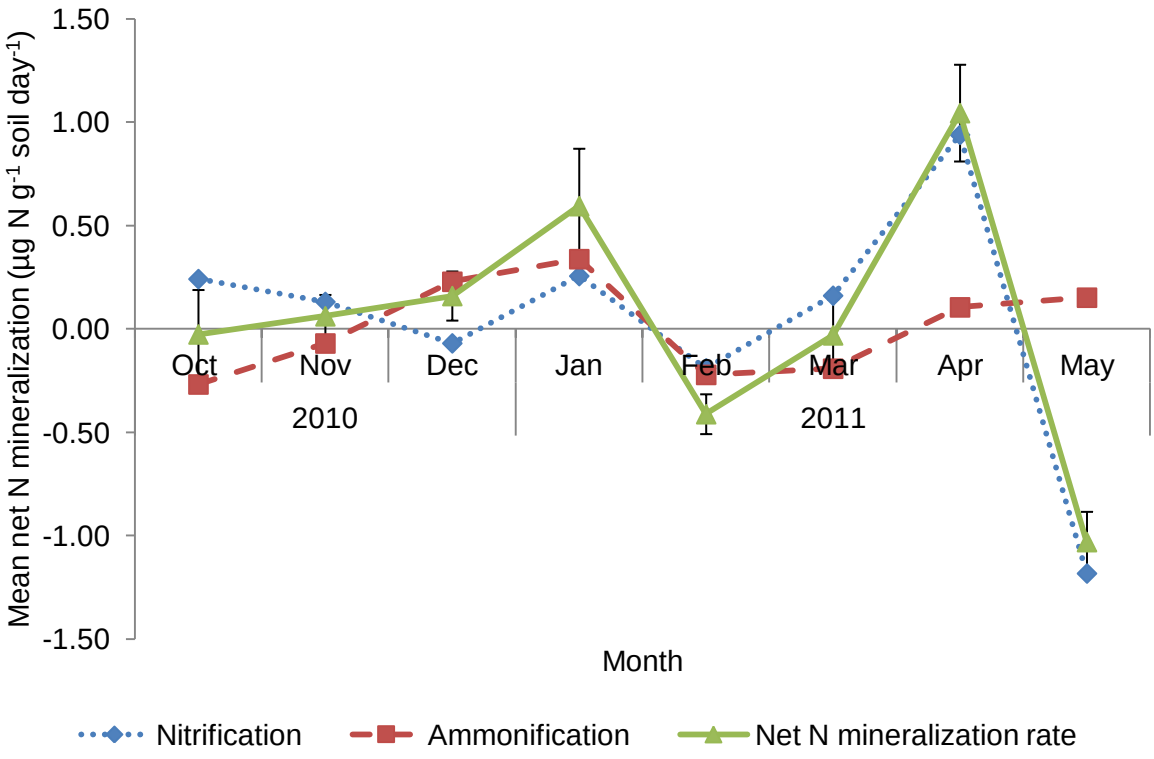

Figure 4.10: Mean ammonification, nitrification and net N mineralization rates (± standard error for net N mineralization only) (µg N g^{-1} soil day^{-1}) from October 2010 to May 2011.

The anaerobic N mineralization index was used to determine the maximum rate (in the laboratory) of N mineralization (Anderson & Ingram, 1996). The mean anaerobic N mineralization rate was calculated to be 5.0 ± 1.0 µg N g^{-1} soil day^{-1} (Table 4.4), almost five times faster compared to the fastest field measured rate of 1.05 µg N g^{-1} soil day^{-1} (Figure 4.10). The anaerobic N mineralization rate translated to a potential annual mineralization rate of 3 997.0 ± 769.1 kg N ha^{-1} yr^{-1}.

Table 4.4: The mean anaerobic N mineralization rate (± standard error) for October 2010, February and March 2011 and the mean anaerobic N mineralization index (± standard error).

Mean anaerobic N mineralization (µg g^{-1} soil day^{-1})	
October 2010	3.76 ± 0.41
February 2011	3.50 ± 1.29
March 2011	7.70 ± 0.97
Mean anaerobic N mineralization index	5.00 ± 1.34

The average soil moisture content (T0) in the tubes for any given month was compared to the average soil moisture content in the tubes for the following month (T1) to analyze whether moisture had increased or decreased in the soil during that month. As the tubes were securely covered with plastic from December 2010 to June 2011, it was assumed that an increase in soil moisture would be depicted by higher soil moisture in T1 samples of any month compared to T0 samples of the previous month. This would suggest a greater rate of upward water movement by capillary movement compared to the combined rates of leaching, microbial respiration and water use in decomposition.

The mean soil moisture ranged between 2.6 ± 0.2% in T0 tubes in March 2011 and 17.3 ± 1.4% in T1 tubes in January 2011 (Figure 4.11). Moisture was lost from the tubes during the months of October 2010 and February 2011, months which coincided with the second and third slowest N mineralization rates over the eight month sampling period (Figure 4.11). Moisture increased in the soil during all the remaining months (November, December 2010, February, March, April, May 2011). The highest soil moisture measurement (January 2011) coincided with the second fastest N mineralization rate of 0.60 ± 0.28 µg g^{-1} soil day^{-1} (Figure 4.11). These associations can be explained by the weak positive correlation between N

mineralization rate and T1 soil moisture (R = 0.2456). The net N mineralization rate appeared to show a de-coupling from the soil moisture from April to May 2011.

The largest increase in soil moisture was from December 2010 (4.5%) to January 2011 (17.3%) with an increase of 12.8% (Figure 4.11). The smallest increase in soil moisture occurred from January 2011 (14.2%) to February 2011 (14.5%) with an increase of 0.4% (Figure 4.11). Soil moisture decreased from October to November 2010 by 9.6% and from February to March 2011 by 7.6% (Figure 4.11). Significant month and T0-T1 effects were calculated ($F = 37.0680$, $p = 0.0000$; $F = 1149.1460$, $p = 0.0000$ respectively) with soil moisture in January 2011 significantly higher than soil moisture in all other months except April 2011 ($p < 0.05$ in all cases). A significant month-T0-T1 interaction was also calculated ($F = 39.6190$, $p = 0.0000$) with soil moisture in October 2010 T0 samples significantly higher or lower than soil moisture in all other variable combinations ($p < 0.001$ in all cases).

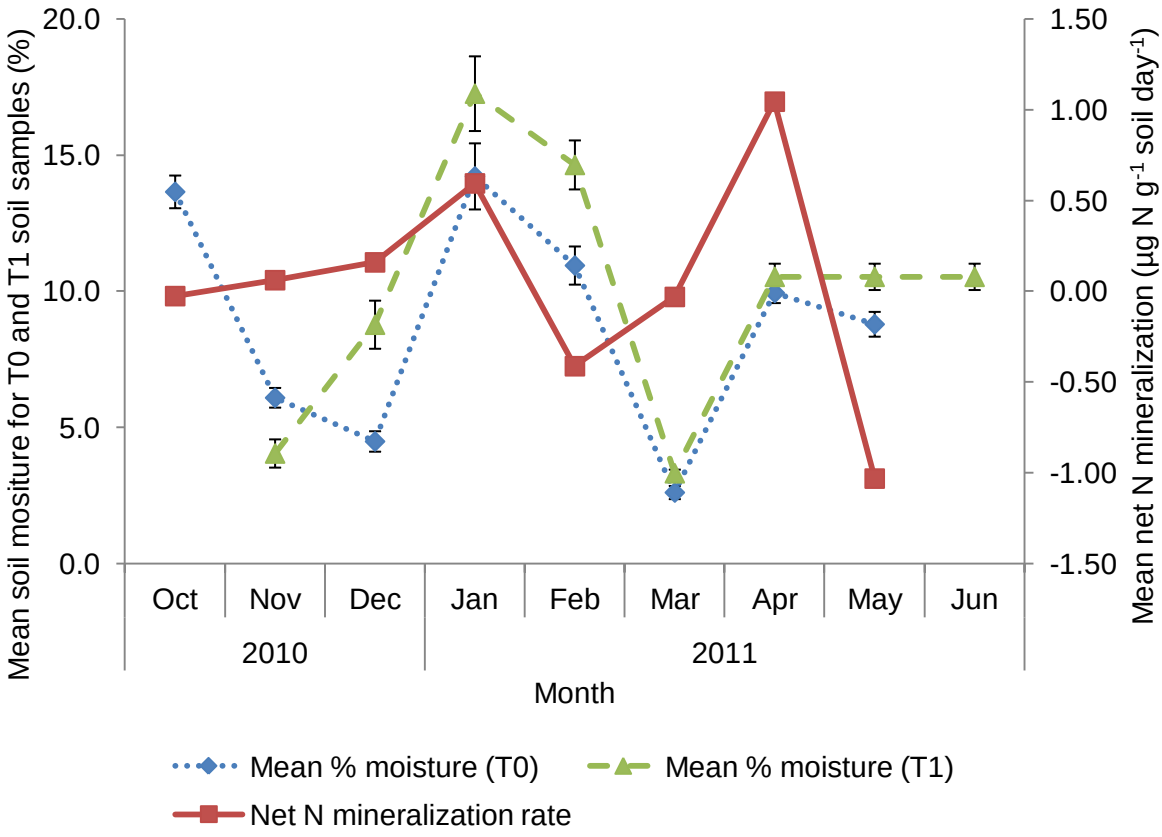

Figure 4.11: Mean soil moisture (%) for Time 0 (T0) and Time 1 (T1) samples (± standard error) and mean net N mineralization rate (µg N g^{-1} soil day^{-1}) from October 2010 to June 2011.

4.3.2 Sulfate Mineralization

The net SO_4^{2-} mineralization rate was determined monthly from October 2010 to May 2011 and SO_4^{2-}mineralization are positive slopes and SO_4^{2-} immobilization are negatives slopes in Figure 4.12. The SO_4^{2-} mineralization rate ranged between -1.06 ± 0.20 µg S-SO_4^{2-} g^{-1} soil day^{-1} in December 2010 and 0.91 ± 0.21 µg S-SO_4^{2-} g^{-1} soil day^{-1} in November 2010 (Figure 4.12). Sulfate was mineralized from October to November 2010 as well as from December 2010 to May 2011 and immobilized from November to December 2010 (Figure 4.12). The SO_4^{2-}mineralization rate slowed from February to April 2011 and appeared to

plateau from April to May 2011 (Figure 4.12). The mineralization rate was significantly affected by the month in which sampling was performed ($F = 6.0683$, $p = 0.0000$) with the mineralization rate in November 2010 being significantly higher than the mineralization rate in December 2010 and January 2011 ($p < 0.05$ in both cases). The mineralization rate in December 2010 was significantly lower than in November 2010, February, March, April and May 2011 ($p < 0.05$ in all cases).

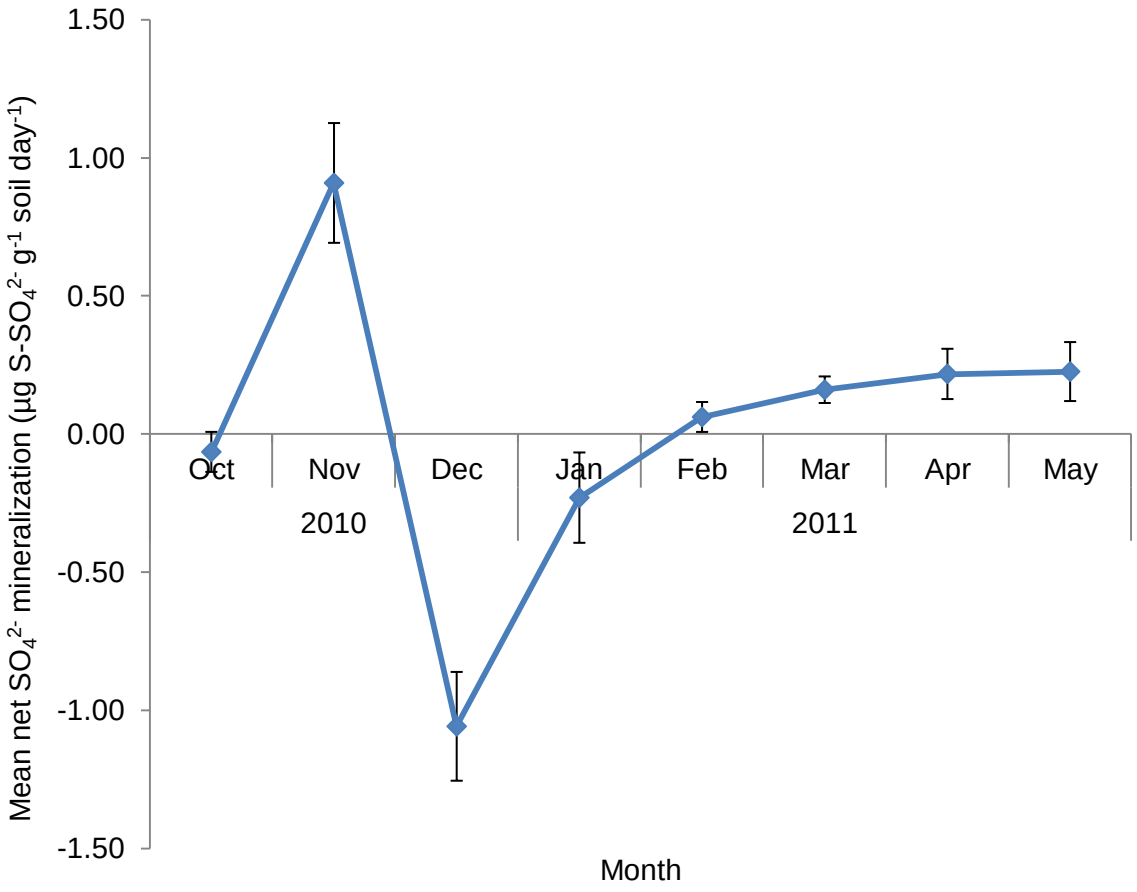

Figure 4.12: Mean (± standard error) net SO_4^{2-} mineralization rate (µg $S\text{-}SO_4^{2-}$ g^{-1} soil day^{-1}) from October 2010 to May 2011.

The net SO_4^{2-} mineralization rate showed no correlation with T0 or T1 soil moisture although it increased along with soil moisture from December 2010 to February 2011 and from April to May 2011 (Figure 4.13). The net SO_4^{2-}mineralization rate appeared to show a de-coupling from the soil moisture from October to December 2010 and in March 2011 (Figure 4.13). Significant month and T0-T1 effects were calculated ($F = 9.1060$, $p = 0.0000$; $F = 855.5370$, $p = 0.0000$ respectively) with soil moisture in December 2010 significantly lower than soil moisture in October 2010, January, April and May 2011 ($p < 0.05$ in all cases). A significant month-T0-T1 interaction was calculated ($F = 35.5380$, $p = 0.0000$) with soil moisture in October 2010 T0 samples significantly higher or lower than soil moisture in all other variable combinations ($p < 0.01$ in all cases).

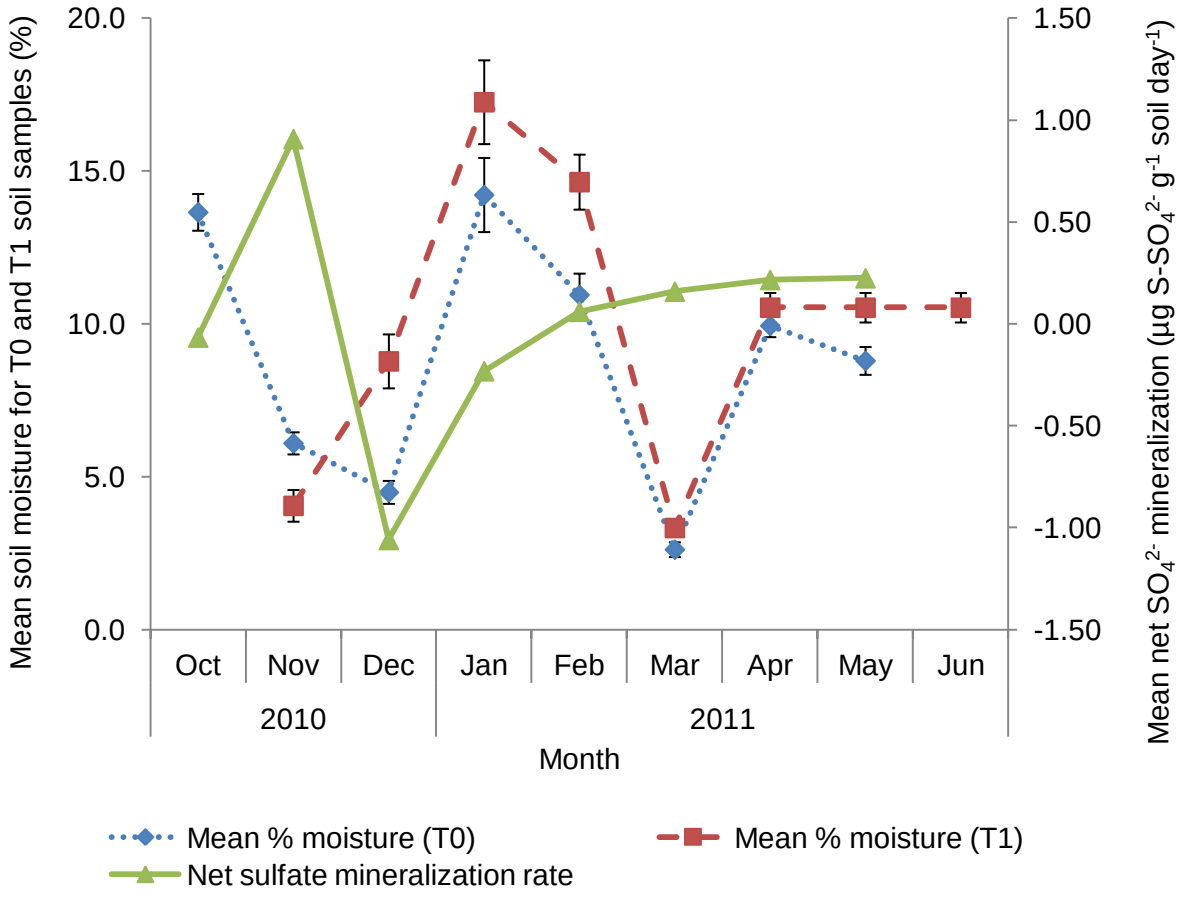

Figure 4.13: Mean soil moisture (%) for Time 0 (T0) and Time 1 (T1) samples (± standard error) and mean net SO_4^{2-} mineralization rate (µg S-SO_4^{2-} g^{-1} soil day^{-1}) from October 2010 to June 2011.

4.3.3 Selected Soil Nutrient Pools and Properties

Soil pool sizes and properties for this study site were compared with values from various literature sources to gauge their comparability with similar sites in South Africa. The location and conditions of Kathawaroo (2005) and Land Type Survey Staff (2002) were previously discussed. The values used from du Toit & du Preez (1995) were chosen from a site located closest geographically to this study site. du Toit & du Preez (1995) sampled at 50 different locations all within the summer rainfall region west of the Drakensberg mountains in South Africa in Gauteng, Free

State and Mpumalanga provinces. The values for total S in the soils (%) were from the Morgenzon district and were sampled between February and April 1991 in a site not used for agricultural purposes (du Toit & du Preez, 1995).

The average concentrations (%) of N, S, C and P in the soil were calculated to a depth of 0.15 m and were 0.21, 0.02, 2.70 and 0.03% respectively (Table 4.5). The average concentrations of N and C in the soil were fairly similar to the literature values of 0.19 and 2.53% respectively (Kathawaroo, 2005) (Table 4.5). The literature P concentration of 0.04% in the soil (Kathawaroo, 2005) was equal to the maximum amount of P in the soil in this study site (Table 4.5). The average pH of the soils was 5.4, slightly more acidic than the literature value of 5.9 for the Fa land type across South Africa (Land Type Survey Staff, 2002). The literature values of the amounts of Ca^{2+} (8.50 cmol (+) kg^{-1}) and Mg^{2+} (3.90 cmol (+) kg^{-1}) for the Fa land type fell within the range of the amounts of Ca^{2+} and Mg^{2+} measured for this study (Land Type Survey Staff, 2002) (Table 4.5). The average amount of Na^{+} in this study, 0.31 cmol (+) kg^{-1}, was higher than the literature value of 0.10 cmol (+) kg^{-1} and the average amount of K^{+} in this study, 0.69 cmol (+) kg^{-1}, was less than the literature value of 1.10 cmol (+) kg^{-1} (Land Type Survey Staff, 2002) (Table 4.5). The measured Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} followed the expected ratios of Na < K < Mg < Ca as there are no additional sources of Na^{+} and K^{+} in this system and the mudstone and sandstone parent material provide Ca^{2+} and Mg^{2+} to the soils. The amount of extractable Al^{3+} was expected to increase with a decrease in pH but was calculated to be between 0.00 and 0.01 cmol (+) kg^{-1}, within the range of the literature value expected for this land type (Land Type Survey Staff, 2002) (Table 4.5). The contributions (%) of the three soil fractions to texture were analyzed to be 51.2% clay, 43.4% silt and 5.4% sand (Table 4.5).

Table 4.5: Minimum, average and maximum measured and literature values of various soil chemical elements, texture and pH of soil samples collected in October 2010.

Element / Chemical Property Measured	Measured Value			Literature Value	Source
	Minimum	Average	Maximum		
Total S - Soil (%)	0.02	0.02	0.03	0.03	du Toit & du Preez (1995)
Total N - Soil (%)	0.14	0.21	0.30	0.19	Kathawaroo (2005)
Total C - Soil (%)	1.94	2.70	3.93	2.53	
Total P - Soil (%)	0.02	0.03	0.04	0.04	
pH (in water)	5.10	5.43	5.80	5.90	Land Type Survey Staff (2002)
Na (cmol (+) kg^{-1})	0.26	0.31	0.36	0.10	
K (cmol (+) kg^{-1})	0.49	0.69	0.90	1.10	
Ca (cmol (+) kg^{-1})	8.17	10.55	12.06	8.50	
Mg (cmol (+) kg^{-1})	2.82	3.34	4.35	3.90	
Al (cmol (+) kg^{-1})	0.00	0.00	0.01	0.01	
Texture - Clay (%)		51.2		46.2	
Texture - Silt (%)		43.4		14.5	
Texture - Sand (%)		5.4		36.3	

The soil C:N ratio of 13:1 calculated for the soils in this study was less than the literature value of 18:1 calculated by du Toit & du Preez (1995) (Table 4.6). The C:P ratio however was greater (92:1) for the soils in this study compared to the literature value of only 56:1 (Kathawaroo, 2005) (Table 4.6). The C:N:S ratio was used to compare the N:S ratio as well as the C:S ratio for the soils in this study with the literature ratios. The C:S ratio of 138:1 in the soils in the research site was lower than the expected literature value of 146:1 calculated by du Toit & du Preez (1995) although the difference between the two ratios was relatively small (Table 4.6). The N:S ratio calculated by Kathawaroo (2005) in similar grassland soils was 11:1, slightly higher than the calculated N:S ratio in this study of 8:1 (Table 4.6).

Table 4.6: Ratios of C, N, P and S in soils from this study (collected in October 2010) compared to literature values (du Toit & du Preez, 1995 - highlighted pink; Kathawaroo, 2005 - highlighted green).

	C:N	C:P	C:N:S
Calculated Value (this study)	13:1	92:1	138:11:1
Literature Value	18:1	56:1	146:8:1

4.4 Nitrogen and Sulfur Input-Output Budgets for the Sandspruit System

The N and S input-output budgets for the Sandspruit system were compiled using the findings from this study as well as values from published literature from similar grassland ecosystems. These literature sources included Scholes & Walker (1993), Snyman (2005), Bird (2011), Scholes *et al.* (2003), Blight *et al.* (2009), du Preez *et al.* (1983) and Edwards (1998). Scholes & Walker (1993) conducted research in the Nylsvley Nature Reserve which is located NE of Johannesburg and is in the savanna biome. The area receives a total mean annual rainfall of 623.0 ± 134.0 mm and is located in the summer rainfall region of South Africa (Scholes & Walker, 1993). The soils are mainly comprised of low activity clays and contain a clay percentage of between 4.5 and 12.4% in the A horizon (Scholes & Walker, 1993). du Preez *et al.* (1983) also conducted research in the Nylsvley Nature Reserve, where study sites are located in the broad-leaf savanna on sandy soils. Samples were collected for S determination in *Digitaria eriantha* Stued. and *Eragrostis pallens* Hack. every two weeks from August to December 1980. Snyman (2005) conducted research in Bloemfontein which is located in the semi-arid, summer rainfall region of South Africa and receives a mean annual rainfall of 560.0 mm. The study site is situated in the Highveld grasslands with soil clay content of approximately 10% in the A horizon (Snyman, 2005). Bird (2011) conducted research in 11 sites in the Mpumalanga Highveld grasslands from January 2008 to January 2009 during which the mean annual rainfall was 734.0 mm. All 11 sites are known to receive N and S deposition from nearby

Eskom power stations (Bird, 2011). Scholes *et al.* (2003) conducted research in the Kruger National Park which is in the savanna biome and falls within two climate zones and as such, mean annual rainfall is between 300.0-700.0 mm. Blight *et al.* (2009) modelled N and S deposition for the Mpumalanga Highveld grasslands. Deposition estimates were dependent on the rainfall scenario, creating three possibilities of either a below-average, average or above-average rainfall year. These models incorporated N and S deposition from both fossil fuel burning for electricity generation and biomass burning (Blight *et al.* 2009).

The minimum, average and maximum pools sizes and fluxes were calculated using data from vegetation, litter and soil in this study site (Table 4.7 & 4.8) and used in three N and three S input-output budgets that could occur under minimum, average (6.2 kg N ha^{-1} yr^{-1} and 21.5 kg S ha^{-1} yr^{-1} respectively) and maximum levels of deposition (Figures 4.14-4.19). Not all values presented in Table 4.7 & 4.8 were used in the N and S budgets although values not used were useful in giving an indication of how the measured values compared with published literature from similar grassland ecosystems.

Table 4.7: Calculated minimum, average and maximum pools (kg ha^{-1}) and fluxes (in italics, kg ha^{-1} $year^{-1}$) used to compile the N input-output budgets for the Sandspruit system under minimum, average and maximum levels of deposition.

Variable Measured	Calculated Pools (kg ha^{-1}) or Fluxes (kg ha^{-1} yr^{-1}) in this study			Literature Pools (kg ha^{-1}) or Fluxes (kg ha^{-1} yr^{-1})		Source for minimum and maximum literature pools and fluxes
	Minimum	Average	Maximum	Minimum	Maximum	
Vegetation and Litter						
Vegetation (kg ha^{-1} in March 2011)	1 706.4	2 549.7	3 808.8	1 570.0	2 341.0	Scholes & Walker (1993) (min.) Snyman (2005) (max.)
Litter (kg ha^{-1} in March 2011)	373.6	685.1	936.0	392.5	585.3	Assumed to be 25% of vegetation
Nitrogen						
Average % N in vegetation	1.7			2.0		Scholes & Walker (1993)
Above-ground biomass (kg N ha^{-1} March 2011)	28.5	42.6	63.6	31.4	46.8	Calculated: vegetation (March 2011) x average % N in vegetation
Average % N in litter	1.5			1.7		Assumed to be 87% of average % N in vegetation
Litter (kg N ha^{-1} March 2011)	5.5	10.0	13.7	6.8	10.2	Calculated: litter (March 2011) x average % N in litter
Below-ground - biomass	-	-	-	2.5	4.1	du Preez et al. (1983)
Total N - Soil (to depth of 0.15 m)	3072.3	4718.2	6583.5	3060.0	6630.0	Scholes & Walker (1993) (min.) Bird (2011) (max.)
Inorganic N-NH_4^+ - Soil	0.5	21.6	41.6	0.2	120.7	Bird (2011)
Inorganic N-NO_3^- - Soil	0.0	10.2	63.7	0.6	68.5	Bird (2011)
Microbial N - soil	-	-	-	16.6	115.9	Scholes & Walker (1993)
Ammonification	*-130.5*	*2.3*	*158.5*	*-34.5*	*47.5*	Bird (2011)
Nitrification	*-88.5*	*44.1*	*273.3*	*-10.0*	*80.5*	Bird (2011)
Mineralization	*10.0*	*131.3*	*287.9*	*3.3*	*161.5*	Bird (2011)
Immobilization	*-15.3*	*-85.1*	*-141.5*	*-5.1*	*-99.0*	Bird (2011)
Plant uptake	*31.0*	*45.9*	*67.7*		*41.1*	Above-ground vegetation N + below-ground-living N Scholes & Walker (1993)
Losses through burning	*25.5*	*39.4*	*58.0*		*8.7*	Scholes *et al.* (2003)
Denitrification	*0.0*	*2.2*	*13.7*			Scholes & Walker (1993) Assumed to be 5% of nitrification
Deposition	*-*	*-*	*-*	*3.9*	*8.5*	Blight *et al.* (2009)

Table 4.8: Calculated minimum, average and maximum pools (kg ha^{-1}) and fluxes (in italics, kg ha^{-1} $year^{-1}$) used to compile the S input-output budgets for the Sandspruit system under minimum, average and maximum levels of deposition.

Variable Measured	Calculated Pools (kg ha^{-1}) or Fluxes (kg ha^{-1} yr^{-1}) in this study			Literature Pools (kg ha^{-1}) or Fluxes (kg ha^{-1} yr^{-1})		Source for minimum and maximum literature pools and fluxes
	Minimum	Average	Maximum	Minimum	Maximum	
Vegetation and Litter						
Vegetation (kg ha^{-1} in March 2011)	1 706.4	2 549.7	3 808.8	1 570.0	2 341.0	Scholes & Walker (1993) Snyman (2005)
Litter (kg ha^{-1} in March 2011)	373.6	685.1	936.0	392.5	585.3	Assumed to be 25% of vegetation
Sulfur						
Average % S in vegetation	0.1			0.1		du Preez et al. (1983)
Above-ground vegetation (kg S ha^{-1} March 2011)	1.9	2.8	4.2	1.9	2.8	Calculated: Vegetation (March 2011) x Average % S in vegetation
Average % S in litter	0.1			0.1		du Preez et al. (1983)
Above-ground litter (kg S ha^{-1} March 2011)	0.3	0.5	0.7	0.3	0.4	Calculated: Litter (March 2011) x Average % S in litter
Below-ground - living	-	-	-	0.3	0.4	du Preez et al. (1983)
Total S - Soil (to depth of 0.15m)	364.1	462.1	631.8	62.4	555.8	du Toit & du Preez (1995)
Inorganic S (SO_4^{2-}) - Soil	43.0	59.1	120.2	7.1	88.6	Bird (2011)
Microbial S - soil	16.1	23.1	31.6		23.4	5% of organic S pool Edwards (1998) Bird (2011)
Mineralization	4.1	28.5	124.5	0.6	29.7	Bird (2011)
Immobilization	-18.8	-47.3	-84.4	-5.4	-80.0	Bird (2011)
Plant uptake	2.2	3.2	4.6	0.3	0.4	Above-ground vegetation S + below-ground-living S Scholes & Walker (1993)
Losses through burning	1.1	1.6	2.4	1.1	1.6	Assume 50% loss in annual burns (Scholes & Walker, 1993)
Deposition	-	-	-	8.0	35.0	Blight et al. (2009)

The minimum, average and maximum budgets for the Sandspruit system were created under the assumption that the amount of N gained through N fixation and the amount of S lost from the system as H_2S gas were negligible. The values for the below-ground living component of N and S, the microbial N and S in the soil and the N and S deposition were used from the presented literature sources (Table 4.7 & 4.8). The remaining values from the literature were used as comparisons and were chosen to be as close to what would be occurring within the study site so that the budgets created could be assumed to be an accurate representation of the N and S cycling in the Sandspruit system.

4.4.1 Nitrogen Budget Assuming Minimum Modelled Deposition

The total above-ground N pool was composed of N present in the above-ground vegetation (28.5 kg N ha^{-1}) and above-ground litter (5.5 kg N ha^{-1}) (Figure 4.14). Nitrogen lost to the atmosphere when the vegetation and litter burnt in this system was estimated at 75.0% of the N stored in the above-ground pools. This calculation was based on the loss of N through fires in savanna ecosystems where the fuel loads are made up of approximately 80.0% grass (Scholes *et al.*, 2003). In this budget, it amounted to 25.5 kg N ha^{-1} yr^{-1} (Figure 4.14). Nitrogen may also have been lost through denitrification as nitrogen dioxide (NO_2) and nitrous oxide (N_2O) gases, especially under any anaerobic soil conditions when the tubes were uncovered in October and November 2010. This was estimated as 5.0% of the nitrification rate (Scholes & Walker, 1993) and in this budget was calculated to be 0.0 kg N-NO_3^- ha^{-1} yr^{-1} as the nitrification rate was negative (Figure 4.14). The incoming N deposition composed of both wet and dry deposition included the N from fossil fuel combustion as well as from biomass burning and amounted to a minimum of 3.9 kg N ha^{-1} yr^{-1} (Figure 4.14) (Blight *et al.*, 2009). In this scenario, the amount of N deposition is fairly small compared to the 25.5 kg N ha^{-1} yr^{-1} lost through biomass burning.

A very large proportion of the N in this system is stored in the soil in organic form with the total N pool (3 072.3 kg N ha^{-1}) composed of 0.01% inorganic N and 99.99% organic N (Figure 4.14). Nitrate and NH_4^+ contribute to the soil inorganic N pool and were calculated to be 0.0 kg N-NO_3^- ha^{-1} and 0.5 kg N-NH_4^+ ha^{-1} respectively (Figure 4.14). The nitrification and ammonification rates, that add NO_3^- and NH_4^+ to their respective pools, were calculated to be -88.5 kg N-NO_3^- ha^{-1} yr^{-1} and -130.5 kg N-NH_4^+ ha^{-1} yr^{-1} respectively in this budget (Figure 4.14). The ammonification rate was negative and smaller than the nitrification rate although the NH_4^+ pool of 0.5 kg N-NH_4^+ ha^{-1} was larger than the NO_3^- pool of 0.0 kg N-NO_3^- ha^{-1} (Figure 4.14). Nitrate and NH_4^+ plant uptake was estimated to be equal to the N in the above-ground vegetation biomass and the below-ground root stock combined (Scholes & Walker, 1993). In this budget, the plant N uptake amounted to 31.0 kg N ha^{-1} yr^{-1} that would have been taken up metabolically and incorporated into the plant tissue (0.36% into leaf tissue, 0.1% into root tissue and 0.66% into litter) (Scholes *et al.*, 2003a).

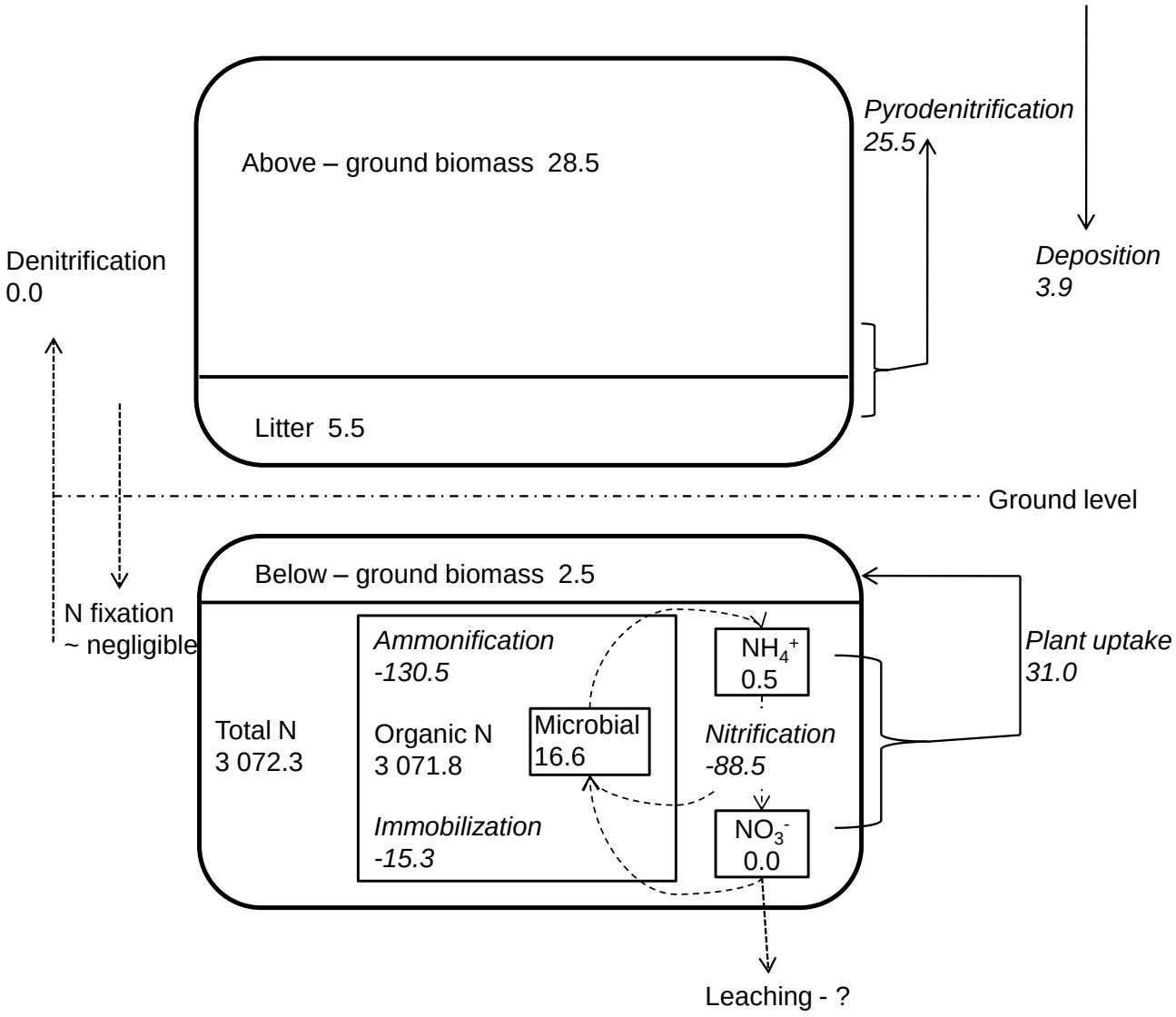

Figure 4.14: The N budget for the Sandspruit system assuming minimum pool sizes, fluxes and deposition. The unit for pool size is kg ha^{-1} and for flux (in italics) is kg ha^{-1} yr^{-1}.

4.4.2 Nitrogen Budget Assuming Average Modelled Deposition

The total above-ground N pool amounted to 52.6 kg N ha^{-1}, composed of 42.6 kg N ha^{-1} in the vegetation and 10.0 kg N ha^{-1} in the litter (Figure 4.15). The amounts of N lost from the system to the atmosphere through pyrodenitrification and denitrification in this budget followed the same assumptions as the minimum N budget. Pyrodenitrification in this average budget amounted to 39.4 kg N ha^{-1} yr^{-1} and the loss of N through denitrification amounted to only 2.2 kg N ha^{-1} yr^{-1} (Figure 4.16). The incoming N deposition in this budget amounted to 6.2 kg N ha^{-1} yr^{-1} (Figure 4.15) (Blight *et al.*, 2009). In this average budget as well, the amount of N deposition is fairly small compared to the total of 41.7 kg N ha^{-1} yr^{-1} lost through biomass burning and denitrification (Figure 4.15).

The organic soil N pool in this budget was 4 686.4 kg N ha^{-1}, forming part of the total N pool of 4 718.2 kg N ha^{-1} (Figure 4.15). Relatively large amounts of NO_3^- and small amounts of NH_4^+ were produced with nitrification and ammonification rates of 44.2 kg N-NO_3^- ha^{-1} yr^{-1} and 2.3 kg N-NH_4^+ ha^{-1} yr^{-1} (Figure 4.15). Although the nitrification rate was larger than the ammonification rate in this budget, the NH_4^+ pool of 21.6 kg N-NH_4^+ ha^{-1} was larger than the NO_3^- pool of 10.2 kg N-NO_3^- ha^{-1} (Figure 4.15). In this average budget, the plant N uptake amounted to 45.9 kg N ha^{-1} yr^{-1} that was incorporated into above- and below-ground vegetation and litter (Figure 4.15).

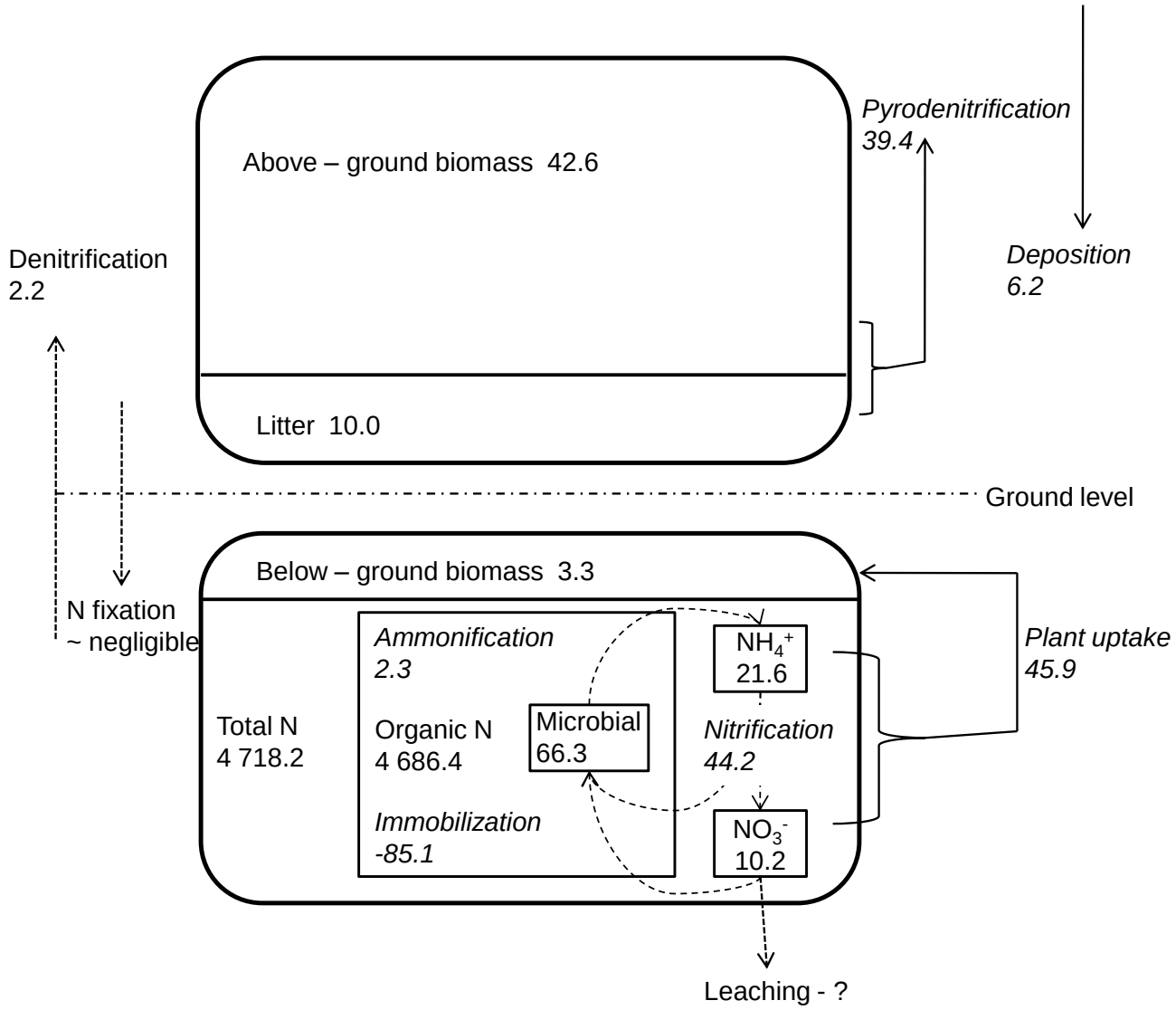

Figure 4.15: The N budget for the Sandspruit system assuming average pool sizes, fluxes and deposition. The unit for pool size is kg ha^{-1} and for flux (in italics) is kg ha^{-1} yr^{-1}.

4.4.3 Nitrogen Budget Assuming Maximum Modelled Deposition

The total above-ground N pool amounted to 77.3 kg N ha^{-1}, composed of 63.6 kg N ha^{-1} in the vegetation and 13.7 kg N ha^{-1} in the litter (Figure 4.16). The N lost from the system to the atmosphere through pyrodenitrification and denitrification in this budget followed the same assumptions as the minimum N budget. Pyrodenitrification in this maximum budget amounted to 58.0 kg N ha^{-1} yr^{-1} and the loss of N through denitrification amounted to only 13.7 kg N ha^{-1} yr^{-1} (Figure 4.16). The incoming N deposition in this budget amounted to an average of 8.5 kg N ha^{-1} yr^{-1} (Figure 4.16) (Blight *et al.*, 2009). In this maximum budget as well, the amount of N deposition is fairly small compared to the total of 71.6 kg N ha^{-1} yr^{-1} lost through biomass burning and denitrification (Figure 4.16).

The organic soil N pool was 6 478.2 kg N ha^{-1}, contributing to the total soil N pool of 6 583.5 kg N ha^{-1} (Figure 4.16). Large amounts of NO_3^- and NH_4^+ were produced with nitrification and ammonification rates of 273.3 kg N-NO_3^- ha^{-1} yr^{-1} and 158.5 kg N-NH_4^+ ha^{-1} yr^{-1} (Figure 4.16). In this budget, the nitrification rate and the NO_3^- pool were larger than the ammonification rate and NH_4^+ pool. Through the process of nitrification (at a rate of 273.3 kg N-NO_3^- ha^{-1} yr^{-1}), a soil NO_3^- pool of 63.7 kg N-NO_3^- ha^{-1} was created in this site and through the process of ammonification (at a rate of 158.5 kg N-NH_4^+ ha^{-1} yr^{-1}), a soil NH_4^+ pool of 41.6 kg N-NH_4^+ ha^{-1} was created (Figure 4.16). In this maximum budget, the plant N uptake amounted to 67.7 kg N ha^{-1} yr^{-1} that was incorporated into above- and below-ground vegetation and litter.

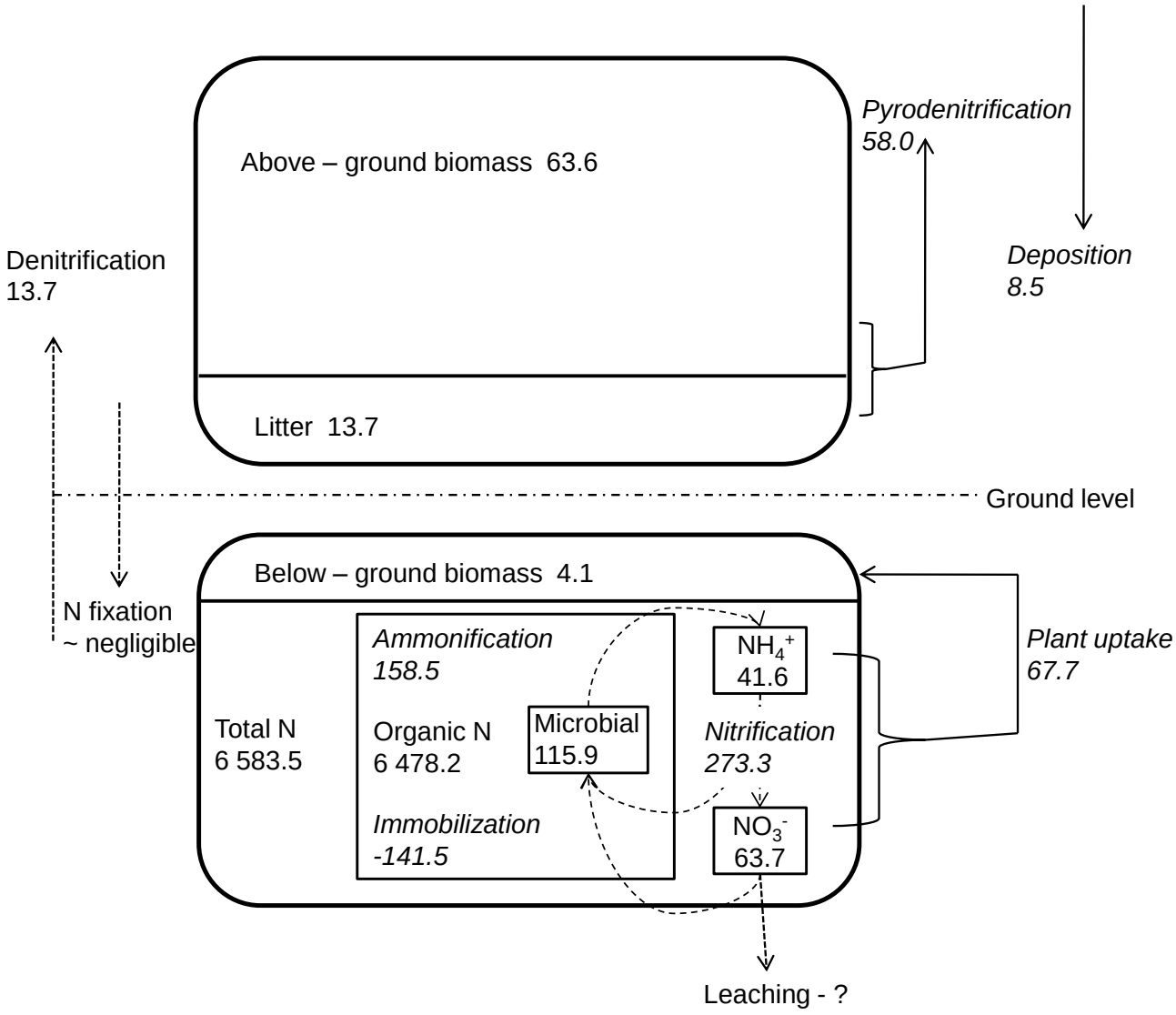

Figure 4.16: The N budget for the Sandspruit system assuming maximum pool sizes, fluxes and deposition. The unit for pool size is kg ha^{-1} and for flux (in italics) is kg ha^{-1} yr^{-1}.

4.4.4 Sulfur Budget Assuming Minimum Modelled Deposition

The above-ground pools in the sulfur budget consisted of the above-ground vegetation and litter. In this budget, the amount of S in these above-ground pools was calculated to be 1.9 kg S ha^{-1} in the vegetation and 0.3 kg S ha^{-1} in the litter (Figure 4.17). Snyman & du Preez (2005) determined that grass roots in semi-arid grasslands are replaced every 20 months. This would have created a turn-over of 0.3 kg S ha^{-1} every 20 months. In this minimum budget, 8.0 kg ha^{-1} yr^{-1} of S was deposited during an average rainfall year and a total loss of S through biomass burning was approximately 1.1 kg ha^{-1} yr^{-1} of S (Figure 4.17). This loss of S was estimated at 50.0% of the total S in the above-ground vegetation and litter pools (Scholes & Walker, 1993).

The largest S pool in this budget was the soil organic S pool and in this budget held 321.1 kg S ha^{-1} (Figure 4.17). By subtracting the organic S pool from the total S pool of 364.1 kg S ha^{-1}, it provided a small inorganic S pool of 43.0 kg S ha^{-1} (Figure 4.17). Annually, a minimum of 4.1 kg S-SO_4^{2-} ha^{-1} was mineralized to SO_4^{2-} with a faster rate of S immobilization at -18.8 kg S ha^{-1} yr^{-1} (Figure 4.17). Plant S uptake was estimated to be equal to S in the above-ground vegetation biomass and the below-ground root stock and was calculated to be 2.5 kg S ha^{-1} yr^{-1}.

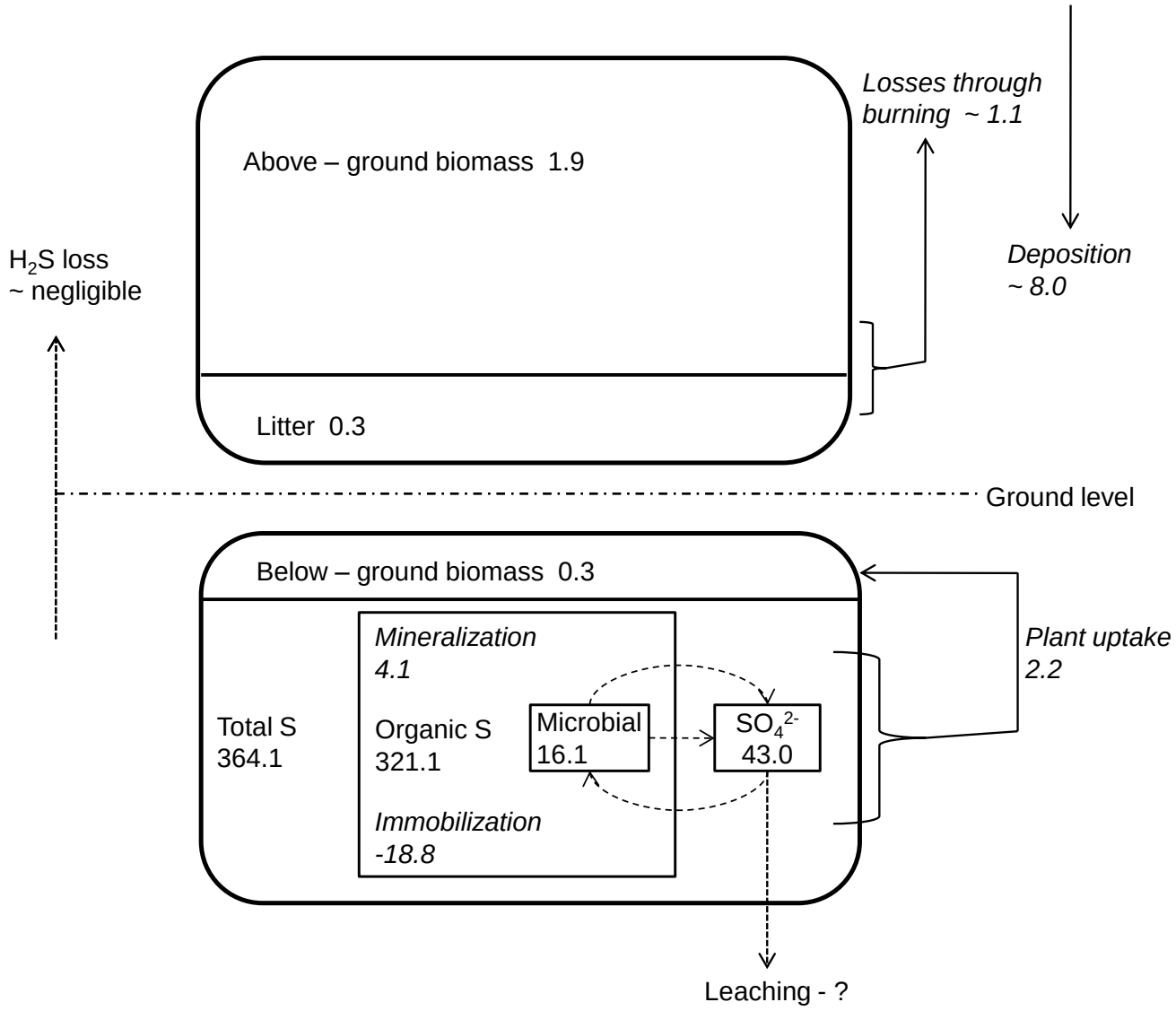

Figure 4.17: The S budget for the Sandspruit system assuming minimum pool sizes, fluxes and deposition. The unit for pool size is kg ha^{-1} and for flux (in italics) is kg ha^{-1} yr^{-1}.

4.4.5 Sulfur Budget Assuming Average Modelled Deposition

In this average budget, the S in the above-ground pools was calculated to be 2.8 kg S ha^{-1} in the vegetation and 0.5 kg S ha^{-1} in the litter (Figure 4.18). The turn-over of S in the root stock of the grasses was calculated to be 0.4 kg S ha^{-1} every 20 months (Figure 4.18). In this average budget, 21.5 kg ha^{-1} yr^{-1} of S was deposited during an average rainfall year with a total of approximately 1.6 kg ha^{-1} yr^{-1} of S lost to the atmosphere through biomass burning (Figure 4.18), 50.0% of the total S in the vegetation and litter (Scholes & Walker, 1993).

The largest S pool in this budget was the soil organic S pool and in this budget held 298.7 kg S ha^{-1} (Figure 4.18). By subtracting the organic S pool from the total S pool of 462.2 kg S ha^{-1}, it provided an inorganic S pool of 163.5 kg S ha^{-1} (Figure 4.18). Annually, an average of 109.5 kg S-SO_4^{2-} ha^{-1} was mineralized to SO_4^{2-} with a slower rate of S immobilization at -47.3 kg S ha^{-1} yr^{-1} (Figure 4.18). Plant S uptake of 3.9 kg S ha^{-1} yr^{-1} was estimated to be equal to S in the above-ground vegetation and the below-ground root stock.

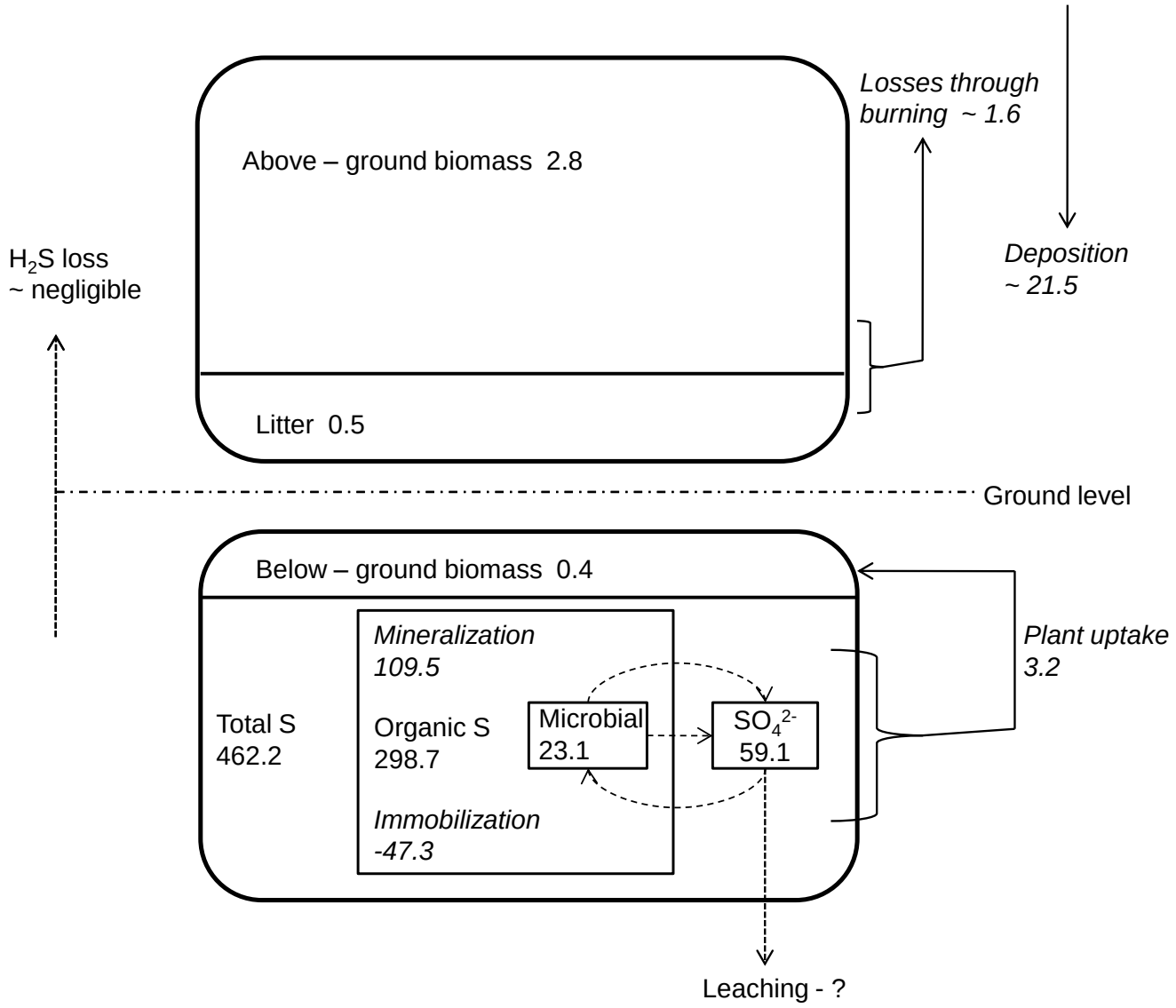

Figure 4.18: The S budget for the Sandspruit system assuming average pool sizes, fluxes and deposition. The unit for pool size is kg ha^{-1} and for flux (in italics) is kg ha^{-1} yr^{-1}.

4.4.6 Sulfur Budget Assuming Maximum Modelled Deposition

In this maximum budget, the S in the above-ground pools was calculated to be 4.2 kg S ha^{-1} in the vegetation and 0.7 kg S ha^{-1} in the litter (Figure 4.19). The turn-over of S in the root stock of the grasses was calculated to be 0.4 kg S ha^{-1} every 20 months (Figure 4.19). In this maximum budget, 35.0 kg ha^{-1} yr^{-1} of S was deposited during an average rainfall year with a total of approximately 2.4 kg ha^{-1} yr^{-1} of S lost to the atmosphere through biomass burning (Figure 4.19), 50.0% of the total S in the vegetation and litter.

Normally, the largest S pool in the budget was the soil organic S pool (145.4 kg S ha^{-1}) although in this budget it was smaller than the inorganic S pool (Figure 4.19). By subtracting the organic S pool from the total S pool of 631.8 kg S ha^{-1}, it provided a large inorganic S pool of 486.4 kg S ha^{-1} (Figure 4.19). Annually, an average of 415.5 kg S-SO_4^{2-} ha^{-1} was mineralized to SO_4^{2-} with a slower rate of S immobilization at -84.4 kg S ha^{-1} yr^{-1} (Figure 4.19). Plant S uptake of 5.8 kg S ha^{-1} yr^{-1} was estimated to be equal to S in the above-ground vegetation and the below-ground root stock.

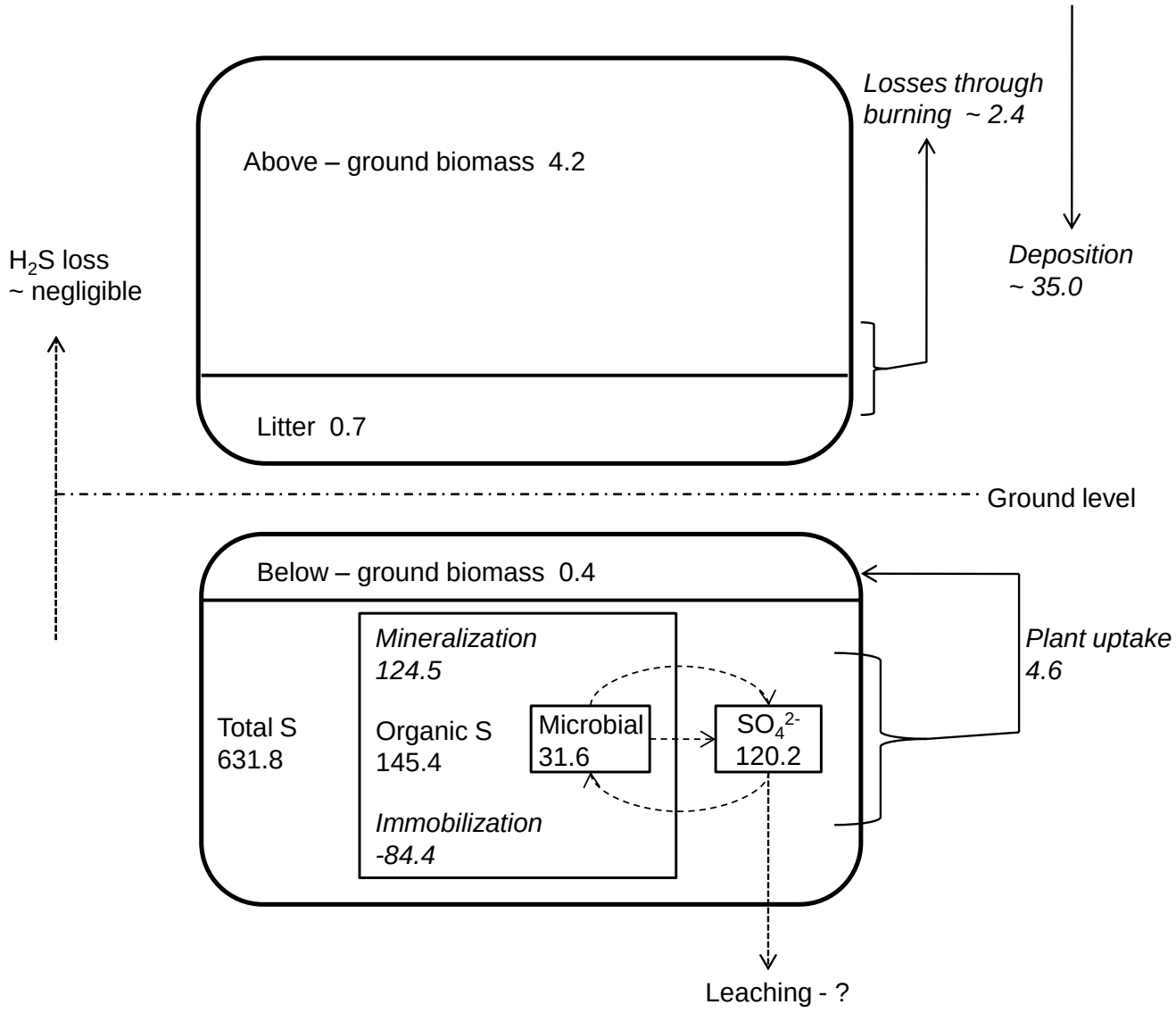

Figure 4.19: The S budget for the Sandspruit system assuming maximum pool sizes, fluxes and deposition. The unit for pool size is kg ha^{-1} and for flux (in italics) is kg ha^{-1} yr^{-1}.

CHAPTER 5: DISCUSSION

5.1 Introduction

The following discussion is based on the inputs, cycling processes and outputs of N and S measured and estimated in the study site of the Sandspruit system. Each section is considered in relation to the overall representation of the N and S input-output budgets and how the amount of each pool and rate of each input and output would affect the potential for NO_3^- and SO_4^{2-} leaching in this system. The overall outcomes of the N and S input-output budgets are briefly discussed before each budget is discussed in detail. The current and potential future N and S deposition rates, the cycling of N and S between the vegetation and soil, the N and S mineralization and immobilization rates, the effects of fire on nutrient cycling and the losses of N and S as a result of biomass burning and denitrification are discussed and compared to a variety of literature values. Finally, the uncertainties apparent in the N and S input-output budgets have been highlighted with regard to the accuracy with which they portray the functioning of the Sandspruit system.

The N and S gaseous losses, the below-ground biomass, the plant N and S uptake and the size of the N and S microbial pools were estimated for this study site. These values presented in Table 4.6 & 4.7 and in the N and S input-output budgets were estimated based on published literature available for similar systems, such as grasslands and savannas with similar climates located in South Africa. The values from other grasslands in South Africa are considered more accurate than values from savannas although where values were not available for grasslands, values from savannas were used. Using data that were not specifically measured at this study site creates some uncertainty about how accurately these values represent certain pools in the soils of the Sandspruit system and outputs from the Sandspruit system. It is also important to note that no control was used for comparison in this study as no similar grassland site exists in South Africa that has not received

elevated N and S deposition from anthropogenic sources for a number of years. The data from this study can therefore be used as baseline data for future impact studies to determine the effects of N and S deposition on the vegetation, soil and water quality in grassland ecosystems in this location in South Africa.

5.2 Nitrogen and Sulfur Input-Output Budgets for the Sandspruit System

The N and S input-output budgets created in this study for the Sandspruit system indicated no potential output of N in the form of NO_3^- leaching and a very small potential for output of S in the form of SO_4^{2-} leaching into nearby surface water. The average current modelled N deposition rate used as an input into the N budget was far smaller than the calculated losses of N during biomass burning. This was the case before considering the gaseous losses from the soil during denitrification and the uptake of N by plants that would have further decreased the available NO_3^- in the soil pool for leaching through the soil profile into surface water. The opposite however was found for the S budget. The average current modelled S deposition rate was greater than the calculated loss of S during biomass burning. The rate of S deposition still exceeded the output of S from the system once accounting for the loss of S through plant uptake, creating a potential for SO_4^{2-} leaching.

Grasslands in the US and Europe have received similar levels of N and S deposition over a longer time period and have recorded and monitored a number of ecosystem impacts. Measured and modelled N deposition from continental-scale studies in Europe and the US ranged from 2.4 to 43.5 kg N ha^{-1} yr^{-1} (Holland *et al.*, 2005; Bowman *et al.*, 2008; Stevens *et al.*, 2010a; van den Berg *et al.*, 2011), greater than the N deposition on certain Highveld grasslands in South Africa that receive up to 25.0 kg N ha^{-1} yr^{-1} (Lowman, 2003). Measured and modelled S deposition from continental-scale studies in Europe and the US ranges from 6.6 to

28.7 kg S ha^{-1} yr^{-1} (EPA, 2008; Stevens *et al.*, 2009; van ben Berg *et al.*, 2011), greater than the calculated average S deposition across 37 sites of 4.5 kg S ha^{-1} yr^{-1} in the northern and eastern regions of South Africa (Josipovic, 2009). If the current levels of N and S deposition were to continue in grasslands in South Africa, including this study site in the Mpumalanga province, studies in the developed world indicate a range of possible impacts to the soil, vegetation and surface water quality of affected ecosystems. This study has highlighted the importance of continued monitoring in areas receiving elevated N and S deposition and has attempted to predict the effects of increased N and S deposition on the cycling and output of N and S in this system.

5.3 Budget Inputs

5.3.1 Current and Potential Future Nitrogen Deposition

The average modelled N deposition of 6.2 kg N ha^{-1} yr^{-1} in this study site was similar to deposition in a site studied by Fenn *et al.* (1996) where 10 kg N ha^{-1} yr^{-1} produced very few measurable impacts and did not result in measurable NO_3^- leaching. Modelled N deposition across a number of sites in the US between 1997 and 2000 ranged between 0.4 and 32.0 kg N ha^{-1} yr^{-1} (Baumgardner *et al.*, 2002; EPA, 2008) while the highest measured N deposition rate between 2004 and 2006 was in the Ohio River Valley at 9 kg N ha^{-1} yr^{-1} (EPA, 2008). Throughout Europe, the modelled and measured N deposition rate was similar to that of the US and ranged between 1.8 and 27.4 kg N ha^{-1} yr^{-1} in the year 2000 (Pieterse *et al.*, 2007; Duprè *et al.*, 2010).

The annual amount of N added to this system as deposition was small compared to the soil N pool of 4 718.2 kg N ha^{-1} and the amount of N stored in the above-ground N biomass pool of 42.6 kg N ha^{-1} (Figure 4.15). The amount of modelled N

deposition was only 19.5% and 14.6% of the total soil inorganic N pool and above-ground N biomass pool respectively (Figure 4.15). When the rate of N deposition, N lost through fires and denitrification and plant uptake were compared, the expectation was that the current N deposition was having a fertilization effect on the system through increasing the soil available NO_3^- pool for plant uptake and incorporation into plant tissue (Stevens *et al.*, 2011). This result was found in a number of other studies where moderate N inputs were not responsible for NO_3^- leaching but rather increased the ecosystem's productivity (Bobbink *et al.*, 1998; Stevens *et al.*, 2011).

Currently, the amount of N that has the potential to be immobilized far exceeds the modelled N input into the system from deposition. When the maximum N input-output budget was analyzed, it showed that the system has the potential to immobilize over 16 times the current maximum rate of modelled N deposition of 8.5 kg N ha^{-1} yr^{-1} (Figure 4.16). It is expected that an increase in N deposition of > 3.8kg N ha^{-1} yr^{-1} in this system in the future may not impact negatively on N cycling and that the added N would be immobilized and become part of the soil microbial pool or become available for plant uptake and have a fertilization effect on the system. The cycling and outputs of N in the system will be discussed in more detail in sections 5.4 and 5.6 respectively.

5.3.2 Current and Potential Future Sulfur Deposition

The average modelled S deposition of 21.5 kg S ha^{-1} yr^{-1} for the Sandspruit system was on a similar scale to the S deposition measured over many of the States in the US between 2004 and 2006 (EPA, 2008). These measurements in the US were made following the implementation of the Clean Air Act and represent decreased levels of deposition following legislation implementation to limit the rate of S

emissions into the atmosphere. Modelled S deposition across a number of sites in the US between 1997 and 2000 ranged between 0.4 and 16.5 kg S ha^{-1} yr^{-1} (Baumgardner *et al.*, 2002) while the highest measured S deposition rate between 2004 and 2006 was in the Ohio River Valley at 21 kg S ha^{-1} yr^{-1} (EPA, 2008). Throughout Europe, the modelled and measured S deposition rate was similar to that of the US and ranged between 1.3 and 15.5 kg S ha^{-1} yr^{-1} in the year 2000 (Pieterse *et al.*, 2007; Duprè *et al.*, 2010).

The annual amount of S added to the system as deposition was relatively small compared to the soil S pool of 462.2 kg S ha^{-1} although the modelled annual S deposition was approximately 13 times larger than the S lost to the atmosphere through biomass burning annually (Figure 4.18). The S lost to the atmosphere during burning was estimated at 50.0% of the S contained in the above-ground biomass and litter (Scholes & Walker, 1993). This loss was estimated in the average S budget to be approximately 1.6 kg S ha^{-1} yr^{-1} (Figure 4.18) under the assumption that the majority of the vegetation in the site burnt annually. If the farmer should decide to burn every second year, the amount of S lost to the atmosphere would halve to 0.8 kg S ha^{-1} yr^{-1}. This change in S output would result in S deposition of approximately 27 times more than the annual S loss from biomass burning.

The budget showing the maximum modelled S deposition represented a prediction of what future S deposition levels may reach in the Sandspruit system and what S deposition levels may currently be during periods of high rainfall (Figure 4.19). Blight *et al.* (2009) modelled peak annual wet S deposition rates averaging 20.0 kg S ha^{-1} yr^{-1} across the Highveld grasslands with between 57.0% and 93.0% of current and future predicted S deposition reaching the ground during precipitation events. The months with the highest precipitation were October 2010

to January 2011 (Figure 4.3) and would have had the highest S deposition rates of between 20.0 and 32.6 kg S ha^{-1} yr^{-1} over the sampling period when compared to the drier months of February to September 2011.

The budget showing the maximum S deposition for the system indicated the soil SO_4^{2-} pool was 120.2 kg S-SO_4^{2-} ha^{-1} and was more than 3 times larger than the microbial pool of 31.6 kg S ha^{-1} and the SO_4^{2-} taken up by plants (4.6 kg S ha^{-1}) combined (Figure 4.19). This large soil SO_4^{2-} pool could become a concern, especially if S deposition were to increase beyond the maximum modelled rate of 35.0 kg S ha^{-1} yr^{-1}. An increase in S deposition would be expected to increase the SO_4^{2-} mineralization and immobilization rates and the soil SO_4^{2-} pool. The increase in the immobilization rate however, would most likely not be sufficient to immobilize the added SO_4^{2-} from the increased level of deposition. Increased S deposition may create an increased potential for plant S uptake and SO_4^{2-} leaching should the soil SO_4^{2-} pool increase without an increase in the SO_4^{2-} immobilization rate.

5.4 Nutrient Cycling in the Sandspruit System

5.4.1 Nutrient Cycling Between the Vegetation and the Soil

5.4.1.1 Plant nitrogen and sulfur uptake and return to the soil

Plant N and S uptake were estimated in this study as the total N and S in the above-ground biomass and the estimated literature below-ground biomass values combined. The plant N and S uptakes in the average modelled N and S deposition budgets were estimated at 45.9 kg N ha^{-1} yr^{-1} and 3.2 kg S ha^{-1} yr^{-1} respectively (Figures 4.15 & 4.18). The estimated plant N uptake was over seven times larger than the modelled N deposition of 6.2 kg N ha^{-1} yr^{-1}. Conversely, the modelled S deposition of 21.5 kg S ha^{-1} yr^{-1} was over six times larger than the estimated plant

S uptake in this system. Plant N and S uptake is important for the removal of NH_4^+, NO_3^- and SO_4^{2-} from soil pools. The modelled S deposition was over six times higher than the plant S uptake in the average S budget and is a cause for concern as excess SO_4^{2-} increases the potential for SO_4^{2-} leaching into surface water.

Litter fall and subsequent decomposition were inputs of nutrients into the soil N and S pools. Decomposition would readily occur in this system as the soil pH of 5.4 supports organisms responsible for decomposition (Miller & Donahue, 1990). Decomposition occurs in these soils as *Nitrobacter* and *Nitrosomonas*, two bacteria that contribute to decomposition, thrive in soils when the ambient temperature is approximately 28 °C and soil pH levels are between 5.8 and 8.5 (Holt & Hendricks, 1994). Most litter decomposition in the Sandspruit system occurred during the warmer, wetter months of October 2010 to May 2011. This is supported by Epstein *et al.* (2002) who found > 30.0% of the variability in the decomposition rate in the US Great Plains was explained by precipitation. In this study, the increases in the net N mineralization rate from October 2010 to January 2011 (peak 0.60 µg g^{-1} soil day^{-1}) and from February to April 2011 (peak 1.05 µg g^{-1} soil day^{-1}) (Figure 4.10) are one indication of the change in the decomposition rate over the eight month sampling period. Bird (2011) found similar peaks in the net N mineralization rate in the soil of 11 grassland sites in the Mpumalanga Highveld. The net N mineralization rate peaked in February 2008 at 0.25 µg g^{-1} soil day^{-1} and in May 2008 at 0.52 µg g^{-1} soil day^{-1} (Bird, 2011).

The addition of N from deposition in the study site would aid in the rate of decomposition. During the months of March to May each year, there is sufficient moisture, C and N available in the system for decomposition to occur as C and N are provided by the senescing litter and moisture is provided by the rainfall still experienced during the autumn and early winter months. The decomposition rate in

grasslands has been shown to depend on the initial litter N concentration and will be faster when the initial N concentration is higher (Moretto *et al.*, 2001; Parton *et al.* 2007). Parton *et al.* (2007) found grass litter with an initial N concentration of 2.0% decomposed quicker than litter with an initial N concentration of 0.4%. In this study, the initial litter N concentration in October 2010 was relatively high at 1.5% (Figure 4.6). When N is limited, the soil organic microbes decompose the dead organic matter and the N is immediately immobilized by the microbes before it has had the chance to become available for plant uptake, reducing the initial litter N concentration. However, if N deposition exceeded levels that allowed for increased Al^{3+} mobility in certain systems, the resultant toxic ion release would reduce the decomposition rate (Mulder *et al.*, 2001; Horwath, 2007), slowing the return of nutrients to the soil. Mulder *et al.* (2001) found that acid deposition inputs to forest soils decreased the decomposition rate by 30.0-40.0%. They attributed the effect of deposition on decomposition to increased binding of Al to SOM, decreasing the SOM decay and decomposition rates (Mulder *et al.*, 2001). A decrease in decomposition rate of up to 40.0% is not expected in the Sandspruit system as a result of the high buffering capacity of the soils.

The increase in the N:S ratio in the vegetation and litter from October 2010 to March 2011 may have been a result of decomposition and the S leaching out of the litter prior to the completion of litter decomposition as majority of the S in plant leaves is in a simple amino acid form (Millard *et al.*, 1985). The increase in the vegetation N:S ratio from 3:1 in October 2010 to 17:1 in March 2011 (Table 4.3) may be the result of a decrease in S availability and an increase in vegetation N content (Figure 4.6) (Millard *et al.*, 1985). Bardsley & Jordan (1957) found S deficient plants had a N:S ratio between 20:1 and 30:1 and plants that were supplied with S had N:S ratios between 10:1 and 17:1 with a mean of 14:1. Similarly, Werner & Monteiro (1988) found the N:S ratio in forage grasses was normally between 12:1 and 17:1 in areas that were well supplied with N and S. A

low N:S ratio of < 5:1, as was measured in the vegetation in October 2010 in the Sandspruit system, is indicative of plants that are supplied with N at low rates in combination with medium or high S rates (Werner & Monteiro, 1988; Batista & Monteiro, 2007).

5.4.1.2 Change in plant species diversity

The availability of certain nutrients is one of many key factors that determine plant species composition in this system. The levels of N and S deposition currently modelled for the Highveld grasslands are expected to have a fertilization effect on the grasses and forbs growing in the Sandspruit system, especially if the deposition remains at current modelled levels in the future. Changes in plant species diversity in a number of grasslands receiving N and S deposition at levels higher than those experienced in this site have been thought to be a result of the changes in soil chemistry brought about by the deposition (Bobbink *et al.*, 1998; van den Berg *et al.*, 2011). Changes in soil chemistry and resultant changes to plant species diversity are not expected for the Sandspruit system for the foreseeable future as deposition levels are considered relatively low compared to similar grasslands across Europe and North America. Should future N and S deposition rates rise above their modelled maximum levels, the resultant changes to the pH, ANC and the concentrations of toxic elements such as Al^{3+} in the soil may lead to changes in the plant species diversity. Studies performed in regions where N deposition or experimental addition exceeds the levels modelled for the Sandspruit system by up to 133.8 kg N ha^{-1} yr^{-1} showed changes in plant species diversity including an overall decrease in species richness, dominance of a few plant species that were adapted to higher soil N and S levels and a significant decrease in the number of forb species in each region (Carroll *et al.*, 2003; Bobbink, 2004; Emmett, 2007; Stevens *et al.*, 2010a).

The study site was dominated by a single grass species (*H. hirta*) (Table 4.1) that may not be highly impacted by an increase in N and S deposition in the future. Disturbed areas often have *H. hirta* and *S. africanus* as part of their grass composition and are known to increase in abundance under increased disturbance (Tainton, 1972; Hurt *et al.*, 1993). The use of the study site for cattle grazing for the past 60 years would have contributed to the dominance of *H. hirta* that is palatable in the early growing season. The majority of the Sandspruit catchment and the Mpumalanga Highveld however, would most likely have higher plant species diversity than shown by this study site with a larger number of grass and forb species. In a study by O'Connor *et al.* (2001) in a grassland site near Bloemfontein in the neighbouring Free State province of South Africa, a total of nine grass species were present in a good quality rangeland. Elsewhere in the world, high altitude Himalayan grasslands contained six grass species (Ram *et al.*, 1989), European grasslands contained 14 plant species (Hector *et al.*, 1999) and acid grasslands across Great Britain contained up to 26 plant species (Stevens *et al.*, 2004). Land-use can impact on plant species diversity of grasslands and the Sandspruit system would be affected by the past crop agricultural use and current cattle rangeland use. The study by O'Connor *et al.* (2001) found a decline from nine to six grass species when good quality rangeland was degraded to poor quality rangeland. A study in Australian grasslands by Fensham *et al.* (1999) found a decline in the number of grass species with increased herbivore grazing although in an ungrazed site, a reduction in the plant species density was found in a Mediterranean grassland (Montalvo *et al.*, 1993).

If N and S deposition were to increase in the Highveld grasslands, deposition gradient studies by Bobbink (2004) and Stevens *et al.* (2004) predict a decrease in plant species diversity of up to 40.0%. The low grass species diversity in this study site may mean an increase in deposition would result in a change in the dominant grass species and a change in the dominant forb species or a decrease in the

number of forb species. This was found to be the case in studies by Carroll *et al.* (2003), Maskell *et al.* (2010), Stevens *et al.* (2010a) and Stevens *et al.* (2010b) in UK grasslands across a N deposition gradient.

5.4.2 Nutrient Cycling in the Soil

5.4.2.1 Nitrification and the soil nitrate pool

The nitrification rate is primarily regulated by NH_4^+ supply and is a source of acidity in many soils as H^+ ions are produced during the conversion of NO_2^- to NO_3^- by oxidizing autotrophic bacteria present in the soil. Nitrification therefore, would be a contributing factor to the soil pH of 5.4 in the soils of the Sandspruit system, especially as nitrification is responsible for the larger of the two processes making up the net N mineralization rate (Figure 4.10). The contribution of nitrification to soil acidity has previously been found in forested ecosystems where NH_4^+ input increased the nitrification rate, increasing soil acidity and decreasing the downstream water quality (Driscoll *et al*., 2001). The nitrification rate of 44.2 kg N ha^{-1} yr^{-1} in the average N deposition budget could have contributed to a decline in soil pH over time, especially when combined with the H^+ ions that were released into the soil during acid deposition. A decrease in soil pH would not be the only consequence of a rapid nitrification rate but may also be linked to an increased soil NO_3^- pool, an increased potential for NO_3^- leaching and a decrease in the buffering capacity of the soil. If the buffering capacity of the soils in this system decreases, the ability of the soil to deal with incoming acid deposition decreases, leading to a further drop in pH of the soil.

In the budget incorporating the average modelled N deposition of 6.2 kg N ha^{-1} yr^{-1}, the process of nitrification created a soil NO_3^- pool of 10.2 kg N-NO_3^- ha^{-1} (Figure 4.17). This suggests a strong possibility that some of the N entering the system as

deposition was added to the soil NO_3^- pool via the process of nitrification and then the majority of the soil NO_3^- was quickly immobilized into the soil microbial pool or taken up by plants. When attempting to determine the potential for NO_3^- leaching, the size of the soil NO_3^- pool is important as the NO_3^- anion is highly mobile, more so than the NH_4^+ cation (Groffman, 2000). The larger the soil NO_3^- pool, the greater the potential for leaching becomes in the system, especially from out of the rooting zone in the soil when the precipitation exceeds evapotranspiration in the system. This is not likely to be of concern in this system as precipitation rarely exceeds evapotranspiration. Moisture increased in the incubation tubes in six of the eight sampling months indicating water had moved up into the tubes by capillary action when evapotranspiration exceeded precipitation in the system.

5.4.2.2 Net nitrogen mineralization and soil moisture

The range of the daily net N mineralization rate measured in the soils of the Sandspruit system was similar to a number of N mineralization ranges from laboratory incubations of grassland soils. The range of the net N mineralization rate in the Sandspruit system (-1.03 to 1.05 µg N g^{-1} soil day^{-1}) was similar to the range in Mongolian grasslands (-0.01 to 1.10 µg N g^{-1} soil day^{-1}) (Zhang *et al.*, 2008; Lui *et al.*, 2010) and Pakistani grasslands (0.15 to 1.50 µg N g^{-1} soil day^{-1}) (Abbasi *et al.*, 2001). However, the range of the net N mineralization rate in the Sandspruit system was greater than the range measured by Yahdjian & Sala (2008) in Patagonian grasslands (-0.03 to 0.04 µg N g^{-1} soil day^{-1}) and in other studies of the Highveld grasslands of South African (-0.60 to 0.52 µg N g^{-1} soil day^{-1}) (Bird, 2011).

The weak positive correlation between the N mineralization rate and soil moisture suggests the N mineralization rate increased with soil moisture which may have temporarily increased the soil NO_3^- pool, especially during the months of maximum

precipitation (October 2010-January 2011). This finding is in agreement with the knowledge that soil moisture and temperature are two of the most critical factors that regulate soil biological activity (Robertson & Groffman, 2007). Barrett *et al.* (2002) found a similar result in the central grasslands of the US where net N mineralization rates were weakly influenced by the average soil moisture content and temperature. The seasonal trends in rainfall recorded in the US grasslands explained up to 24.0% of the seasonal variation in the net N mineralization rate (Barrett *et al.*, 2002).

During the winter months of May to September in the Sandspruit system when the soils are drier and temperatures are lower, moisture becomes the controlling factor determining the rate of N mineralization. Barrett *et al.* (2002) found a negative correlation between the N mineralization rate and the average monthly temperature and a decrease in the contribution that the nitrification rate made to the net N mineralization rate under the condition of increased soil moisture. This same trend was found in the soils of the Sandspruit system with increased rainfall from October to December 2010 (Figure 4.3) coupled with an increase in the nitrification rate (Figure 4.10). During the months of little or no rainfall (May-September), the lack of moisture in the soils of the Sandspruit system limited the nitrification and ammonification rates and decreased the net N mineralization rate and addition of NO_3^- into the soil NO_3^- pool.

5.4.2.3 Sulfate mineralization and the soil sulfate pool

The range of the daily SO_4^{2-} mineralization and immobilization rates in the soils of the Sandspruit system was dissimilar to a number of incubation studies that tested the SO_4^{2-} mineralization rates in other grassland soils. The SO_4^{2-} mineralization rate in the Sandspruit system (0.91 µg S g^{-1} soil day^{-1}) was greater than the

mineralization rate measured by Bird (2011) in a number of soils in the Highveld region (0.32 µg S g^{-1} soil day^{-1}) but was less than the average mineralization rate of 1.63 µg S g^{-1} soil day^{-1} measured in laboratory incubation studies in grassland soils in the US (Pirela & Tabatabai, 1988), New Zealand (Ghani *et al.,* 1991) and Italy (Riffaldi *et al.*, 2006). The SO_4^{2-} immobilization rate in the Sandspruit system (-1.06 µg S g^{-1} soil day^{-1}) was less than the immobilization rate measured by Bird (2011) in a number of soils in the Highveld region (-2.38 µg S g^{-1} soil day^{-1}) but was more than the average immobilization rate of -0.04 µg S g^{-1} soil day^{-1} measured in laboratory incubation studies in grassland soils in the US (Pirela & Tabatabai, 1988), New Zealand (Ghani *et al.,* 1991) and Italy (Riffaldi *et al.*, 2006).

Sulfate immobilization has the potential to decrease the size of the soil SO_4^{2-} pool and transfer some of the incoming S deposition into the soil microbial pool and is an important indication of the potential for SO_4^{2-} leaching in any system. In this study site, the annual SO_4^{2-} immobilization rate was measured at -47.3 kg S ha^{-1} yr^{-1} (Figure 4.12). The SO_4^{2-} mineralization rate however, was measured to be over twice the rate of immobilization in the system (109.5 kg S ha^{-1} yr^{-1}). This comparison, coupled with the rate of S deposition created a small potential for SO_4^{2-} leaching in the average modelled S budget. The relatively small S immobilization rate and SO_4^{2-} uptake rate by plants could have left a large soil SO_4^{2-} pool that increased the potential for SO_4^{2-} leaching into nearby surface water. However, SO_4^{2-} is known to move particularly slowly through clayey soils compared to sandy soils as the small pore space restricts SO_4^{2-} movement (Barrow, 1975). Soils with a higher clay content have also been shown to have a slower SO_4^{2-} mineralization rate (Riffaldi *et al.*, 2006). This may possibly explain the slower SO_4^{2-} mineralization rate in the Sandspruit system compared to a number of other grassland studies globally as the clay content in the soils of the study site was particularly high (51.2%) (Table 4.4). The high clay content of the soils in this system may have prevented SO_4^{2-} leaching up to a point. This

threshold however, may be crossed if S deposition increases in the future to levels above the maximum modelled deposition rate.

In areas that do not receive S deposition, the main S input comes from soil weathering although a large percentage of the world's soils currently receive S deposition (Plante, 2007). On average, most agricultural soils globally contain between 20-2 000 µg S g^{-1} soil and the organic S pool makes up approximately 90.0% of the total soil S pool (Plante, 2007). In this study site, the soil contained 200.0 µg S g^{-1} soil and the organic S pool made up 64.6% of the total soil S pool, fairly low considering the global average. The majority of the 35.4% of S in the inorganic S pool may be present in the SO_4^{2-} pool, increasing the potential for SO_4^{2-} leaching from the soil into nearby surface water.

5.5 The Effects of Fire on Nutrient Cycling in the Sandspruit System

Fire is a vital component of grassland ecosystems and much of the Sandspruit catchment undergoes planned burning. On average, the farmer at this study site burns every two years or once he has decided enough above-ground biomass has accumulated. The effects of fires on the below- and above-ground systems depend on the intensity of each fire, although grasslands rarely experience very intense fires as a result of the lack of woody fuel. The fire that burnt in November 2010 would have had a number of effects on the nutrient cycling processes of the system.

The fire that occurred in the study site in November 2010 changed certain soil conditions and removed the majority of the standing vegetation and litter (Figure 3.10). The surface soil temperature would have increased slightly depending on

the intensity of the fire and the amount of insulation provided by the clayey soil. The loss of vegetation and litter cover would have been of greater significance to the soil temperature and would have increased the amount of radiation received by the soil surface. The loss of vegetation and litter cover may also have increased the potential for surface run-off to occur, especially during heavy precipitation events (Snyman, 2002). Nitrogen would have been lost to the atmosphere during the fire as it volatilizes at 200.0 °C although during lower intensity burns, soil organic N can be converted into inorganic NH_4^+ and NO_3^- that may be lost through denitrification, leaching or plant uptake (Kucera et al., 1967; Weast, 1988; Neary et al., 1999).

The nitrification results obtained by Romanyà *et al.* (2001) validated the nitrification results obtained in this study as both showed a decline in the nitrification rate within two months following the fire (Figure 4.10). Romanyà *et al.* (2001) further postulated that a measured delay in plant N uptake in the burnt plots had resulted from a decrease in N availability following the fire. This decrease in N availability was thought to be a result of a decreased N mineralization rate and increased NO_3^- leaching soon after the fire, both linked to an increase in soil surface temperature (Romanyà *et al.*, 2001). The N mineralization rate did not show a measurable decrease following the fire in this study site (Figure 4.10) however if the rate of NO_3^- leaching and the soil surface temperature were measured before and after the fire, increases in these two measurements may have been found following the fire. The initial increase in NO_3^- leaching would have slowed and eventually been prevented by the small pore space in the soil (Neary *et al.*, 1999).

Fires in grasslands frequently cause soil compaction, especially as the fire often removes the majority of the above-ground vegetation (Neary *et al.*, 1999; Snyman, 2003). No measure of soil compaction was made in this study following the fire

although the high clay content of the soil and the elevated ridges and depressions from previous tillage may have resulted in soil compaction which was observed during sampling.

5.6 Budget Outputs

5.6.1 Nitrogen and Sulfur Lost Through Biomass Burning

Pyrodenitrification in the Sandspruit system was calculated to be 39.4 kg N ha^{-1} yr^{-1} and was based on the assumption that 75.0% of the N in the above-ground biomass and litter is lost during a fire (Scholes *et al.*, 2003). When applying the same calculation to recorded above-ground biomass and litter from similar ecosystems in South Africa, it provided similar N losses of between 28.7 and 42.8 kg N ha^{-1} yr^{-1} (Scholes & Walker, 1993; Snyman, 2005). This output of N from the system acts to offset incoming N deposition although it is over six times larger than the average modelled N deposition of 6.2 kg N ha^{-1} yr^{-1} in the Sandspruit system (Figure 4.15). The loss of S through biomass burning in the Sandspruit system amounted to approximately 1.6 kg S ha^{-1} yr^{-1} and was based on the assumption that 50.0% of the S in the above-ground biomass and litter is lost during a fire (Scholes & Walker, 1993). When applying the same calculation to recorded above-ground biomass and litter from similar ecosystems in South Africa, it provided similar S losses of between 1.1 and 1.6 kg S ha^{-1} yr^{-1} (Scholes & Walker, 1993; Snyman, 2005; Bird, 2011).

5.6.2 Denitrification

The high proportion of clay in the soils of this study site (51.2%) and the evidence of standing water in the depressions of the site from December 2010 to February 2011 may have been responsible for a denitrification rate greater than that

estimated as a result of anaerobic microsites within the soil. The formation of anaerobic microsites generally only occur after precipitation events and begins when the water-filled pore-spaces are 60.0% filled (Robertson & Groffman, 2007). Rainfall on soil that is already moist or wet leads to a faster denitrification rate than when rain falls on dry soil (Groffman & Tiedje, 1988; Bergsma *et al.*, 2002). If the denitrification rate was to be measured in this study site, it would be expected to be highest during October to January annually, during months of highest rainfall (Figure 4.3) and in soil microsites where compaction had caused anaerobic soil conditions.

5.6.3 The Potential for Nitrate and Sulfate Leaching

When comparing the magnitude of all the N and S inputs and outputs in the Sandspruit system considered so far, the budgets generally suggest very little chance for NO_3^- leaching under current deposition rates (Figures 4.15 & 4.18). A number of studies have measured the rate of NO_3^- and SO_4^{2-} leaching from grassland and forested regions under a gradient of N and S deposition up to 120.0 kg N ha^{-1} yr^{-1} and 50.0 kg S ha^{-1} yr^{-1} respectively.

In a study carried out by Graffman *et al.* (2009) in four urban grasslands in Baltimore (US) from 2001 to 2005, NO_3^- leaching was found to be small compared to the total soil N pool. Nitrate leaching ranged from 0.5 kg N ha^{-1} yr^{-1} to 4.1 kg N ha^{-1} yr^{-1} and the grasslands with the greatest rate of N fertilization did not always show the highest NO_3^- leaching rate (Graffman *et al.*, 2009). The small amount of NO_3^- leached in those grasslands was thought to be a result of the high C cycling rates between the vegetation and soil that drove N immobilization and the high NO_3^- retention capacity of the soils (Hart *et al.*, 1994; Lovett *et al.*, 2002; Raciti *et al.*, 2008; Graffman *et al.*, 2009). This retention capacity depends on the

rate of N input into the system via deposition. An N deposition gradient study in Britain conducted by Pilkington *et al.* (2005) measured the amount of NO_3^- leaching occurring under N deposition rates ranging from 40.0 to 120.0 kg N ha^{-1} yr^{-1}. They found NO_3^- leaching only in systems that received > 96.0 kg N ha^{-1} yr^{-1} and all study sites were able to retain 95.0% of the added NO_3^- up to 80.0 kg N ha^{-1} yr^{-1} of deposition (Pilkington *et al.*, 2005). Dise & Wright (1995), Fenn *et al.* (1996) and Pilkington *et al.* (2005) all found a positive correlation between the amount of NO_3^- leaching in their study sites and the N deposition occurring when above the threshold of 10.0 kg N ha^{-1} yr^{-1}. Nitrate leaching is not currently expected to be of concern in this system as the N deposition was modelled at a maximum of 8.5 kg N ha^{-1} yr^{-1}, slightly below the threshold deposition level that has caused NO_3^- leaching in other grassland systems. An increase in N deposition of 1.5 kg N ha^{-1} yr^{-1} or more may begin to impact on the rate of NO_3^- leaching in the Sandspruit system.

In a study carried out by Eriksen *et al.* (2002) in three Danish soils between 1997 and 2000, the amount of SO_4^{2-} leached was recorded at 29.0 kg S ha^{-1} yr^{-1} and was found to be lower in the soils of grasslands compared to soils cultivated for crop agriculture. A similar SO_4^{2-} leaching rate was found in UK grassland soils studied over a three year time period (Riley *et al.*, 2002). They found SO_4^{2-} leaching up to 35.0 kg S ha^{-1} yr^{-1} in sandy loam soils receiving S deposition of between 6.7 and 7.8 kg S ha^{-1} yr^{-1} (Riley *et al.*, 2002). Sulfate leaching may possibly be of concern in the Sandspruit system as the maximum modelled S deposition was 35.0 kg S ha^{-1} yr^{-1}, over five times the minimum S deposition rate that resulted in SO_4^{2-} leaching in UK grassland soils.

5.7 Budget Estimations

The uncertainties of the N and S input-output budgets created for the Sandspruit system were greatly influenced by the values estimated for the study, the time period selected for data collection and the burning regime of the study site. The uncertainty created as a result of the use of modelled data contributing to the budgets is discussed as the accuracy, performance and limitations of the CALPUFF model in Blight *et al.* (2009).

The N in the below-ground biomass and the plant N uptake values were most likely underestimated in the average N budget. The estimated N in the below-ground biomass (3.3 kg N ha^{-1}) is small compared to the above-ground biomass measurement (42.6 kg N ha^{-1}) (Figure 4.15) and may be on a similar scale to the above-ground biomass measurement. Snyman (2005) found the root biomass in a good quality rangeland was 2 956.0 kg ha^{-1}, similar to the above-ground biomass in the study site. If the below-ground biomass is increased in the budget, the plant N uptake value increases with the same amount (Figure 4.15). The plant N uptake value of 45.9 kg N ha^{-1} yr^{-1} is similar to the literature value supplied by Scholes & Walker (1993) and a small increase in plant N uptake would be insignificant in the functionality of the budget. The gaseous losses of N in denitrification and pyrodenitrification in the average N budget were possibly underestimated (Figure 4.15). Standing water found in the tubes and the high clay content of the soil may have created anaerobic microsites that would have increased the denitrification rate in the system. The loss of N during biomass burning was estimated at 75.0% of the N in the above-ground biomass and litter, based on a savanna ecosystem where grasses account for 80.0% of the fuel of the fire (Scholes *et al.*, 2003). In this grassland, the grass fuel component would be closer to 100.0%, increasing the loss of N in pyrodenitrification by up to 20.0%. Unlike the average N budget, the below-ground biomass S and the plant S uptake were possibly overestimated. The

concentration (%) of S in the vegetation and litter was significantly higher at the beginning of the growing season (October 2010) compared to the end of the growing season (March 2011) (Figure 4.7) suggesting S was not actively removed from the leaves and stems before they began senescing and were dropped. The plant S uptake was calculated as the total S in the above- and below-ground biomass (Scholes & Walker, 1993). However, using this calculation provided a plant S uptake value eight times larger than the maximum plant S uptake in Scholes & Walker (1993). The gaseous loss of S during biomass burning in the average S budget may also be overestimated based on the possible overestimation of the amount of plant S uptake (Figure 4.18). If the amount of S taken up by plants is decreased in the budget, the amount of S lost through the burning of the above-ground biomass and litter would decrease by 50.0% of the decrease in plant S uptake.

The period of sampling in the study site and the seasonal variation in rainfall and temperature would have influenced some of the variables measured. All samples were collected from October 2010 to June 2011, incorporating one growing season. Cycling processes in the soil from June to September 2011 were estimated. The ammonification, nitrification and SO_4^{2-} mineralization rates from October 2010 to May 2011 were scaled up to create annual rates (Figure 4.10 & 4.12). It was assumed that rainfall and soil moisture would be at a minimum from June to September 2011 and that the mineralization and immobilization rates would not deviate outside the ranges measured from October 2010 to May 2011.

The final uncertainty in the N and S budgets involved the gaseous losses from the system during biomass burning. These were estimated as annual rates with the assumption that the study site burns annually. However, the study site is often burned every two years. If the majority of the Sandspruit system is also burnt every

two years, the N and S losses from biomass burning are halved in the budgets. The decrease in the amounts of N and S lost to the atmosphere do not have large impacts on the outcomes of the budgets. In the average N budget, the amount of N lost to the atmosphere through biomass burning is still far greater than the incoming N deposition. In the average S budget, the decrease in the loss of S to the atmosphere through biomass burning is small compared to the total S deposition and would not greatly decrease the soil SO_4^{2-} pool or the potential for SO_4^{2-} leaching.

5.8 Conclusion

Assuming current N and S deposition rates continue in the Sandspruit system, SO_4^{2-} leaching poses the largest threat. The relatively small soil NO_3^- pool and the indication that the system is N limited would result in the input of N from deposition acting as fertilizer for the grassland. The large soil SO_4^{2-} pool coupled with the large current modelled S deposition rate compared to the amount of S lost from the system indicate a potential for SO_4^{2-} leaching. The high proportion of clay in the soil however, may increase the buffering capacity of the soil against SO_4^{2-} leaching on a large scale throughout the year. Monitoring the rate of NO_3^- and SO_4^{2-} leaching throughout the year may give insight into to the potential for leaching, especially during the months of maximum precipitation from October to February annually.

5.8.1 Key Questions Answered

Key Question 1 - What are the rates of N and S mineralization and immobilization in the soils of the Sandspruit catchment over the 2010-2011 growing season?

The net N immobilization rate ranged between -0.03 µg N g^{-1} soil day^{-1} and -1.03 µg N g^{-1} soil day^{-1} and the net N mineralization rate ranged between

0.06 µg N g^{-1} soil day^{-1} and 1.05 µg N g^{-1} soil day^{-1}. The net N mineralization rate peaked in April 2011 and the net N immobilization rate was greatest in the following month of May 2011. The S immobilization rate ranged between -0.07 µg S g^{-1} soil day^{-1} and -1.06 µg S g^{-1} soil day^{-1} and the S mineralization rate ranged between 0.06 µg S g^{-1} soil day^{-1} and 0.91 µg S g^{-1} soil day^{-1}. The S mineralization rate peaked in November 2010 and the S immobilization rate was greatest in the following month of December 2010.

Key Question 2 - What are the effects of soil moisture on N and S mineralization rates in the soils of the Sandspruit catchment?

There was a weak positive correlation found between the net N mineralization rate and the soil moisture in soils of the study site. This finding suggests the net N mineralization rate increased with soil moisture, especially during the months of maximum precipitation (October 2010-January 2011). During the colder, winter months (May-September 2011), the soils would have been drier and the ambient temperature lower during which time soil moisture would have become the controlling factor that determined the net N mineralization rate. No correlation was found between the S mineralization rate and soil moisture.

Key Question 3 - What are the N and S input-output budgets for the selected site in the Sandspruit catchment?

The N input-output budget calculated for the Sandspruit system that included the average modelled N deposition indicated a large pyrodenitrification rate but a small potential for nitrate leaching. The combined loss of N from the soil during plant uptake and from the plants during burning was calculated to be far larger than the expected input of N from deposition. The S input-output budget calculated for the Sandspruit system that included the average modelled S deposition indicated the

loss of S through burning was far less than the incoming S deposition. The S mineralization rate was calculated to be twice as fast as the S immobilization rate forming a soil SO_4^{2-} pool of 59.1 kg SO_4^{2-} ha^{-1} yr^{-1} that provided a small potential for the leaching of SO_4^{2-} through the soil profile.

Key Question 4 - How does the input of atmospheric N and S pollutants affect the potential amounts of N and S available for leaching into nearby streams and rivers?

An increase in the input of N into the Sandspruit system as shown in the N input-output budget with the maximum modelled N deposition produced an increase in the soil NO_3^- pool. This increase however, remained far smaller than the combined loss of N through denitrification and pyrodenitrification, providing a small potential for NO_3^- to leach through the soil profile into nearby surface water. An increase in the input of S into the Sandspruit system as shown in the S input-output budget with the maximum modelled S deposition produced a soil SO_4^{2-} pool twice the size of the average S input-output budget. The input of S from deposition (35.0 kg S ha^{-1} yr^{-1}) was calculated to be over 14 times larger than the loss of S during biomass burning, contributing to a large soil SO_4^{2-} pool of 120.2 kg SO_4^{2-} ha^{-1} that provided a potential for some SO_4^{2-} leaching.

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