INVESTIGATING THE EFFECTS OF MOPANE WORMS (*Imbrasia belina*) ON NUTRIENTS, VENETIA-LIMPOPO NATURE RESERVE, SOUTH AFRICA

By

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DECLARATION

I, Donovan Barry de Swardt (209075753), hereby declare that the dissertation for MTech Nature Conservation is my own work and has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

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ABSTRACT

Ecologists have long been aware that large mammalian herbivores can alter ecosystem functioning in various ways, for example through changing where they defecate and urinate, which consequently affects nutrient cycling. The effects of herbivorous insects on ecosystems, however, have received limited attention until recently. Insects are capable of mass outbreaks, they can consume large volumes of vegetative material and can deposit large quantities of dung (frass), one example being the mopane worm, *Imbrasia belina*. This study looked at the effect of mopane worm frass on soil fertility in the mopane veld of the Venetia-Limpopo Nature Reserve and neighbouring Haakdoring farm. It found that mopane worms are capable of altering soil nutrient dynamics beneath the trees where they are browsing in three ways. Firstly, they increase the potential amount of nutrients deposited when compared with that deposited through conventional leaf litter. Secondly, they alter the rate of nutrient recycling by depositing nutrients in frass which decomposes more rapidly than conventional leaf litter. Lastly, they increase the amounts of potassium and phosphorus in the soil. While there is no conclusive data from this study that the mopane veld would become extinct if mopane worms were harvested to extinction, it has shown that there is relatively little evidence on how herbivorous insects could affect ecosystem functioning in the landscapes in which they occur. Further research is recommended that investigates how mopane worms influence soil fertility in more detail and over multiple geographical locations.

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CHAPTER 1: INTRODUCTION AND LITERATURE

1.1. INTRODUCTION

Herbivores play a vital role in nutrient cycling, and studies indicate that insect herbivores may play as important a role as their larger mammalian counterparts (Hunter, 2001, Picker, 2012, Chew, 1974). Certain insect species frequently reach outbreak proportions; some population outbreaks erupt into densities far in excess of normal population densities while others emerge *en masse* from dormancy (Berryman, 1987). Lepidoptera that reach outbreak proportions in the United States of America (USA) have received particular attention due to their impacts on pine (*Pinus* spp.) forests, oak (*Quercus* spp.) forests and savannas (Ritchie et al., 1998). Some of these Lepidoptera are introduced species (e.g., *Lymantria dispar*) whilst others are indigenous (e.g., *Malacosoma disstrium*) (Christenson et al., 2002). Defoliation by insects during an outbreak results in inputs of nutrients in the form of large volumes of frass and abscised leaf material (Frost and Hunter, 2004). The impact of outbreaks on nutrient cycling has been the subject of many studies, which are reviewed in the following section, as changes to the nutrient cycle can have profound consequences for ecosystem organisation and functioning.

A number of African Lepidoptera species display outbreak dynamics, the most well-known being the mopane worm (*Imbrasia belina*, Westwood 1849). Mopane worms are considered a valuable natural resource in rural communities where they are harvested as food (Ditlhogo, 1996). Traditionally the worms were harvested for use in local homesteads but rapid African urbanisation has created a contemporary market for dried mopane worms (Illgner and Nel, 2000). The South African market was worth £57m in 1994 and has been increasing steadily since then (Stack et al., 2003). This market has had an adverse effect on mopane worm populations due to increased harvesting and unsustainable harvesting practices (Akpalu et al., 2009). Recently there have been reports of mopane worm population declines and a number of areas which used to have regular outbreaks have not experienced one for several years (Gondo et al., 2010). Although the loss of mopane worms can potentially be a substantial perturbation to nutrient cycling and ecosystem functioning, this dynamic has not previously been investigated.

The following section is a literature review concerning mopane worms and herbivore-induced changes to nutrient cycling that forms the basis of this study.

1.2. BACKGROUND TO MOPANE WORMS

The mopane 'worm' is the larval stage of the anomalous Emperor moth (*Imbrasia belina*) within the order Lepidoptera. Mopane worms can be classed as an outbreak species due to their occurring in very large numbers over short periods of time (Ditlhogo, 1996). Outbreaks of mopane worms are usually restricted to mopane veld, which is largely dominated by near mono-specific stands of the host mopane tree (*Colosphospermum mopane*) (Ditlhogo, 1996). The adult moth normally appears in late November to early December, which usually coincides with the onset of the summer rains. Female moths (Plate 1.2.1) locate suitable host plants, generally mopane trees, but other tree species are also used (Hrabar et al., 2009), and then release a pheromone to attract males (Plate 1.2.1). After mating, the females can lay between 30-300 eggs on the leaves, branches or trunk of the trees. Eggs hatch in approximately 10 days (Plate 1.2.2), and the larvae then undergo five instar stages of development. Once the larvae have developed fully (Plate 1.2.3), they will burrow underground to pupate. Depending on weather, newly developed moths may emerge for a second time around late March or early April and follow the same developmental process. The second generation will go through a stage of diapause during winter to emerge as the first generation at the start of the following summer (Gaston et al., 1997).

The five instar stages of the worm take five to six weeks during which the worms can gain 4000 times their original mass (Akpalu et al., 2009). Styles (1994) found that a worm will consume an average of 41 g dry mass leaf material over its larval stage. An outbreak, which can number in the millions, is capable of completely defoliating large tracts of mopane trees. Styles (1994) calculated that during an outbreak of mopane worms (n = 19 million) on a 4000 ha property, worms were capable of consuming nine and a half times (779 t) the amount of mopane tree leaf material in six weeks than 14 elephant bulls could in 12 months (83 t). He also calculated that the same worms were capable of producing 665 tons dry mass of dung in six weeks, three and half times more than the same elephants would

produce in a 12 month period (178 t). The calculations were based on existing available data rather than a field experiment but nonetheless demonstrate the influence mopane worms can have on ecosystem functioning.

Plate 1.2.1: Adult form of *Imbrasia belina*, female pictured here above male moth (Source: Google Images).

Plate 1.2.2: Recently hatched mopane worms. First instar worms are less than 1 cm long (Source: Google Images)

Worms are harvested in the fifth instar stage (Plate 1.2.3). The gut content is expelled and the worm is boiled in a salt water solution. After being boiled the worms are either sun- dried or roasted over coals and in this state can be stored for a number of months (Akpalu et al., 2009). The appearance of the first emergence in the beginning of summer normally coincides with a time when subsistence farmers have depleted their winter stocks of dried mopane worms and have not yet harvested their summer crops, making the worm an ideal alternative food (Ditlhogo, 1996).

Plate 1.2.3: Mopane worm in its fifth instar stage shortly before pupation (Source: J. Donahue)

1.3. THE MOPANE VELD

Colophospermum mopane is a legume, but does not form nodules or fix nitrogen (N) (Burbano et al., 2015). It is a broad-leafed deciduous, small to large sized tree that often forms monospecific stands termed mopane veld (Palgrave et al., 2002, Rutherford et al., 2006). Mopane trees can tolerate a wide variety of soil conditions but most often occur on alluvial or alkaline, poorly drained soils which it apparently tolerates better than other species (Palgrave et al., 2002). Across much of its distribution, stands of mopane trees are either uniformly stunted and shrubby, uniformly dominated by medium sized trees, or uniformly dominated by tall trees attaining a height of up to 25 m (termed cathedral mopane) (Sebego, 1999). These differences in growth form can often be abrupt along a landscape gradient. Mopane occurs at elevations between 300-1000 m in areas of low to moderate summer rainfall (400 mm to 1000 mm per annum). Mopane trees are able to tolerate water stress making them tolerant of drought conditions and long dry winters (Van Voorthuizen, 1976).

The mopane veld covers extensive areas from southern Angola and northern Namibia, through Botswana, Zimbabwe and southern Zambia to Malawi, Mozambique and the northern parts of South Africa (Smit, 2014), resulting in one of the largest savanna types in Africa (Du Plessis, 2001). The total area covered by mopane veld in southern Africa is approximately 555 000 km^2 (Rutherford et al., 2006). Much of the area covered by mopane is utilized for conservation, cattle or game farming. Intense grazing pressure may lead to an increase of mopane tree density, with the consequence that the trees suppress the herbaceous layer (Smit, 2014).

Mopane veld is a valuable source of natural resources for rural communities. Mopane trees are heavily utilized for making tools, fences, fuelwood, and poles for building and their leaves provide fodder for domestic animals. Heavy utilization of mopane resources has led to an increased awareness of the need for correct management of this resource (Flower et al., 1996).

1.4. NUTRIENT CYCLING

'Nutrients' is the term used to describe the 22 elements essential for growth in all organisms. These elements can exist as gas, liquid or solid in either an organic form or inorganic form (Lavelle et al., 2005). Nutrients are mostly utilized by plants in their ionic inorganic form where they are then bound into various carbon-based compounds. Animals obtain most of their nutrients in the organic form by consuming either plants or other animals (Lavelle et al., 2005). Micro-organisms are capable of utilizing nutrients in either inorganic or organic form and are capable of converting nutrients into forms to be utilized by plants.

Nutrient cycling describes how nutrients move through systems. The movement of nutrients is typically described as going from the physical environment to living organisms and then back to the physical environment again (Chapin et al., 2011). The cycle is complex and can be influenced by various factors including parent material, soil texture, vegetation, climate, herbivory, and micro-organisms (Brady and Weil, 2008). Of particular interest to this study is the mechanism of herbivory which is explored in detail below. One factor that will be discussed in less detail but is also relevant to the study is that of soil texture.

Soil texture can have a profound effect on how soils react to disturbance or affect vegetation in an ecosystem. For example, soil texture often dictates the overlaying plant species composition and vegetation type (Högberg, 1986). Soil texture describes the particle size of the soil and the manner in which particles aggregate. Soil particles can be broadly classified into four major groups, namely silt, clay, sand or gravel. Soil texture describes the relative representation of these components. When investigating nutrient dynamics, an important first step is to determine soil texture (Brady and Weil, 2008).

Sandy soils are made up of aggregates greater than 0.05 mm and smaller than 2 mm and particles are generally visible to the naked eye. Coarse sands often consist of several minerals, derived from the parent rock, but they generally contain primarily silicates, usually quartz (Brady and Weil, 2008). The relatively large particle sizes of sandy soils dictates that these soils have relatively large spaces between particles and thus are easily drained. Due to the large particle sizes, plants on sandy soils may be more prone to drought and these soils are considered nutrient poor as nutrients are rapidly leached from the soil profile. Sandy soils are also associated with poor cation exchange capacity (CEC) and can thus greatly affect the way in which micro-organisms and plants obtain nutrients from the soil profile (Brady and Weil, 2008). The poor CEC of sandy soils is a result of the relatively small surface area an individual sand particle has compared to clay or carbon-rich organic material. The small surface area has fewer negative charges with which cations can bind. In areas of sandy soils the organic layer beneath trees plays an important role in how plants obtain nutrients. Campbell et al. (1994) conducted a study of two different soil types, namely a sandy dystrophic soil and a eutrophic silt soil in a semi-arid savanna in Matopos, Zimbabwe, to assess the nutrient dynamics beneath trees and in adjacent open areas. They found that beneath trees on sandy soils the organic inputs from the trees in the form of leaf litter greatly increased the exchange capacity. The organic input beneath trees on silt-rich soils had no significant effect, presumably as silt soils have a greater CEC to start with and thus organic material has a less influential role on these soils.

Whilst it is important to consider the various effects other mechanisms may have on nutrient dynamics, the scope of this study is largely interested in the effect of

herbivory. The various actions of herbivores can strongly influence the decomposer component of soil ecosystems, which in turn affect the soil mineralisation processes and nutrient content of soil profiles, which in turn affects plant productivity and composition (Bardgett and Wardle, 2003). The decomposer component is made up of various forms of bacterial and fungal micro-organisms that are responsible for decomposing organic matter which results in nutrients becoming available to plants (Chapin et al., 2011).

Much research has investigated the effect of large vertebrate herbivores on soil fertility over the last five decades, many of it in African savannas (McNaughton et al., 1988, Augustine and Frank, 2001, Sankaran and Augustine, 2004). Mammalian herbivores can have major impacts on plant species composition and nutrient cycling, and are considered important in ecosystem functioning (Augustine and Frank, 2001, Augustine and McNaughton, 1998, Augustine and McNaughton, 2006, McNaughton, 1985, McNaughton et al., 1988, Olff and Ritchie, 1998, Milchunas and Lauenroth, 1993, Chew, 1974, Belsky, 1987). Compared with mammalian herbivores, the effect of invertebrate herbivores on soil fertility has received less attention. Hunter (2001) shows that insects may play as large a role in nutrient turnover and assimilation as larger mammalian herbivores. Much of the research on insects has been limited to irruptive species or species that occur in high densities.

The increases and decreases in nutrient cycling rates caused by herbivores in African savannas have been categorised by McNaughton et al. (1988) as ‘fast’ and ‘slow’. Fast cycles occur in systems with high herbivory where nutrient-rich patches within the ecosystem attract herbivores, which in turn, maintain high nutrient loads through dung and urine deposition (McNaughton et al., 1988). These systems tend to be dominated by plant communities that tolerate high levels of herbivory. In turn, plants have little chance to build up senescent material which favours rapid recycling of nutrients. Slow cycling tends to occur within plant communities that are unpalatable to herbivores. Plants with high levels of ligneous material decompose slowly, which in turn, favour plant communities of species low in nutrients and unpalatable to herbivores. The lack of herbivores means a slower input of easily decomposed dung and thus low nutrient levels. McNaughton et al. (1988) state that any grazing system within the Serengeti, regardless of its current nutrient cycling

status, would eventually favour a plant community indicative of the slow cycle if herbivores were excluded. Elsewhere, however, the presence of herbivores can result in a slow cycle, as sometimes herbivores can remove biomass beneficial to below ground organisms (Harrison and Bardgett, 2004, Ritchie et al., 1998).

A number of reviews (Hunter, 2001, Bardgett and Wardle, 2003) have been written on the role herbivores play in nutrient cycling and this literature review will expand on some of the mechanisms that have been identified. The conclusion will focus on the key mechanisms that may impact the role mopane worms have on nutrient dynamics.

1.4.1. Herbivore induced changes to the quantity of resources

Herbivory, through consumption, can have a number of impacts on soil fertility. First, consumption removes plant matter that would have eventually become leaf litter and leaf litter is an important source of carbon (C) for soil microbes (Bardgett et al., 1998, Bakker et al., 2004) Secondly, consumption of plant leaf material has been shown to affect root density and root exudates (Holland et al., 1996).

Sankaran and Augustine (2004) conducted exclusion experiments in a Kenyan grassland and after two years they noticed a significant increase in microbial biomass within sites that excluded mammalian herbivores. They associated this increase to herbivores depressing the amount of plant C being returned to the soil, indicating that C is a limiting nutrient for microbial action. While decreased inputs of C are common to areas where herbivores are present.

Some studies show that intense herbivory can maintain areas of increased N (Frank et al., 2000, Frank and Groffman, 1998). Grazing lawns are areas of short, highly palatable N-rich stoloniferous grasses that may occur in grasslands dominated by tall bunch grass species (McNaughton, 1984). These lawns are maintained in a state of young growth by intense grazing from large and medium-sized herbivores. Coetsee et al. (2011) found that foliar N was higher on grazing lawns in an African savanna compared to surrounding tall grass areas but soil N was not always higher, suggesting that grazers affect N transformation rates other than mineralisation. Holland and Detling (1990) compared areas of Prairie Dog (*Cynomys ludovicianus*)

colonies to adjacent uncolonised grasslands, in South Dakota. They found that N immobilisation was highest in colonised areas and root biomass was lowest. Decomposing roots are an important source of C for microbial decomposers and a decrease in microbial activity is expected in these heavily grazed areas. Holland and Detling (1990) suggested that decreased root biomass in grazed areas, and the consequent decrease in microbial activity, may explain the increased N mineralisation as plants would not be in direct competition with microbes for available N.

Conversely, studies also indicate that C allocated to root exudation may increase microbial activity, and this allocation may be directly affected by herbivory (Bokhari, 1977, Denton et al., 1998). Holland et al. (1996) conducted a study on maize plants and grasshoppers (*Romalea guttata*), and found that there was a significant positive relationship between browsed plants and increased C allocation to roots as well as increased root exudation. They concluded that this led to an increased C source for microbial activity. An increase in microbial activity can be beneficial to plant growth and thus animals, provided there is sufficient N in the soil profile.

Bakker et al. (2004) showed that herbivore size is important as it affects nutrient cycling. They conducted an exclusion experiment within a wetland in the Netherlands. The wetland was subjected to grazing by three mammals; cattle, rabbits and voles. Where cattle were excluded, the vole population increased, essentially returning N mineralisation rates to pre-exclusion grazing intensity if corrected to metabolic weight. They also found that N distribution by voles was at a fine scale and widespread as opposed to cattle where the distribution was coarser. They found that N mineralisation was greatest in sites where cattle were excluded; here more litter accumulated due to less grazing pressure.

1.4.2. Herbivore-induced changes to resource quality

The nutrient quality of the resource being recycled back to the soil can have significant impacts on soil fertility and soil decomposers (Hunter, 2001, Bardgett and Wardle, 2003, Wardle et al., 2004). Resources rich in labile N and C will speed up the nutrient cycle while senescent ligneous material will slow down the cycle (McNaughton et al., 1988). Herbivores are also capable of influencing secondary

compounds within plants, and in turn, affect the quality of the resource being returned to the soil profile in leaf litter (Findlay et al., 1996).

Grazing lawns may have increased total N and N mineralisation, due to a number of factors. One important factor seems to be an increase in quantities of dung and urine (Ruess and McNaughton, 1987, Frank and Evans, 1997, Ruess and Seagle, 1994). Herbivore waste is rich in nutrients that are easily assimilated by plants and micro-organisms, more so than from leaf litter (Frost and Hunter, 2004, Frost, 2005). Such waste is often cited as the major mechanism in herbivore-induced increases in N availability (Hunter, 2001, Bardgett and Wardle, 2003). Large mammalian herbivores can deposit large quantities of dung and urine, rich in partially digested and non-assimilated nutrients. These deposits can stimulate microbial activity which in turn can have positive effects on plant nutrient uptake and growth. This beneficial effect at a landscape level has been questioned because dung and urine deposition covers a very small percentage of the total area (Augustine and Frank, 2001). This area limitation may be compounded when the volatilization of ammonia from urine is taken into account, although volatilization may be mitigated by gains from rainfall events, as rainfall can reintroduce small amounts of N (Ruess and McNaughton, 1988). Herbivores indirectly affect system processes in ways that lead to N conservation. Ruess and McNaughton (1988) monitored N volatilization in the Serengeti by applying urea to study sites to simulate defecation and urination by the dominant grazer, wildebeest (*Connochaetes taurinus*). Areas of lowest N loss coincided with areas of short grass that attracted large migratory herds during the rainy season. Intensive grazing maintained grasses in an active nutrient-rich growth stage, suggesting that deposits of dung and urine can be rapidly taken up by microbes and plants.

The quality of leaf litter can play an important role in nutrient cycling dynamics, and herbivores are capable of influencing the quality of litter. Chapman et al. (2003) compared the nutrients from litter beneath pinyon pines (*Pinus edulis*) susceptible to attacks from the scale insect *Matsucoccus acalyptus*, and a moth, *Dioryctria albovittella* and those that were resistant. *Matsucoccus acalyptus* and *D. albovittella* usually attack pines less than 50 years old and more than 50 years old respectively. They found that herbivory by both species increased leaf litter N (thereby reducing

the N:lignin ratio), and *M. acalyptus* herbivory increased P. Tree litter decomposition rates were significantly faster in susceptible than in resistant trees, and the litter from resistant trees decomposed faster beneath susceptible trees than beneath resistant trees. These findings were attributed to the inability of susceptible trees to reabsorb nutrients because insects induced premature abscission. Prematurely abscised leaf material had higher nutrients than leaves that were senescent before they abscised.

Conversely in some instances, trees affected by herbivorous attack may produce litter that takes longer to break down than unaffected trees. Plants are known to respond to attack by increasing the production of secondary compounds as a defence against herbivory (Freeland and Janzen, 1974). Secondary metabolites in leaf litter can also inhibit microbial activity which slows down nutrient cycling. Findlay et al. (1996) showed that cottonwood (*Populus deltoides*) subjected to two damage treatments, mechanical and unplanned spider mite, produced litter that decomposed more slowly than litter from undamaged trees. This was because the damaged trees produced phenolic compounds, which decompose more slowly and immobilise available N.

1.4.3. Effects of herbivory on net primary production

The effect of herbivory on soil nutrient measures and net primary production (NPP) has been well studied (Augustine et al., 2003, Bagchi and Ritchie, 2010). Increased palatability is likely to attract herbivores, thus reinforcing the fast cycle, while decreases in N are less attractive to herbivores and thus reinforce the slow cycle (McNaughton et al., 1988). The effect of herbivore-induced increases in NPP is called grazing optimisation (McNaughton, 1979). de Mazancourt et al. (1998) discuss how herbivory may stimulate increases in NPP. They define two possible pathways for nutrient cycling, the plant and herbivore pathways. In the herbivore pathway nutrients are channelled through herbivore consumption and deposition of dung and urine. The plant pathway follows nutrients within plant material not consumed by herbivores. de Mazancourt et al. (1998) calculated the fraction of nutrients lost by dividing the amount of nutrients lost through either pathway by the total amount of nutrients that passed through both pathways. If grazing optimization takes place, the proportion of nutrients lost through herbivory should be smaller than

the proportion lost through the plant pathway; there should also be a large enough input of nutrients in animal wastes for plants to effectively use. They further state that grazing optimization will likely occur in systems where limiting nutrients suffer larger losses during plant litter recycling. In a follow-up study, de Mazancourt et al. (1999) used field collected data to model N budgets in an Ivory Coast humid savanna, where N is considered a limiting nutrient. At the time of the study there were few herbivores, but populations of Kob (*Kobus kob*) and Buffalo (*Syncerus caffer*) were increasing, and the majority of plant biomass was recycled by annual fires. Fires are a major cause of N loss in many grassland systems. Their modelling showed that, within a few decades, the increase in herbivore populations would mitigate the fire-induced losses of N and hence grazing optimization was likely to occur.

Conversely, Ritchie et al. (1998) showed that herbivory can also suppress N and NPP. Small and medium sized herbivores often selectively feed on plants rich in nutrients, particularly N. Intense selective feeding can potentially lead to a situation where nutrient rich plant species are suppressed and are outcompeted by nutrient poor species. Ritchie et al. (1998) excluded insect and mammalian herbivores from sites within a Minnesota Oak savanna for seven years. One of the effects of the treatment was that NPP significantly increased in sites where herbivores were excluded. They concluded this was due to herbivores suppressing leguminous species which usually provided N rich leaf litter. In this study the limiting nutrient, N, suffered greater losses through the 'herbivore pathway'.

Belovsky and Slade (2000) showed that grazing by grasshoppers (*Melanoplus sanguinipes*) in the Palouse prairie (north-western United States) increased plant production. They noticed that N availability increased as grasshopper populations increased. Grasshopper-induced increases in N allowed the grass *Poa pratensis* to dominate over *Elymus smithii*. Furthermore grasshoppers seemed to selectively graze *E. smithii* over *P. pratensis*. *Poa pratensis* decomposed faster than *E. smithii* and had a higher N content that further encouraged greater NPP.

Within a Kenyan rangeland, Augustine and McNaughton (2006) showed that the same suite of herbivores can have both a positive and negative effect on NPP

depending on soil nutrient status. The rangeland where their study took place was dominated by bushlands, clump grasses and woody vegetation with poor soil nutrients, interspersed with grassy glades with high soil nutrients and palatable grasses. Glades were a product of enclosing cattle in bomas overnight, a historic farming method. This farming practice introduced large quantities of nutrient rich dung and urine in a concentrated area, and its effect was sustained despite the method having not been used in over 40 years. Herbivory was shown to reduce NPP in bushland sites regardless of rainfall. During high rainfall years NPP was increased by herbivory in glades and suppressed in low rainfall years. This demonstrates that soil nutrients can be affected by a range of factors apart from herbivory.

Clark et al. (2005) showed that small mammals are capable of depositing similar quantities of N as their larger counterparts. They calculated the volume of N distributed by Hispid cotton rats (*Sigmodon hispidus*) in an old field ecosystem. Their calculations showed that N deposition by cotton rats was comparable to larger herbivores such as cattle. Their results suggest that monitoring N fluxes within ecosystems needs to consider more than just large herbivores, and small mammals need to be accounted for in systems where herbivores increase N at landscape levels.

Insects and insect outbreaks are capable of depositing large volumes of frass, a phenomenon which has received much attention. Hollinger (1986) studied the cycling of N and phosphorus (P) in two Californian oak species, *Quercus agrifolia* and *Quercus lobata,* that had been defoliated by the indigenous California oak moth (*Phryganidia californica*). He observed that the amount of N and P flowing from the canopy to the ground more than doubled during outbreaks. The amount of N and P moving through frass and insect remains was approximately 70% in *Q. agrifolia*, and around 60% of N and 40% of P in *Q. lobate*, the rest moved through plant material. Hollinger (1986) also noted that N was released more quickly from frass compared to the leaf litter of either *Quercus* species. Considering the frequency of oak moth outbreaks (peaking every 5 years), herbivory should have significant effects on nutrient cycling beneath trees in this system.

Frost and Hunter (2004) conducted a laboratory experiment to consider the impact of caterpillar frass on individual red oak trees (*Quercus rubra*). Trees received six treatments; mechanical damage, herbivore damage (eastern tent caterpillar, *Malacosoma americanum*) and undamaged with half receiving frass and half not. Their experiment revealed that frass deposition increased soil total C, total N and soil NH_4. Herbivory increased soil respiration and decreased soil total N when compared to undamaged controls, regardless of frass deposition. They hypothesised that herbivory may increase root exudates which mobilize microbial activity, as indicated by an increase in soil respiration. In conclusion, they mention that frass may enrich soil N and C pools but its ultimate fate may be dictated by other abiotic factors

1.5. LITERATURE SUMMARY

Herbivory has a variable influence on soil nutrient cycling, causing functional differences both within and between ecosystems. Two key themes of this literature review are soil nutrient status and plants' resilience to herbivory. Herbivory has a positive association with high soil nutrient areas but a more negative association with low nutrient areas. Areas of high soil fertility can be considered as a determinant for herbivore diversity, and are often dominated by nutrient rich plants. Plants with a tolerance to herbivory appear to benefit from being kept in a state of N-rich growth, with constant herbivory increasing NPP. Thus, the key mechanism is: nutrients are returned to the soil in a labile form, which has positive effects on micro-organisms and, ultimately, plant growth.

Areas of poor soil fertility are often dominated by plants low in nutrients with slow growth rates. In these systems, selective feeding on nutrient rich plants leads to a community dominated by plants with low nutrients and high levels of secondary compounds. The result is low nutrient inputs to soil micro-organisms which in turn, indirectly, decrease plant growth. Any positive feedbacks such as increased nutrients in dung return is usually localised, except in outbreak situations where turnover rates are faster and in greater volumes, often at times of the year where such inputs are scarce. These pulses of nutrient input may have positive implications for plant growth, especially on nutrient poor soils.

1.6. RATIONALE FOR THIS STUDY

Unlike American Lepidoptera, the majority of mopane worm research focuses on its use as a natural resource, rather than its ecological role. The ongoing depletion of mopane worms raises questions about its importance in supporting ecosystem function in areas where it historically occurred in large numbers but may disappear in future. The poorly understood relationship between mopane worms and their host plant leaves a gaping hole in the understanding of how mopane veld functions. It is well understood that impacts on large herbivores can influence biogeochemical processes of ecosystems. It stands to reason that there could be similar impacts should the ecological functioning of mopane worms be disrupted.

It is hoped that exploring the effect of mopane worm frass on nutrient dynamics of mopane veld will help to further the understanding of the relationship between the two and thus positively influence future management policies concerning protecting mopane worms as a valuable natural resource.

1.7. OBJECTIVES

The main aim of this dissertation was to explore how mopane worms affect soil fertility. This aim is divided into four objectives: a) establish whether the potential nutrient input of mopane worm frass is greater than that of mopane tree leaf litter; b) investigate the difference in rate of decomposition between mopane worm frass and mopane tree leaf litter; c) evaluate nutrient differences between soil beneath trees compared to open areas and; d) assess the difference in soil nutrients beneath trees with and without mopane worms.

1.8. LAY-OUT OF DISSERTATION

Chapter 1 reviewed the key concepts and literature on which this study is based. It provides a synopsis of mopane worm biology, a summary of the factors that influence mopane veld, and discusses the relevant literature concerning the role herbivores play in nutrient cycling. A justification for this research is provided and the research objectives are outlined. The chapter includes both an overview of and a rationale for the research.

Chapter 2 describes the location, topography, geology, soils, vegetation and climate of the study area.

Chapter 3 describes the methods used for field sampling, sample analysis and statistical analysis in this study.

Chapter 4 presents the analyses and results.

Chapter 5 discusses the results in context of the literature synthesis and gives a conclusion.

1.9. CHAPTER SUMMARY

This chapter has provided an overview of what is known about the influence of mopane worms on soil fertility and synthesised the available literature on nutrient cycling relevant to the study. The overview provided a description of the mopane worm life cycle and the habitat in which they occur. The literature synthesis described nutrient cycling, the impact of herbivory on it and how herbivores affect the quality and quantity of vegetation. This study adds to the limited research concerning how insects affect nutrient cycling. The chapter concluded by summarizing the content of the following chapters.

CHAPTER 2: STUDY AREA

2.1. INTRODUCTION

The study was restricted to approximately 27 hectares in the north of the Venetia-Limpopo Nature Reserve (hereinafter referred to as Venetia) and on the neighbouring farm of Haakdoring (Figure 2.1.1). Generally, the study area is described in context of these two areas which are characteristically similar (see sub sections below for details) unless otherwise stated.

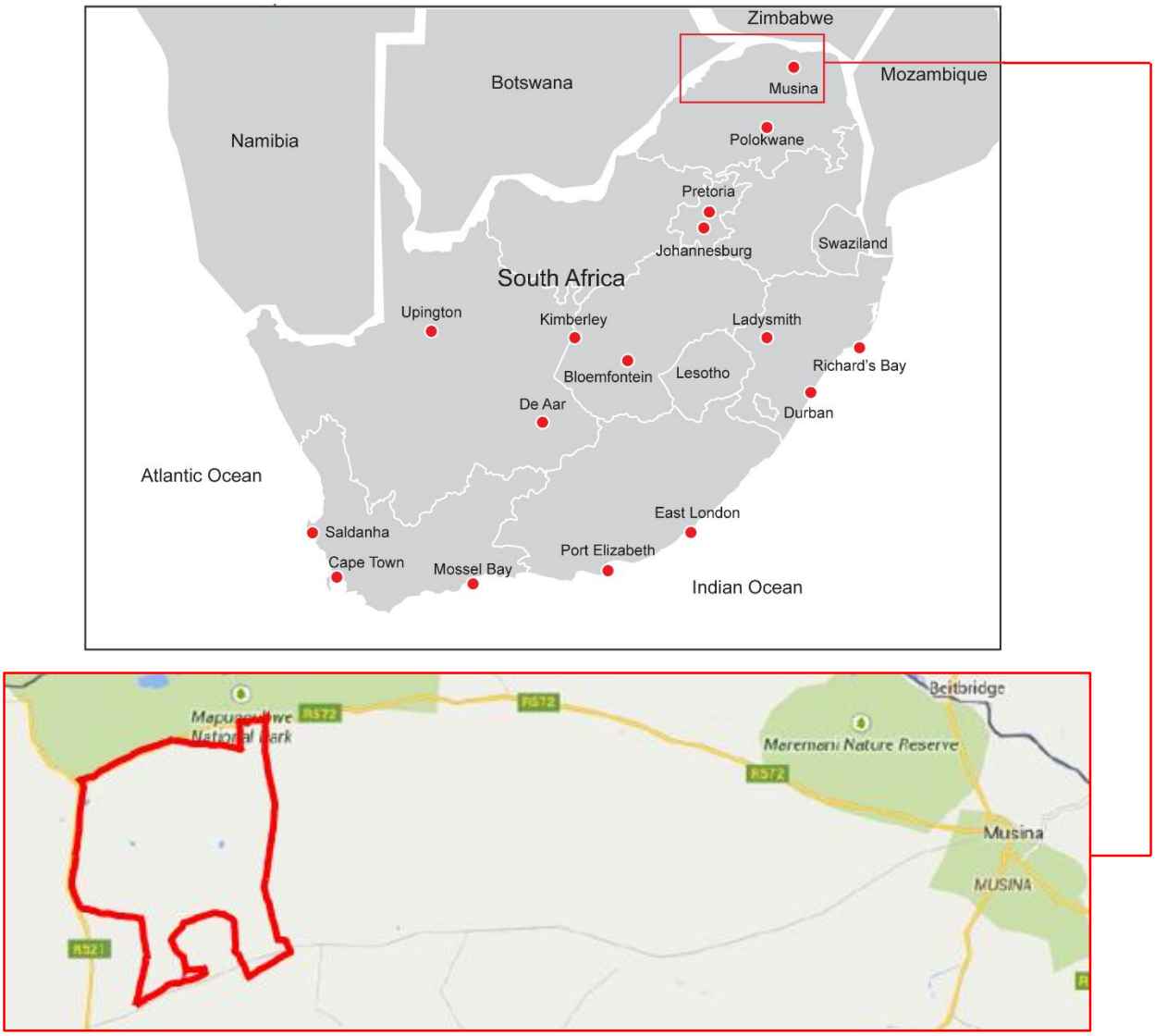


Figure 2.1.1: Location of study area (Venetia-Limpopo outlined in red) within South Africa (adapted from Google Maps and Google Images).

2.2. LOCATION

Venetia is a 31 855 ha privately owned conservation area located in the north of the Limpopo Province of South Africa. It is adjacent to the southern border of the Mapungubwe National Park and the northern boundary fence is about 10km from the borders of Botswana and Zimbabwe (Figure 2.1.1). Haakdoring is a 1500 ha property bordering Venetia along the north eastern fence line and shares geological and vegetative characteristics of Venetia (see sub sections below). The gazetted names of the study area for the areas sampled are Hackthorne 30 MS and Athens 31 MS (known as Haakdoring) while the northern section of Venetia is Hartebeesfontein 35 MS (Figure 3.1.1 p. 22).

2.3. TOPOGRAPHY

The study area is generally flat interspersed with rocky sandstone outcrops (Rutherford et al., 2006). The study area sits at an altitude of approximately 600 metres above sea level. Drainage in the study area is partly endorheic, i.e. with internal drainage ending up in pans but otherwise feeding local tributaries of the Limpopo River. The study area is located on a peneplained plateau that has not yet been removed by cutback of the Limpopo system (O'Connor, 1991).

2.4. GEOLOGY AND SOILS

Generally, the study area has deep red sandy soils of the Hutton soil type (Botha, 1994). The soils for Haakdoring are classified as a Hutton Stella 3100, whose characteristics include a red sandy soil up to 1200 mm in depth with clay content between 6-10 % in the upper horizon. The soils for Venetia are classified as a Hutton Venterdorp 3200, whose characteristics include a red loamy soil, 500-700 mm in depth, with clay content between 8-12 % in the upper horizon. Sands are derived from the Clarens sandstone geology (Botha, 1994). The soils at the study area were more than 85 % sand, with a low CEC and high base saturation (O'Connor, 2014).

2.5. CLIMATE AND WEATHER

Venetia is a semi-arid area and receives summer rainfall (November - March) with dry winters. Mean annual precipitation is 300-400 mm/annum (Hrabar et al., 2009),

though lengthy spells of below average rainfall are not uncommon. Summer rains are frequently experienced as short, intense thunder storms. The area experiences hot summers and mild winters. Mean monthly maximum and minimum temperatures for Musina (80km east of the study area) are 32°C and 20.3°C (December, summer), and 24.7°C and 7.2°C (July, winter) (Smit and Rethman, 1998).

2.6. VEGETATION

The savanna biome is the largest biome in South Africa encompassing 33% of the country, a large proportion of which is mopane veld along South Africa's north eastern boundary (Rutherford et al., 2006). A list of common tree and grass species of the savanna biome that occur on Venetia is given in Table 2.6.1.

Plate 2.6.1: The mopane veld of Venetia-Limpopo Nature Reserve in April 2014, note the defoliated trees (Source: D.B. de Swardt).

The vegetation of the study area is classified by Rutherford et al. (2006) as Musina Mopane Bushveld for which *Colophospermum mopane-Grewia flava* Woodland on Hutton Stella 3100 soils was distinguished from *Combretum apiculatum-Colophospermum mopane* Open Woodland on Hutton Venterdorp 3200 soils (O'Connor, 1991, O'Connor, 1998). The two vegetation types were distinguished by proportional representation of the tree species listed in Table 2.6.1 in the *C. apiculatum-C. mopane* open woodland, as per the Braun-Blanquet cover abundance classification (Braun-Blanquet, 1932, Braun-Blanquet, 1964). The

herbaceous sward of both was dominated by *Schmidtia pappophoroides* and *Stipagrostis uniplumis*.

Table 2.6.1: Some of the more common tree and grass species found in the study area (adapted from O'Connor (1998)).

Botanical Name	Common Name
Perennial grasses	
Bothriochloa radicans	Stinking grass
Digitaria eriantha	Common finger grass
Panicum maximum	Guinea grass
Schmidtia pappophoroides	Blougras
Stipagrostis uniplumis	Bushman grass
Annual/micro-perennial grasses	
Aristida congesta	Cats-tail three-awned grass
Aristida adscensionis	Six-weeks three-awned grass
Enneapogon cenchroides	Common nine-awned grass
Oropetium capense	Haasgras
Common Trees	
Boscia albitrunca	Shepherds tree
Colophospermum mopane	Mopane tree
Combretum apiculatum	Red bush-willow
Commiphora glandulosa	Tall common corkwood
Commiphora mollis	Velvet-leaved corkwood
Lannea schweinfurthii	False marula
Terminalia prunioides	Lowveld cluster-leaf
Adansonia digitata	Baobab

2.7. FAUNA

Before Venetia's inception in 1991, the area consisted of a number of livestock farms (Hrabar et al., 2009). All livestock were removed after 1991 and the reserve was stocked with relatively low densities of indigenous wildlife including giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*), bushbuck (*Tragelaphus*

scriptus) and elephant (*Loxodonta africana*) (O'Connor, 2015). The elephant population reached an estimated 105 individuals in 2013 (O'Connor and Page, 2014). Fire has been excluded from the reserve since 1950 (MacGregor and O'Connor, 2002).

2.8. CHAPTER SUMMARY

The study area location, the north eastern section of Venetia-Limpopo Nature Reserve and the neighbouring farm of Haakdoring, was introduced. A brief outline of the topography, geology and soils, climate and weather, vegetation and fauna was provided. The climatic conditions are relatively harsh given the low rainfall and high temperatures, and this impacts on nutrient dynamics in the area as discussed in the literature (Chapter 1).

CHAPTER 3: RESEARCH DESIGN

3.1. INTRODUCTION

Data were collected in the field during two mopane worm outbreaks that took place during December 2013 and April 2014. The position of the various sampling sites on Venetia and Haakdoring are shown in Figure 3.1.1.

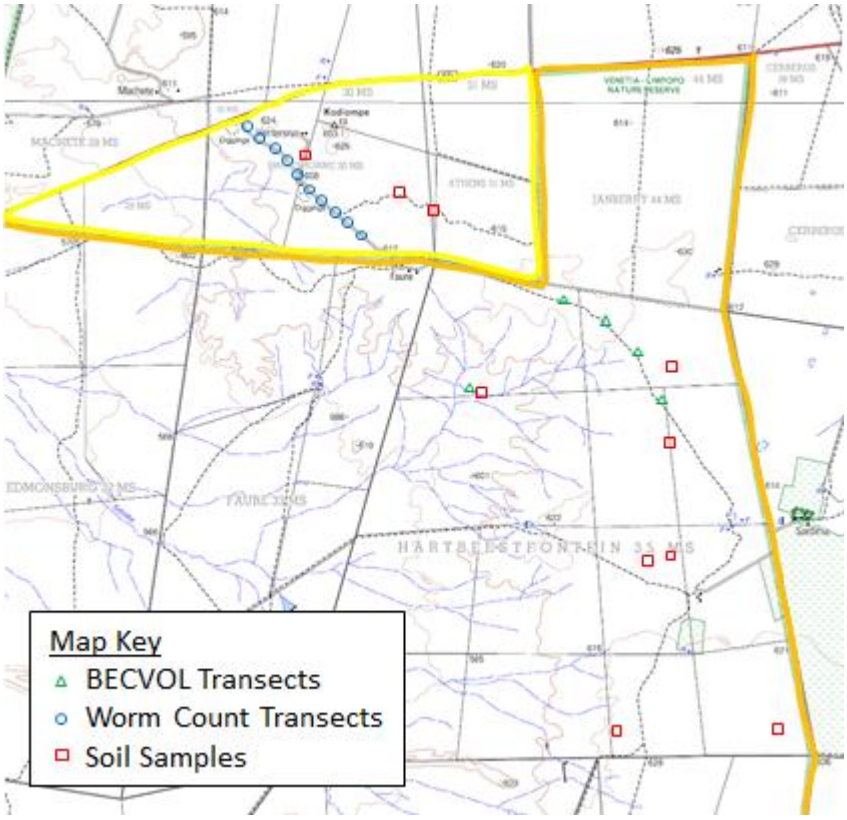


Figure 3.1.1: Topo-cadastral map of northern Venetia-Limpopo Nature Reserve (orange outline) and neighboring Haakdoring farm (yellow outline) showing sampling sites.

3.2. POTENTIAL NUTRIENTS FROM FRASS AND LEAF LITTER

The potential nutrient inputs of frass and leaf litter were calculated by determining the potential production of each. Potential production of frass was calculated by determining the daily production of frass per worm multiplied by the number of worms

per hectare for this study and used as the minimum unit of frass production. The figure of 34 g given by Styles (1994) was used as the maximum frass production per worm, and this was multiplied by the number of worms per hectare in this study to calculate the maximum volume of frass per hectare. The potential biomass of leafy material was calculated using a biomass estimate of canopy volume. Samples of each were collected to determine their respective percentage of macronutrients and C content. These percentages were then extrapolated to the total size of the study area transects to determine the potential nutrient input from frass and leaf litter. The amount of each nutrient per unit area data (kg ha^{-1}) were used to determine whether frass or leaf litter had a higher potential nutrient input. The following section will cover the methods used to collect leaf and frass samples and the additional data required to calculate the percentage extrapolations.

3.2.1. Determining available leaf biomass of mopane trees

Leaf mass estimates were done using the Biomass Estimates from Canopy Volume (BECVOL) method of Smit (1996). Biomass estimates were made by taking a series of measurements from trees along a transect.

The boundary of the mopane worm outbreak area was roughly determined by walking and driving through the study area. Only one road on the reserve traversed an area where the mopane worm outbreak was in progress, a north-west to south-east cutline road. The vegetation along the road was visually similar in structure to the remainder of the study area and it was assumed to be representative of the study area. Five transects were plotted at 500 m intervals along the road in a north to south direction (Figure 3.1.1). All transects were located on the northern side of the road and started 20 m from the road edge. A 100 m tape measure was laid out and followed while holding a 4 m measuring stick perpendicular to the transect. Any mopane tree or part thereof that fell within the boundary of two metres on either side of a transect was measured, thus providing an estimate of their density.

Measurements taken included: a) tree height (A); b) height of maximum canopy diameter (B); c) height of first leaves or leaf-bearing shoots (C); d) maximum canopy diameter on two perpendicular planes (D_1 and D_2); e) basal diameter on two perpendicular planes (E_1 and E_2) as per Figure 3.2.1 (Smit, 1996). All measurement were taken using a 4 m long measuring stick marked at 5 cm intervals and measurements were taken to the nearest 5 cm interval.

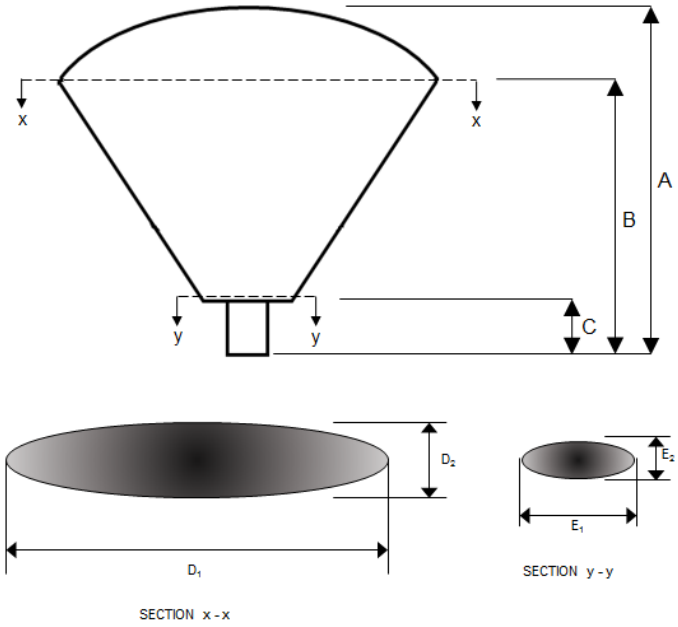


Figure 3.2.1: BECVOL measurements (copied from Smit 1996).

The BECVOL computer program was developed by Smit (1996) to enable the user to input tree measurements collected in the field to determine available leaf biomass. The program is based on regressions of leaf biomass against tree measurements, with separate regressions for different species, of which the mopane tree was one.

3.2.2. Population estimate of mopane worms

The number of mopane worms was determined by transect counts. Counts were conducted by counting all worms present on a mopane tree, this was achieved by two observers visually inspecting the tree for worms and counting all worms present. On

larger trees with many worms, the tree was split into quadrants and each quadrant was counted by two observers until both agreed on the number of worms.

Mopane worm transects were conducted on Haakdoring along a north-west to south-east cutline road in a south to north bearing (Figure 3.1.1, p. 22). Due to the topography of the land, transects were 50 m in length. Transects were located every 100 m along the road, starting on the northern side of the road 20 m from the road edge. A 50 m tape was laid out in a northerly direction and a transect was then followed with a 4 m long measuring stick. Any mopane tree or part thereof that fell within transects was inspected for mopane worms. All trees, and presence or absence of worms were noted and if worms were present, the number of worms was counted. Counting was conducted as previously described.

3.2.3. Mopane worm frass production

Mopane worms occur in five different size classes that are classed as instar stages (developmental stages of mopane worms, described in Section 1.2). Each instar stage produces a different size frass, it was thus necessary to determine frass produced in each of the instar stages.

An attempt to keep the worms in boxes and feed them cut branches of mopane failed and the experiment was abandoned. After consultation with Dr Alan Gardiner (South African Wildlife College, Head of Innovation, Development and Best Practice), I developed a method similar to one he had used as described in Ghazoul et al. (2006). The method involved constructing traps out of shade netting. Traps were 1 m × 1m bags sewn closed on three sides with an opening on one side. This allowed the traps to be placed over a large leafy branch, 50 worms were then introduced to five traps (10 per trap) and the openings closed with a cable tie. The worms were then contained within the trap and all frass deposited collected in the corner of the trap. The traps were set up within the electrified boundary fence of the research facilities at Venetia. This protected the bags from interference from large herbivores and their close proximity allowed for easy monitoring. Bags were inspected twice daily; inspections were

conducted to ensure that all worms were alive and appeared to be feeding. If the available leafy material reached below an estimated 50 % of the original amount, the bag and worms were relocated to a new branch. Frass was collected every three days and weighed. The experiment was conducted over 15 days. Due to an unsuccessful search for newly hatched worms, only 3rd instar worms were used, worms were determined to be 3rd instar using the linear regression model developed by Ditlhogo (1996) where worm size is correlated to weight and instar is plotted. The worms were kept and monitored until they ceased feeding altogether and became restless. This was taken as an indication that worms had reached maturity and were looking for an exit from the trap to find suitable soil to burrow into. This was validated by following the first few worms released from the bags and watching their behaviour. Worms moved approximately 15 m from the tree until they reached soft soil and began burrowing.

The Venetia experiment determined frass production for the last two instar stages as younger worms could not be found, and data on frass production is absent for the first three stages. For this reason it was decided to use the data from this study as a minimum and 34 g as a maximum (Styles 1994).

3.2.4. Determining nutrient content of mopane worm frass

Frass was collected beneath trees defoliated at the same sites as soil nutrients were. Approximately 20 g dry weight of frass (from that season) was collected from below each tree and bagged in paper sample bags, Samples were dried in an oven on NMMU George Campus at 60°C for four days. Dry samples were ground to powder and one sample sent to Bemlab, Somerset West, South Africa (henceforth called Bemlab) to test for N and C while another was sent to Western Cape Government, Department of Agriculture, Elsenberg, Western Cape (henceforth called Elsenberg) to be analysed for P, K, Ca, Mg and Na.

3.2.5. Determining nutrient content of mopane tree leaves

Actively photosynthesizing leaves still on the tree and leaves dropped on the ground were collected for determining mopane tree leaf nutrient content. Leaves were collected

from 10 trees free of mopane worms during soil nutrient sampling. Actively photosynthesizing leaves were collected from the northern side of the tree, for consistency, and litter was collected from the ground beneath the tree canopy. Both sets of leaves were bagged in paper sample bags and air-dried for several days.

A sub-sample of 10 g was then taken from each of the samples and sent to Elsenberg to analyse for Carbon (C), Potassium (K), Phosphorus (P), Calcium (Ca) and Magnesium (Mg). Samples were analysed using the Dry Ashing method. A 2 g sub sample was sent to Bemlab and analysed for Nitrogen using the LECO combustion method (Rossouw, 2014).

3.2.6. Determining nutrient content of mopane worms

Mopane worms were collected from trees in their fourth instar stage and euthanized by placing them in a freezer. Gut content of the worms was not removed and, upon return to NMMU George campus, the mopane worms were placed in a drying oven at 60°C for four days. The dried worms were then ground into powder and two samples of 10 g each were sent to Bemlab for N analysis.

3.3. RATE OF DECOMPOSITION

A decomposition experiment was done to determine the rate of decomposition of frass and leaf litter (adapting the methods of Mlambo and Mwenje (2010)). Whole leaves present in the leaf litter were collected from beneath a variety of mopane trees within the study area that showed no signs of defoliation, leaves were then air dried to a constant mass. Frass was collected from frass traps and air dried to a constant mass. Ten bags, measuring 15 × 10 cm were constructed from fine mesh nylon netting and half received litter (mean 20g) and half frass (mean 50g). The mass of the bags was then noted. As the study could not be conducted in situ, the bags were taken to NMMU George campus where the experiment was processed from February till September 2014 (Figure 3.4.3). Although the climatic conditions at George are different from those experienced at the study site, all bags were subject to the same treatment and therefore measuring rate of decomposition was considered valid. Bags were left outside on the

ground and exposed to the elements. Once a month the bags were oven dried at 50°C until constant mass was reached, and the mass of each bag was recorded.

To make accurate deductions from the decomposition study, meteorological data from Alldays (60km from study site) and George were retrieved from the AccuWeather Inc. (2015) website. These data are presented in the results section (Chapter 4.3 p. 41).

3.4. SOIL NUTRIENTS

Soil samples were collected to determine if mopane worm frass influenced soil fertility. Sampling included bulk density samples. To determine the effect that mopane worms have on soil nutrients, comparisons were made between mopane trees that had been defoliated by mopane worms (referred to as T+), mopane trees that had no defoliation (referred to as T-), and a nearby open area with no trees present (referred to as G). Due to the nature of mopane worm outbreaks, it was easiest to select sample sites by locating mopane trees within the study area that showed no defoliation.

Sites were located by driving and walking through the study area and looking for trees that showed no signs of defoliation (trees showed dull green leaves as opposed to the bright green colour of new growth leaves). Once a tree was identified as showing no signs of defoliation, the leaf litter beneath the tree was inspected for signs of mopane worm frass. If no frass was present the tree was regarded as having had no mopane worm defoliation over that season. A tree was then identified within a 25 m radius that showed signs of mopane worm defoliation. The defoliated tree was only considered if it showed similar visual characteristics, such as size and crown spread, to the un-defoliated tree. Presence of mopane worm frass indicated that defoliation had taken place that season. Thereafter a nearby open area (an area with no trees present within a 10 m diameter) was identified. The open area would only be considered if it was within 25 m of either the defoliated tree or the un-defoliated tree. Soil samples for each of the three treatments (T+,T-, G) within each site were then taken using a soil auger, to a depth of 1 m, to ensure they shared the same soil profile. Sample sites were only

selected based on all these requirements being met, and ten sample sites were selected (Figure 3.1.1, p. 22).

3.4.1. Bulk Density sampling

Bulk density (BD) samples were collected to measure soil health and to convert nutrient sample percentages into a more accurate measure of volume. Bulk density is used to measure soil health as it indicates the level of soil compaction. Soil compaction is important to measure as it influences vital soil processes such as aeration and the ability for water and nutrients to move through the soil profile (Brady and Weil, 2008).

During the course of the first outbreak, three BD samples (T+, T-, G) were taken at each of the ten sample sites. The samples beneath trees were taken on the northern side of the tree, one metre from its base. The layer of leaf litter above the soil and grasses, if present, was carefully removed to expose the soil. A BD sample was taken from the centre of the cleared area.

Bulk density samples were taken by driving a cylinder, with dimensions of 15 cm (h) × 2.8 cm (r), into the ground to a depth of 5 cm. The soil around the cylinder was then removed and a spade was driven in under the cylinder to ensure the content did not fall out. The content of the cylinder was then placed into a brown paper bag on which the site and treatment were noted. Samples were then oven dried at 60°C to a constant mass. Samples were then weighed and the formula for calculating BD was used to obtain the bulk densities for each sub site. The formula for BD is:

$\rho = Ms/Vs$,

where ρ is BD (g cm^{-3}), Ms is mass of oven-dried soil (g), and Vs is the field-moist soil volume (cm^3) (Boone et al., 1999).

3.4.2. Soil nutrient sampling

Soil nutrient sampling was done to compare phosphorus (P), potassium (K), carbon (C) and nitrogen (N) between T+ and T-. This comparison was done to measure the effect

that mopane worms had on soil fertility. Soil nutrient samples were also taken from G to act as a soil nutrient baseline. Samples were taken at the same sites as the bulk density samples.

A 20 cm × 20 cm × 20 cm hole was dug on top of the hole left from the bulk density sampling. This hole was dug to help remove soil samples. Samples were then taken on three sides of the hole using a cylinder (the same cylinder used for BD) with 1 cm and 5 cm indicator lines. Two soil depths, 0-1 cm and 1-5 cm, were sampled. For the 0-1 cm sample, the cylinder was driven into the ground adjacent to the hole to a depth of 1 cm. A steel sheet was then driven under the cylinder from inside the hole to prevent soil dropping out of the cylinder on removal. This was repeated six times along the perimeter of the hole on three sides. These samples were placed in the same paper sample bag on which the site and treatment were noted. The remaining top 1 cm of soil was carefully removed using the steel sheet. To collect the remaining soil, the cylinder was then driven into the ground adjacent to the hole to a depth of 5 cm. The steel sheet was then used to prevent soil from falling out of the cylinder on removal. This was repeated three times on three sides of the hole. These samples were also lumped together. This process was repeated for all ten sites and the three different treatments at each site.

Samples were air dried for 72 hours and sieved through a 1 mm sieve to remove large organic material and stones. Samples were divided into two equal sub samples and sent to Bemlab (total N) and Elsenberg (organic C, K and P) for analysis. N and C were analysed using the LECO total combustion and the Walkley-Black methods respectively, while P and K were analysed using Inductively Coupled Plasma in 1 % Citric Acid as the extractant (Rossouw, 2014). Bulk density was used to convert all nutrient data from concentration to content (g m^{-2}).

3.4.3. Nitrate and ammonium soil sampling

During the second mopane worm outbreak of the season, ten new sample sites were identified using the approach previously explained and samples were collected to test

for inorganic N. Before sampling was done, 30, 50 ml plastic test tubes were prepared with a solution of KCl and distilled water. Each test tube then received 40 ml of the solution. At each site, three samples were taken (T+, T- and G). All humus and leaf litter were carefully collected and placed in a sieve together with the top 1 cm of soil. The sample was then carefully sieved through a 1 mm mesh sieve. Once sieved, the mixture was thoroughly blended and a 10 g sub-sample removed and added to the test tube with the KCl solution. The mixture was agitated for one hour and frozen. Upon return to Nelson Mandela Metropolitan University (NMMU) George campus, the test tubes were thawed, agitated and left for the sediments to settle over 24 hours. Using a syringe the supernatant was removed from the test tube, and the sample refrozen.

Frozen samples were sent to Bemlab to be analysed for nitrate (NO_3^-) and Ammonium (NH_4^+) using the LECO combustion method. Ammonium and nitrate was extracted from the soil with 1N KCl. NH_4^+-N was determined colorimetrically on a SEAL Auto-Analyser 3 after reaction with a sodium salicylate, sodium nitroprusside and sodium hypochlorite solution that was buffered at a pH of 12.8 to 13.0. Nitrate (NO_3^--N) concentration in the extract was determined colorimetrically on a SEAL AutoAnalyzer 3 through reduction of NO_3^- to NO_2^- using a copper-cadmium reduction column, after which the nitrate reacts with sulfanilamide under acidic conditions, using N-1-naphthylethylenediamine dihydrochloride (Christenson et al., 2002).

3.5. STATISTICAL ANALYSIS

All data were analysed using Statistica 12 (Stat Soft Inc., 2013). Data were tested for normality using Shapiro-Wilk's W test and non-normal data, data more than one, were transformed. When testing for significant differences between two treatments, a student's t test was used when the data was normal, otherwise a Mann-Whitney U-test was used. When testing for differences among more than two treatments, a One Way Analysis of Variance (ANOVA) or Kruskal-Wallis ANOVA was used for normal and non-parametric data respectively. The post-hoc Tukey HSD and multiple comparisons by group tests were used respectively to determine specific differences among treatments.

3.6. CHAPTER SUMMARY

This chapter presents this study's research design. The methods for data collection and statistical analysis are described. Data collected to achieve results for objective one included BECVOL transects, frass collection, worm counts and leaf litter collection. For the second objective, leaves were collected from two treatments and subjected to a decomposition experiment. Soil sampling was done to collect data for objectives three and four. The next chapter presents the results of the experiments and samplings described above.

CHAPTER 4: RESULTS

4.1. INTRODUCTION

This chapter presents the results of the study. Soil nutrients, potential available nutrients and decomposition are presented separately.

4.2. POTENTIAL NUTRIENTS FROM LEAVES, FRASS AND WORMS

Calculations done using BECVOL (Chapter 3.2.1) on mopane tree dimensions estimated that the trees had an average of 329062 g (329.1 kg) of dry leaf biomass in the five transects sampled. This equates to 1974.37 kg (SE ±132.47 kg; n = 5) of leaf biomass per hectare within the study site. Mopane worm count transects (see Chapter 3.2.2) estimated an average of 579 worms per transect (SE ±109 worms; n = 10) equating to 28 945 worms per hectare or 91.5 kg of wet worm per hectare. Data collected during transects is displayed in Table 4.2.1.

From frass collected in the frass traps (Chapter 3.2.3) it was estimated that the average mopane worm produces 1.42 g of frass (dry) per day, over the last two instar stages and this equates to 22.1 g per worm or 615 kg in total for the period 31 March 2014 to 24 April 2014. This figure was used as the minimum potential nutrient deposition from frass at 639kg/ha (dry). Styles (1994) calculated that each worm produced 34 g (dry) over their cycle (five instar stages); this figure was used to determine the maximum potential nutrient deposition from frass of 1013 kg/ha (dry). Carbon and macronutrient content of leaf samples and frass are shown in Table 4.2.1.

Table 4.2.1: Data collected during transects showing (per transect) the number of trees, number of trees with worms, number of worms, average number of worms per tree, number of worms per hectare, and amount of frass dropped. Also shown is the amount of frass dropped per hectare.

Transect	# of trees	# of trees with worms	% trees with worms	# of worms	average number of worms per tree	# worms/ha	Frass dropped (g)	frass/ha (g)
1	34	12	35%	492	41	24600	10873	543660
2	34	12	35%	777	65	38850	17172	858585
3	10	5	50%	284	57	14200	6276	313820
4	15	6	40%	265	44	13250	5857	292825
5	15	7	47%	340	49	17000	7514	375700
6	13	5	38%	139	28	6950	3072	153595
7	30	16	53%	562	35	28100	12420	621010
8	21	10	48%	684	68	34200	15116	755820
9	36	18	50%	1113	62	55650	24597	1229865
10	26	12	46%	1133	94	56650	25039	1251965
Mean	23	10	44%	579	54	28945	12794	639685

Table 4.2.2: Mean carbon and macronutrient content of leaf litter and dry frass samples (mg g^{-1}).

Treatment	Mg	C	Ca	N	K	P
Leaf Litter (n = 10)	0.78	527	26.4	9.97	0.91	0.60
Frass (n = 5)	1.97	544	18.5	14.8	12.4	3.50

Using the above data, the potential amounts of carbon and macronutrients that could be added to the soil were calculated using two assumptions: a) normal outbreak conditions where mopane worms eat all available biomass and produce frass and; b) if no outbreak were to occur and all available leaf biomass was to fall and become leaf litter. The latter was tested at a minimum frass input and a maximum frass input. These amounts are given in Table 4.2.3.

Table 4.2.3: Estimated average amounts (kg ha^{-1}) of carbon and macronutrients that could potentially be added to the soil in the study area.

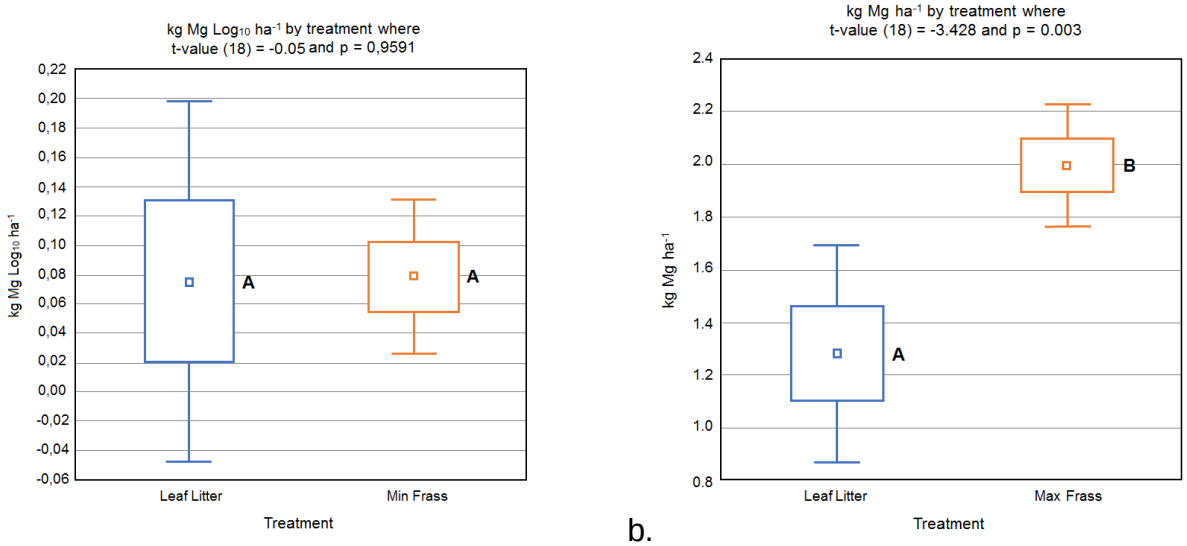
Treatment	Mg	C	Ca	N	K	P
Leaf Litter	1.28	867	43.5	16.4	1.50	0.99
Minimum Frass	1.21	335	11.4	9.12	7.64	2.16
Maximum Frass	2.00	552	18.8	15.0	12.6	3.55

Statistical analysis was done to compare the potential input of nutrients between leaf litter and minimum frass and leaf litter and maximum frass (all g). The results from Student's t-test are presented in Table 4.2.4 and Figure 4.2.1 a-g, and from the Mann Whitney U-test in Table 4.2.5 and Figure 4.2.2 a-e.

Table 4.2.4: Student's t-test results for amounts of carbon and macronutrient that could potentially be added to the soil in the study area by treatment (LL = leaf litter; Min F = minimum frass; Max F = maximum frass).

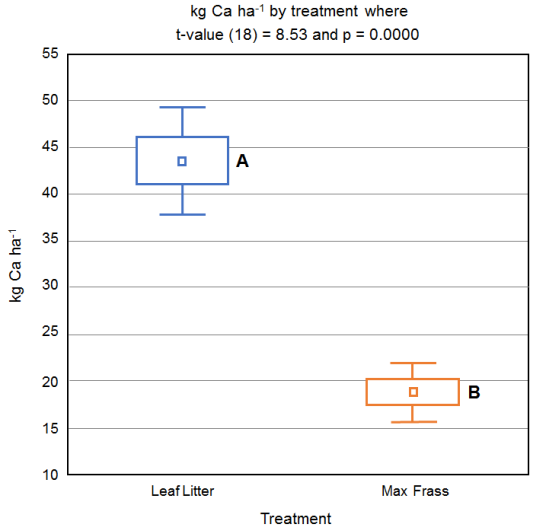
Factor	Treatment	df	t	P
Magnesium (Mg)	LL vs Min F	18	-0,05	0.960
	LL vs Max F	18	-3.43	0.003
Calcium (Ca)*	LL vs Max F	18	8.53	< 0.0001
Nitrogen (N)	LL vs Min F	18	5,80	< 0.0001
	LL vs Max F	18	0.96	0.350
Phosphorous (P)	LL vs Min F	18	-3,39	0.003
	LL vs Max F	18	-5.71	< 0.0001

degrees of freedom (df); critical t-values (t); probabilities (P). *Results for LL vs Min F in Table 4.2.5 as analysed by non-parametric statistics

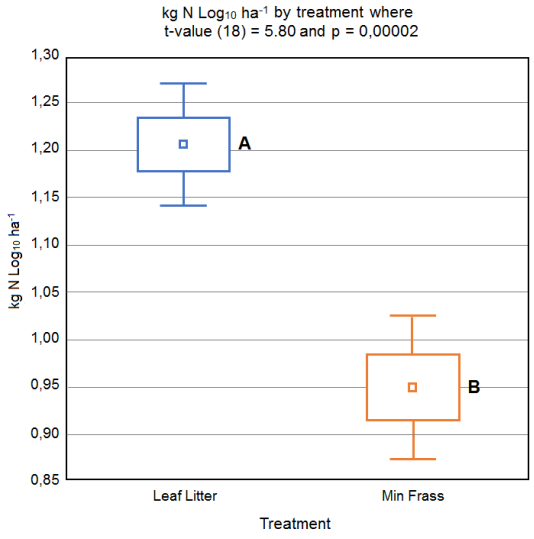


a. b.

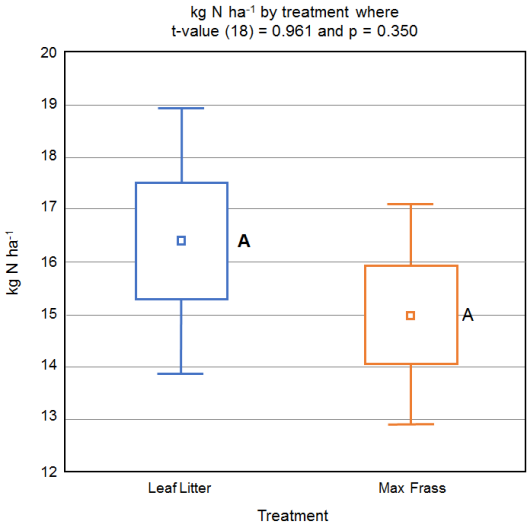
Figure 4.2.1: Graphs showing comparisons between macronutrient potential input by leaf litter vs. minimum and maximum frass inputs (a. Mg in leaf litter vs minimum frass, b. Mg in leaf litter vs. maximum frass input). Different uppercase letters denote statistically significant differences (from student's t-test results). Box-whisker plots shows mean, standard error and 95% confidence intervals.



c.

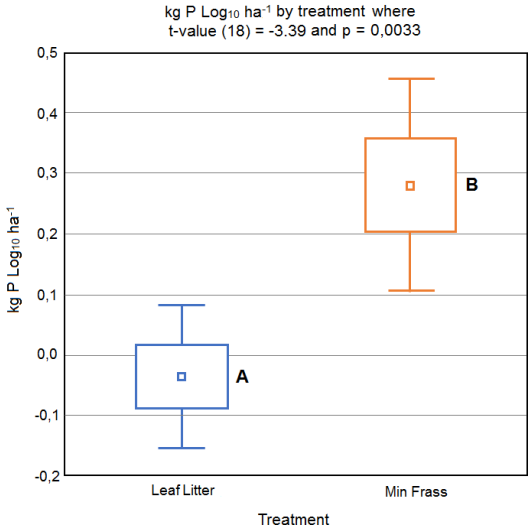


d.

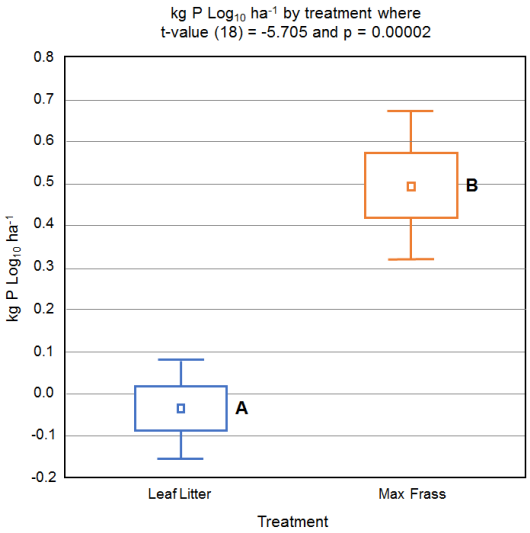


e.

Figure 4.2.2 (cont.): Graphs showing comparisons between macronutrient potential input by leaf litter vs. minimum and maximum frass inputs (c. Ca in leaf litter vs. maximum frass, d. N in leaf litter vs. minimum frass, e. N in leaf litter vs. maximum frass, f. P in leaf litter vs. minimum frass, and g. P in leaf litter vs. maximum frass). Different uppercase letters denote statistically significant differences (from student's t-test results). Box-whisker plots shows mean, standard error and 95% confidence intervals.



f.



g.

Figure 4.2.3 (cont.): Graphs showing comparisons between macronutrient potential input by leaf litter vs. minimum and maximum frass inputs (f. P in leaf litter vs. minimum frass, and g. P in leaf litter vs. maximum frass). Different uppercase letters denote statistically significant differences (from student's t-test results). Box-whisker plots shows mean, standard error and 95% confidence intervals.

Table 4.2.5: Mann Whitney U-test results for amounts of carbon and macronutrient that could potentially be added to the soil in the study area by treatment (LL, leaf litter; Min F, minimum frass; Max F, maximum frass).

Factor	Treatment	n_1; n_2	z	P
Carbon (C)	LL vs Min F	10; 10	3,74	< 0.0001
	LL vs Max F	10; 10	3.74	< 0.0001
Calcium (Ca)*	LL vs Min F	10; 10	3,74	< 0.0001
Potassium (K)	LL vs Min F	10; 10	-3.74	< 0.0001
	LL vs Max F	10; 10	-3.74	< 0.0001

Sample size by treatment (n_1; n_2) critical z-values (z) and probabilities (P).

*Results for LL vs Min F in Table 4.2.4 as analysed by parametric statistics

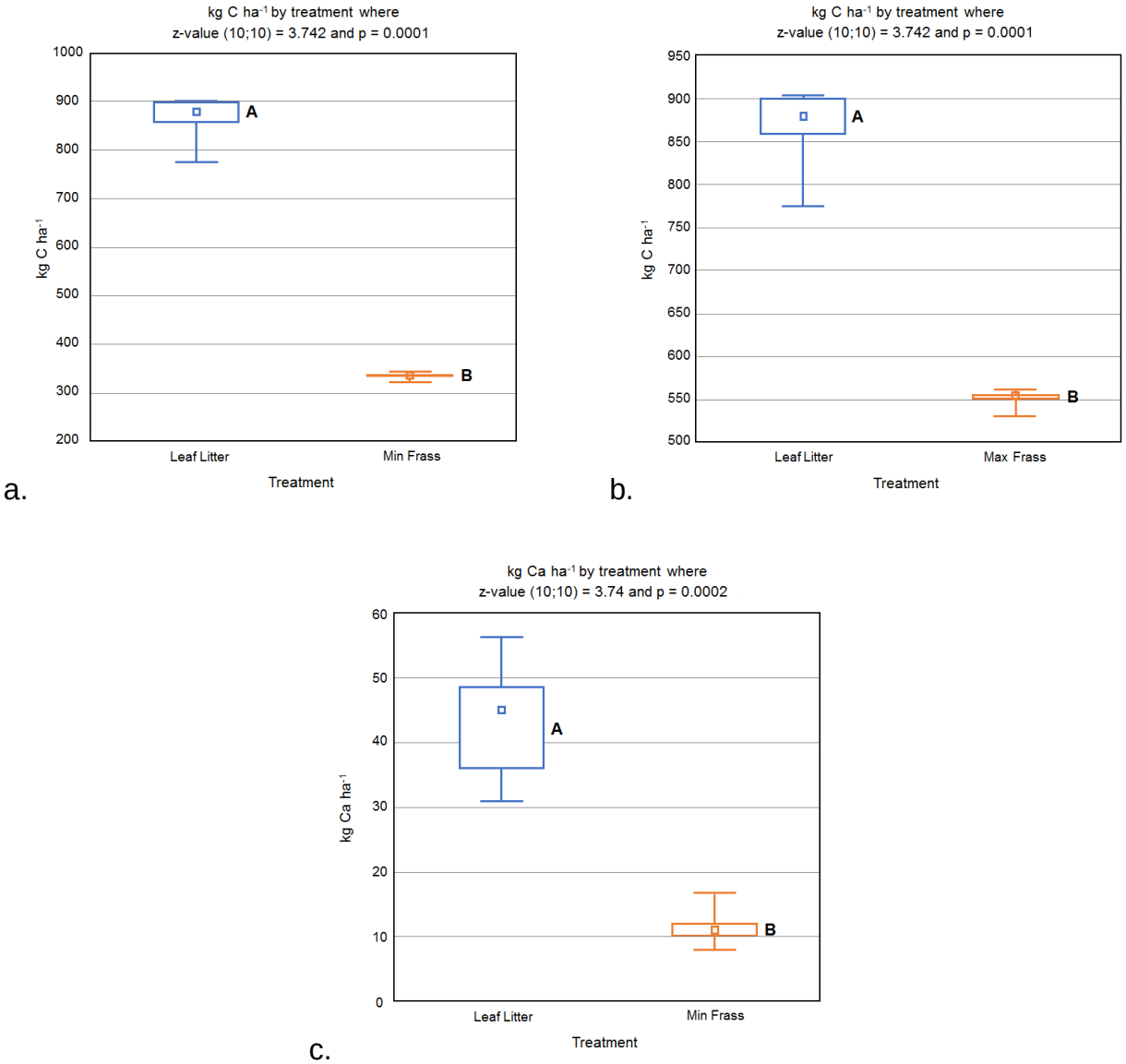
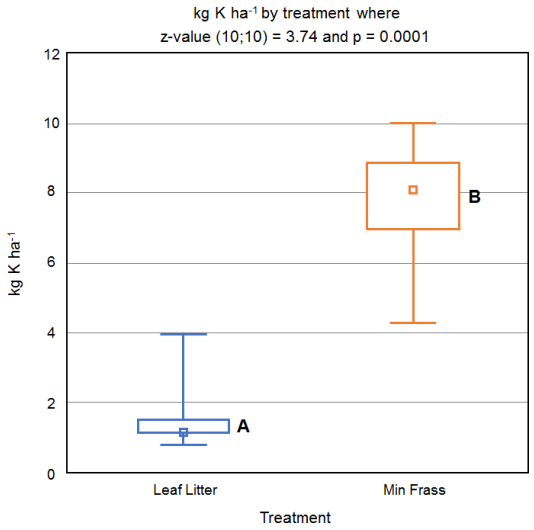
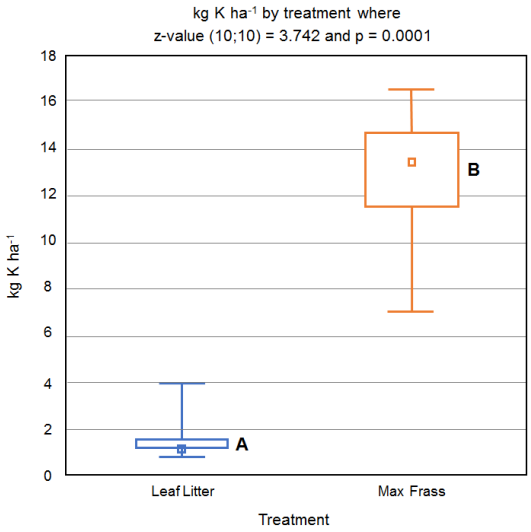


Figure 4.2.4: Graphs showing comparisons between macronutrient potential input by leaf litter vs. minimum and maximum frass inputs (a. C in leaf litter vs minimum frass, b. C in leaf litter vs. maximum frass, c. Ca in leaf litter vs. minimum frass). Different uppercase letters denote statistically significant differences (from Mann Whitney U-test results). Box-whisker shows median, range of values and percentages.



d.



e.

Figure 4.2.5 (cont.): Graphs showing comparisons between macronutrient potential input by leaf litter vs. minimum and maximum frass inputs (d. K in leaf litter vs. minimum frass, e. K in leaf litter vs. maximum frass). Different uppercase letters denote statistically significant differences (from Mann Whitney U-test results). Box-whisker shows median, range of values and percentages.

For minimum potential nutrients from frass, Mg was the only macro-nutrient which did not show a significant difference between input by leaf litter versus input by frass ($p = 0.960$), all other macro-nutrients as well as C showed a significant difference (Table 4.2.4 and Table 4.2.5). C ($p < 0.0001$), Ca ($p < 0.0001$) and N ($p < 0.0001$) inputs were statistically higher from leaf litter than from frass, while inputs of K ($p < 0.0001$) and P ($p = 0.003$) were higher from frass than from leaf litter (Figure 4.2.1 and Figure 4.2.2).

Increasing the amount of potential frass deposition changed the significance of the results. For the maximum potential nutrients from frass, N was the only nutrient to show no significant difference between input by leaf litter versus input by frass ($p = 0.350$). C ($p = 0.0001$) and Ca ($p < 0.0001$) inputs from leaf litter were significantly higher compared to frass, the opposite being true of Mg ($p = 0.003$), K ($p = 0.0001$) and P ($p < 0.0001$).

4.3. RATE OF DECOMPOSITION

Temperature and precipitation data collected at both NMMU George Campus and Venetia-Limpopo Nature Reserve are displayed below. The difference in the average maximum temperatures between the two locations was no more than 5°C during the eight months of the experiment (Figure 4.3.1). Average minimum temperatures were never more than 3°C different during any given month (Figure 4.3.2). There was a large difference in the precipitation at each of the locations with George receiving higher monthly precipitation on average than Venetia during the experiment (Figure 4.3.3).

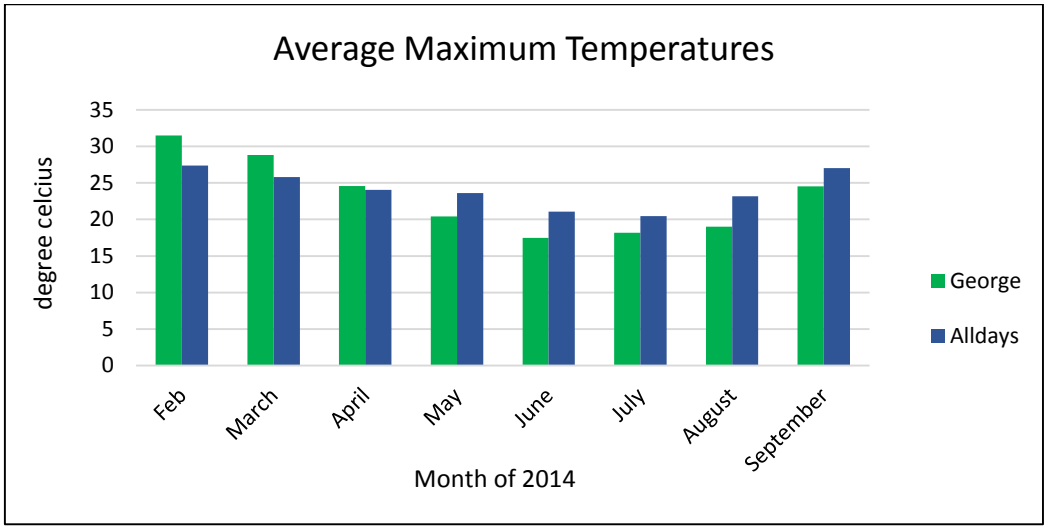


Figure 4.3.1: Average monthly maximum temperatures (˚C) for George (green) and Alldays (blue) during the experimentation period in 2014 (Source: AccuWeather Inc, 2015).

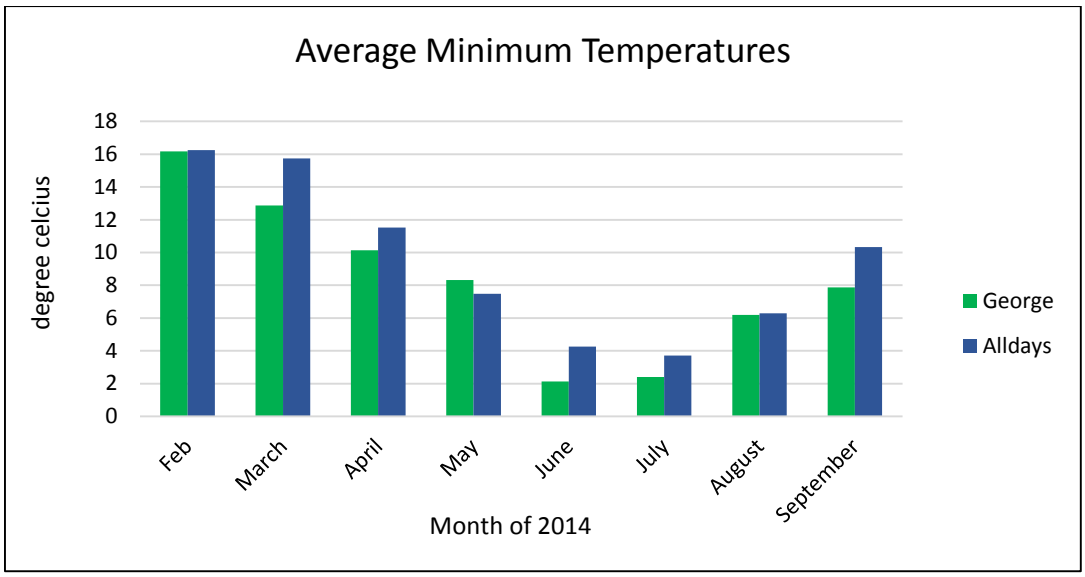


Figure 4.3.2: Average monthly minimum temperatures (˚C) for George (green) and Alldays (blue) during the experimentation period in 2014 (Source: AccuWeather Inc, 2015).

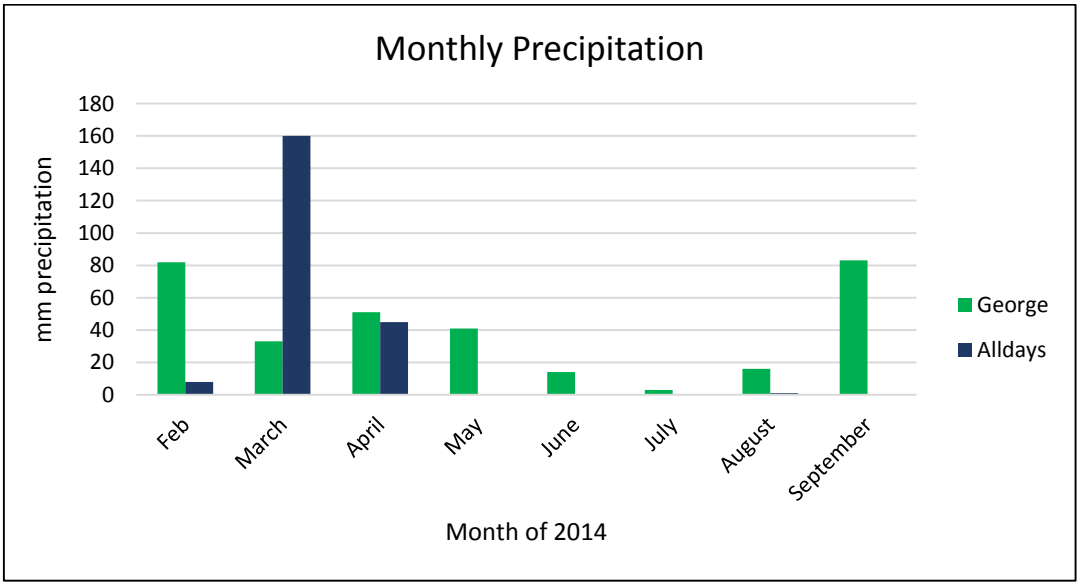


Figure 4.3.3: Monthly precipitation (mm) for George (green) and Alldays (blue) during the experimentation period in 2014 (Source: AccuWeather Inc, 2015).

Frass decomposed significantly faster than leaf litter (p = 0.01) (Table 4.3.1 and Figure 4.3.4). Frass weight loss in grams was higher at each weighing (Figure 4.3.5) and the C:N ratio was significantly lower in frass (Figure 4.3.6).

Table 4.3.1: Results of Student's t-test comparing the effect of different treatments on decomposition (g).

Factor	Treatment	df	MS	P
Decomposition	LL vs F	8	7.7525	0.01

degrees of freedom (df), Mean Square-values (MS) and probabilities (P)

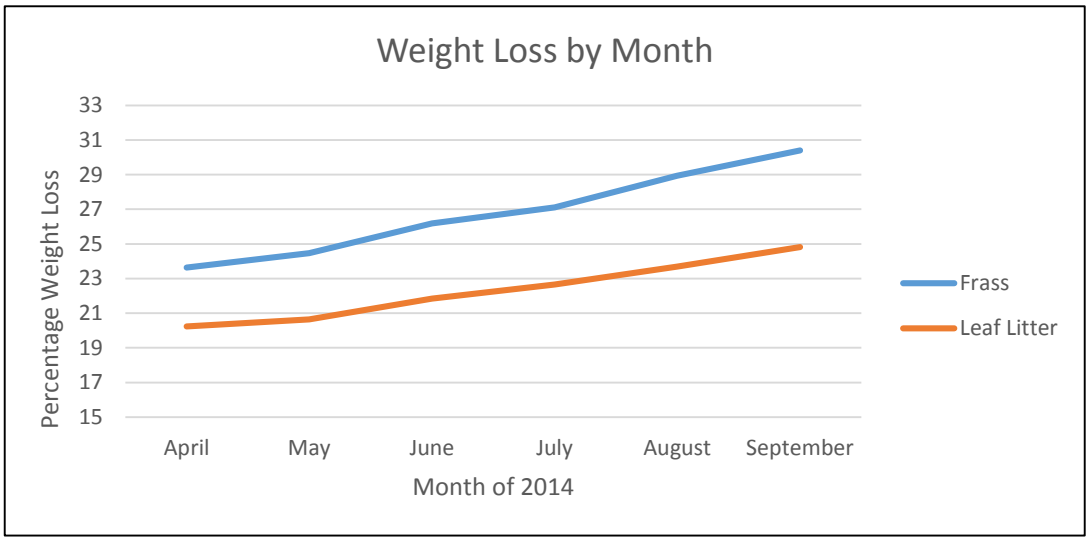


Figure 4.3.4: A graphic representation of frass and leaf litter decomposition represented by comparing the monthly decomposition means of weight loss (g) per treatment.

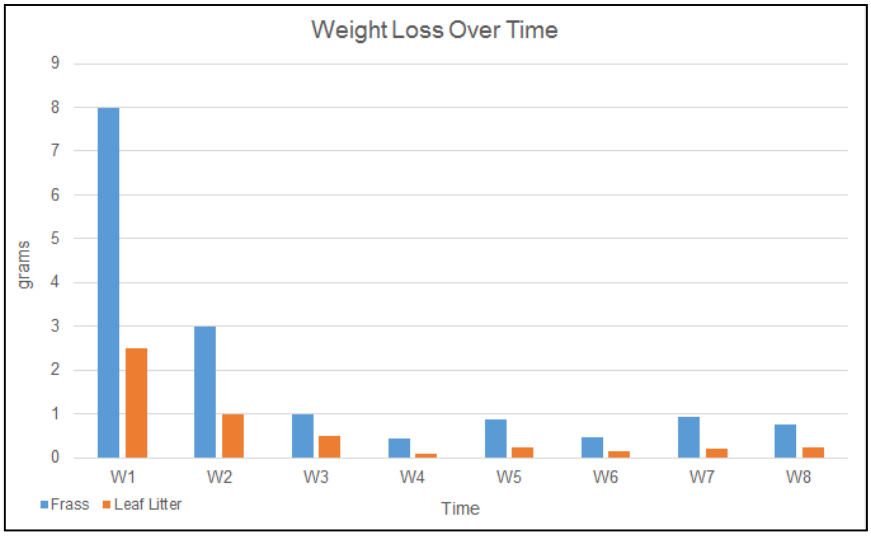


Figure 4.3.5: Weight loss (g) of frass and leaf litter over time.

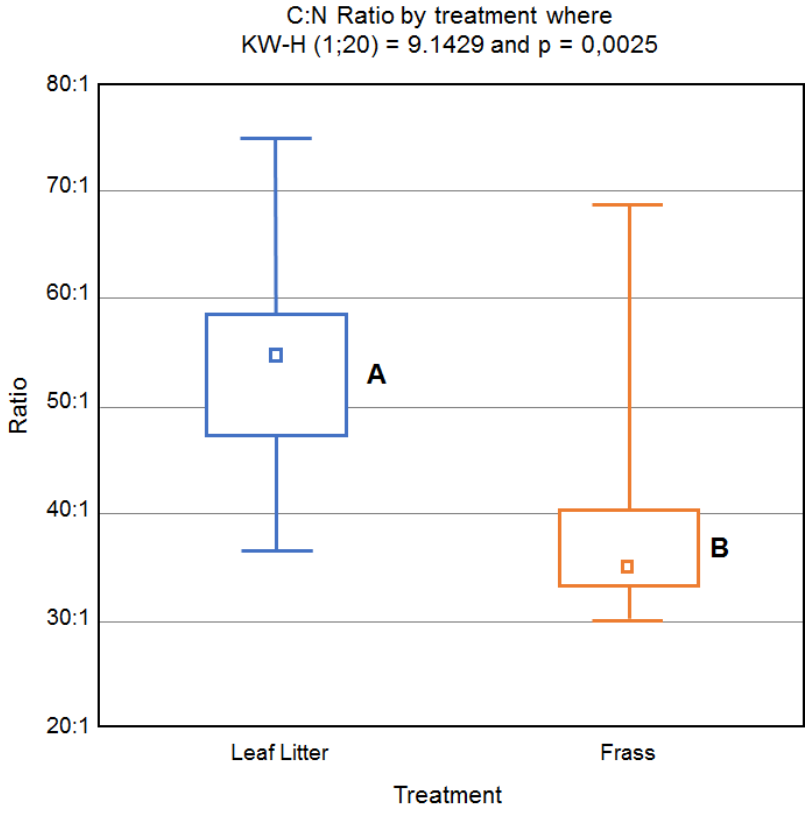


Figure 4.3.6: C:N ratio of leaf litter and frass. Different uppercase letters denote statistically significant differences (from Student t-test results). Box-whisker plots shows mean, standard error and 95% confidence intervals.

4.4. BULK DENSITY AND SOIL NUTRIENTS

Bulk density of soil was significantly higher in open areas than in T+ ($p = 0.002$) and T- ($p = 0.027$) treatments. There was no significant difference in bulk density between T+ and T- treatments ($p = 1.000$) (Figure 4.4.1).

There was a significant difference between treatments with trees and in the open for all nutrients at 0-1 cm depth; the presence of trees increased nutrients. Whether trees had mopane worms present or not had no effect on N ($p = 0.840$) and C ($p = 0.101$). However, trees where worms were present had higher soil P ($p = 0.006$) and K ($p = 0.025$) (Table 4.4.1 and Figure 4.4.2 a-d).

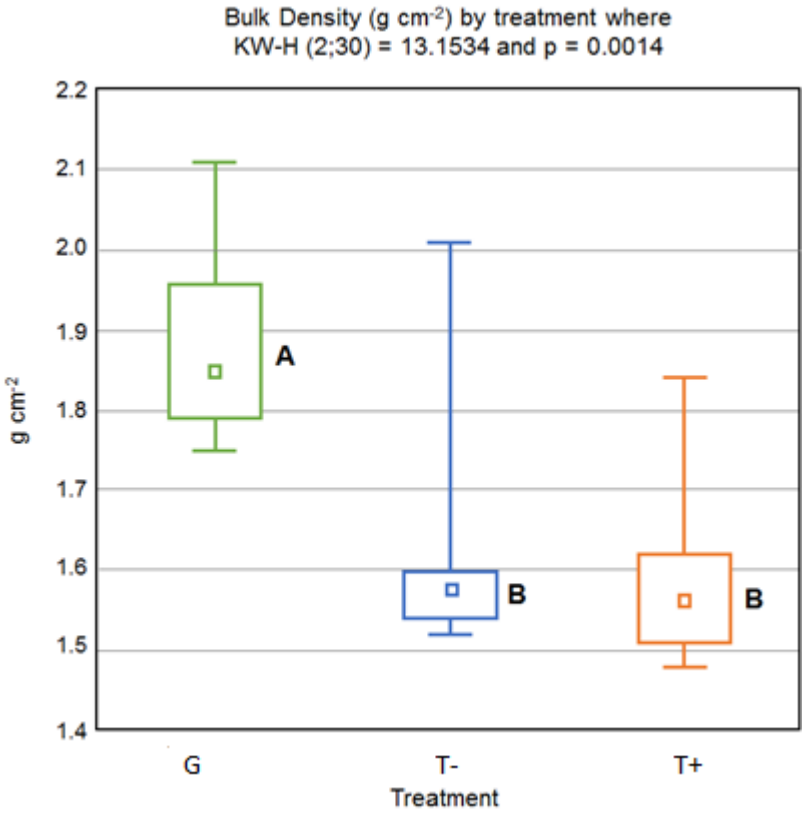


Figure 4.4.1: Graphs showing Kruskal-Wallis results for bulk density by treatment. Different uppercase letters denote statistically significant differences (from Kruskal-Wallis results). Box-whisker shows median, min-max and percentages.

Table 4.4.1: Results of Tukey HSD Test that compared the effect of different treatments (G, open ground; T+, trees with worms present; T- trees without worms present) on nutrient levels in the soil (g m^{-2}) at 0-1 cm.

Factor	Treatment	df	MS	P
Soil N	G vs T-	27	0.19	0.006
	G vs T+	27	0.19	0.002
	T- vs T+	27	0.19	0.840
Soil C	G vs T-	27	0.03	0.000
	G vs T+	27	0.03	0.000
	T- vs T+	27	0.03	0.101
Soil P	G vs T-	25	0.01	0.011
	G vs T+	25	0.01	0.000
	T- vs T+	25	0.01	0.006
Soil K	G vs T-	27	0.41	0.004
	G vs T+	27	0.41	0.000
	T- vs T+	27	0.41	0.025

degrees of freedom (df), Mean Square-values (MS) and probabilities (P)

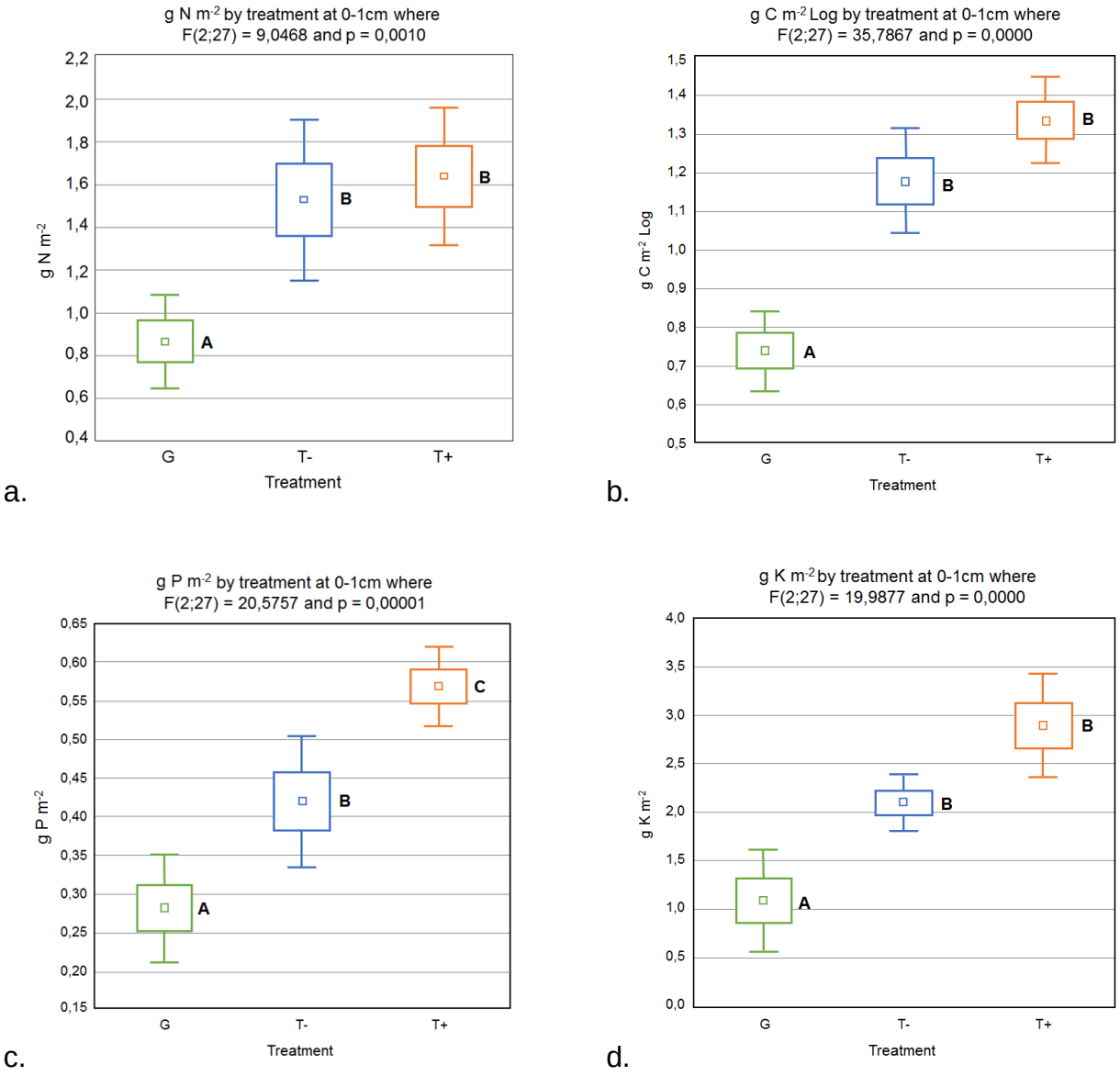


Figure 4.4.2: Graphs showing comparisons between macronutrients present at 0-1cm by open ground vs. trees with worms vs trees without worms (a. N present in soil between treatments, b. C present in soil between treatments, c. P present in soil between treatments, d. K present in soil between treatments). Different uppercase letters denote statistically significant differences (from ANOVA results). Box-whisker shows mean, standard error and 95% confidence intervals.

At 1-5 cm there were no significant differences across all treatments for soil N and P. Soil C and K under trees had significantly higher levels when compared to open areas but no difference was found between T- and T+ treatments (Table 4.4.2 and Figure 4.4.3 a-d).

Table 4.4.2: Results of Tukey HSD Test that compared the effect of different canopy positions (G, open ground; T+, trees with worms present; T- trees without worms present) on nutrient levels in the soil (g m^{-2}) at 1-5 cm.

Factor	Treatment	df	MS	P
Soil N	G vs T-	27	1.56	0.102
	G vs T+	27	1.56	0.520
	T- vs T+	27	1.56	0.565
Soil C	G vs T-	27	0.03	0.002
	G vs T+	27	0.03	0.002
	T- vs T+	27	0.03	0.999
Soil P	G vs T-	27	6.35	0.816
	G vs T+	27	6.35	0.113
	T- vs T+	27	6.35	0.326
Soil K	G vs T-	25	0.08	0.034
	G vs T+	25	0.08	0.001
	T- vs T+	25	0.08	0.246

degrees of freedom (df), Mean Square-values (MS) and probabilities (P)

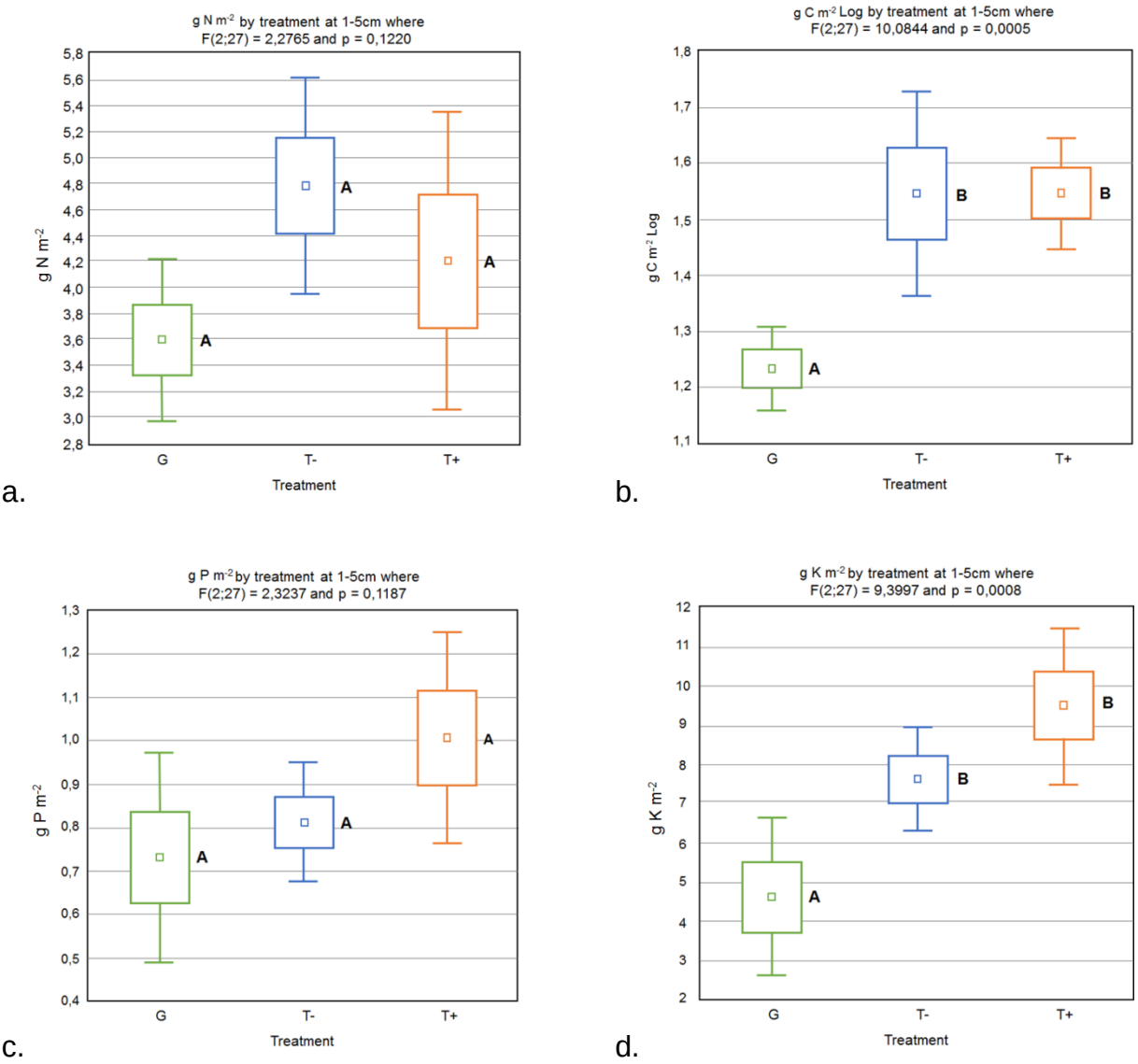


Figure 4.4.3: Graphs showing comparisons between macronutrients present at 1-5 cm by open ground vs. trees with worms vs trees without worms (a. N present in soil between treatments, b. C present in soil between treatments, c. P present in soil between treatments, d. K present in soil between treatments). Different uppercase letters denote statistically significant differences (from ANOVA results). Box-whisker shows mean, standard error and 95% confidence intervals.

Ammonium content for T- was significantly higher than in G ($p = 0.003$). Ammonia content was higher beneath trees with worms than those without worms ($p < 0.0001$) but there was no significant difference between T- and T+. For Nitrate there was no

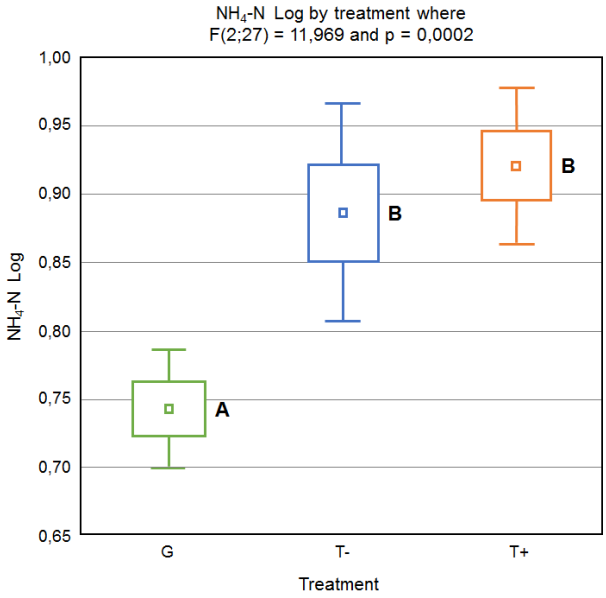
significant difference between G and T- , but T+ had significant higher levels of ammonium than G ($p = 0.008$) and there was no significant difference between T- and T+ in ammonium (Table 4.4.3 and Figure 4.4.4 a-b). Inorganic N, however, tended to be higher under trees with rather than without worms.

Table 4.4.3: Results of Tukey HSD Test that compared the effect of different treatments (G, open ground; T+, trees with worms present; T- trees without worms present) on ammonium and nitrate in the soil (mg kg^{-1}).

Factor	Treatment	df	MS	P
NH_4-N	G vs T-	27	0.01	0.003
	G vs T+	27	0.01	< 0.0001
	T- vs T+	27	0.01	0.661
NO_3-N	G vs T-	27	0.10	0.163
	G vs T+	27	0.10	0.008
	T- vs T+	27	0.10	0.369

degrees of freedom (df), Mean Square-values (MS) and probabilities (P)

a.



b.

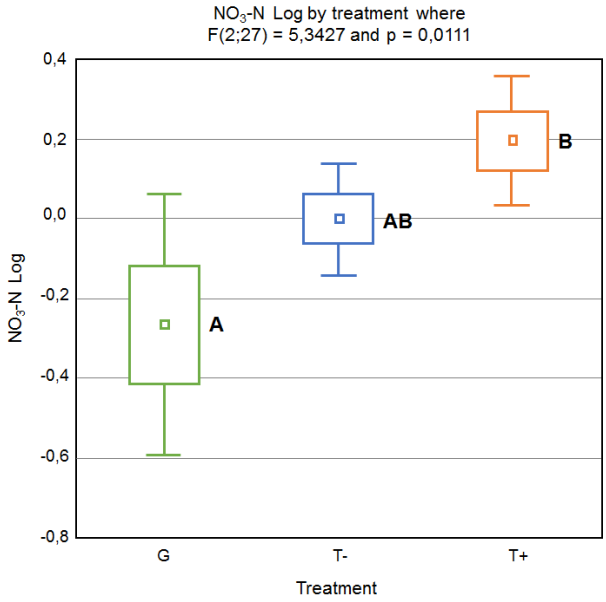


Figure 4.4.4: Graphs showing comparisons for ammonium and nitrate present in the soil by open ground vs. trees with worms vs trees without worms (a. NH_4-N present in soil between treatments, b. NO_3-N present in soil between treatments). Different uppercase letters denote statistically significant differences (from ANOVA results). Box-whisker shows mean, standard error and 95% confidence intervals.

4.5. CHAPTER SUMMARY

This chapter showed the results for the effects of different treatments on soil nutrients, potential nutrient inputs and rate of decomposition. The results (Table 4.2.1) suggest that mopane worms add between 154 and 1252 kg frass per ha per year in the study area. Whether the amount of nutrients going back to soil is higher in frass than litter depends on the nutrient, with P and K returned being higher in frass. The higher P and K levels are also reflected in the top soil layer under trees where worms were present. Whether or not more nutrients are added in frass, frass decomposes faster than litter.

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1. INTRODUCTION

This chapter will discuss implications of the results and put them in context of the relevant literature. Soil nutrients will be discussed independently but results from potential nutrients will be discussed in conjunction with decomposition.

5.2. POTENTIAL NUTRIENTS AND RATE OF DECOMPOSITION

This section deals with the first and objectives of establishing whether the potential nutrient input of mopane worm frass is greater than that of mopane tree litter and investigating the difference in rate of decomposition between mopane frass and mopane tree leaf litter. My results show that more K and P were returned to soil in frass than in leaf litter. The high inputs of K and P from frass were also reflected by the higher levels of soil K and P. Magnesium was no more in leaf litter than in minimum frass, however maximum frass showed higher Mg than leaf litter. There was significantly more N, C and Ca in leaf litter than in frass. Other studies look at how frass effects soil nutrients when compared to leaf litter, but none of these studies conducted nutrient analysis on the actual frass or leaf litter samples, only on the soil once decomposition had taken place (Fogal and Slansky Jr, 1985, Frost and Hunter, 2004, Hollinger, 1986).

Despite my extensive literature search few studies address population densities of mopane worms; only one study gives population density estimates (Styles, 1994). Styles (1994) estimated a population density of 4750 worms ha^{-1}. This study estimated population density as 28 945 worms ha^{-1}, seven times that of Styles. Ditlhogo (1996) considered a dense infestation to be one that left the majority of trees defoliated at the end of an outbreak. In this study 44 % of the trees within transects had worms present (Table 4.2.1. pg. 35), and these worms would have continued to consume leaves on any available tree until pupation. At the time transects were sampled, there were worms present from the third to the fifth instar stage, but the presence of worms on trees does not indicate number of trees that would have been defoliated and thus the density of infestation could not be compared with Ditlhogo (1996). The method Styles used to estimate the population of worms in his study is not mentioned. Hence, differences between estimates of this study and Styles' (1994) study may be

attributable to a number of factors such as the difference in tree densities between the two study sites or the sampling method (e.g. in this study density was based on the presence of an outbreak, whereas in Styles' (1994) study density may have been based on total area). Although the mopane worm larval cycle is estimated to last approximately six weeks, worms do not hatch simultaneously. It can therefore be assumed that a more accurate population size can be determined by conducting multiple transects over multiple days, preferably over the course of the entire outbreak. This would then ensure that counts allowed for worms not yet hatched, worms too small to be noticed in one count and worms that die during the outbreak.

The only study that mentions frass production (Styles 1994) suggests that frass production is 35 g per worm. My study found an estimated 21.27 g per worm over a 15 day period. However, I failed to find worms before the third instar and could only measure frass produced over a 15 day period. Although Styles (1994) does not mention whether 35 g was produced over a six week period (the period it would take for a mopane worm to complete its entire life cycle), the lower amount recorded in this study could be related to dung that might have been produced by the missing instar stages. Determining frass production in the field by laying out frass traps is extremely difficult. Personal observation showed that traps in the field solicited too much unwanted attention from wild animals, like baboons, which are attracted by the mopane worm outbreak. Frass production reported by Styles (1994) did not necessarily equate to double the amount of frass production recorded in this study as worms in the first three weeks are smaller and thus produce smaller frass balls. Ditlhogo (1996) showed that consumption by mopane worms can be influenced by a number of variables, such as ambient temperature and leaf moisture. I used results from this study to represent the minimum frass production whilst Styles's (1994) estimates represent the maximum.

To determine the differences between potential nutrient inputs from an outbreak compared to when no insects are present, some studies simply compare data from before and after an outbreak event (Hollinger, 1986). Others compare data from within and adjacent to exclusion plots (Frost and Hunter, 2004). The method used for this study ensured there was no variation in nutrients over time, that normal nutrient functioning was not prohibited by excluding other herbivores and this was done by comparing data from within the same immediate area. This study assumed that, without an outbreak, all leaves occurring on dryland areas would be shed and become

leaf litter within the study site, based on phenological observations of Dekker and Smit (1996).

Generally, there were higher nutrient loads in frass than in leaf litter, which corresponds with findings in other studies (Fogal and Slansky Jr, 1985, Frost and Hunter, 2004, Hollinger, 1986). It was only when the potential nutrients from each medium (frass versus litter) were extrapolated to the size of the study area that leaf litter offered higher potential nutrients compared to frass. A possible reason for the latter is spatiotemporal variability in mopane worm outbreaks. This variability may mean that the data collected in this study did not accurately depict frass quantity dropped. Furthermore the first three weeks of frass production was not accounted for, although this would, presumably, have accounted for a small proportion of frass, and the comparisons done using Styles' data confirms this. Despite certain nutrient inputs being potentially higher from leaf litter, potential frass input of K and P was still significantly higher than it was from leaf litter, making mopane worm frass an important source of both. Potential input of nutrients by frass and leaves into the soil were based on calculations of quantity per hectare, another possible reason explaining the higher nutrient content in leaf litter (1645 kg/ha leaf litter, 1013 kg/ha maximum frass and 639 kg/ha minimum frass). If the weights of frass and leaf litter were equal per hectare, results may have been different and is worthy of future study.

The rate of decomposition is an important aspect of the nutrient cycle. The quicker a material can decompose, the quicker nutrients are available to plants and micro-organisms (Graécas et al., 2005) The C:N ratio is closely linked to the rate of decomposition (Hodges, 2010). The lower the ratio, the quicker the material will decompose. Mopane tree leaves whilst high in N are also relatively high in secondary compounds, which may slow down the rate of decomposition (Styles and Skinner, 1997). The results of this study were consistent with those of Chapman et al. (2003) and Uselman et al. (2011). Chapman et al. (2003) found that the combined effects of the mesophyll-feeding scale insect (*Matsucoccus acalyptus*) and the stem-boring moth (*Dioryctria albovittella*) decreased C:N ratios of aboveground litter, while Uselman et al. (2011) showed that frass from the northern tamarisk beetle (*Diorhabda carinulata*) also decomposed significantly faster than the leaf litter from the invasive *Tamarix* sp tree.

The results of the decomposition experiment in this study were also potentially skewed in favour of leaf litter, perhaps due to the experimental design. Frass placed into decomposition bags appeared to have a larger quantity of frass per area than that observed in the field. It can therefore be assumed that under field conditions more of the surface area of each frass ball would be exposed to the elements and thus break down faster. During the course of the study it was noted that after a single heavy rainfall event at the study site, exposed surface frass became difficult to locate. At the site of the decomposition experiment, there were numerous rainfall events during the course of the experiment and frass balls seemed to deteriorate more slowly here, than those observed in the field. Despite this, it was clear that frass decomposed faster than leaves, thereby potentially releasing nutrients into the soil more quickly.

Another important aspect potentially affecting decomposition is one associated with semi-arid savannas and the microclimates that exist beneath and between tree canopies. The shaded environments beneath tree canopies are cooler and retain moisture better than the open areas between tree canopies (Campbell et al., 1994). Mlambo & Mwenje (2010) investigated the influence of mopane tree cover over leaf litter decomposition in the southern Highveld of Zimbabwe. The experiment included three treatments, namely litter decomposition beneath large mopane trees (8.3 m crown diameter), beneath small mopane trees (2.3 m crown diameter) and in open areas (no crown). Their results showed that during the wet season (summer), when trees are in full leaf, it was wetter beneath trees than in open areas, and wettest beneath large trees. In the dry season (winter), when leaves are largely absent, moisture content was similar across treatments. Soil temperature was lowest beneath large trees and highest in open areas during the wet season but was similar across treatments during the dry season (Mlambo and Mwenje, 2010). Micro-organismal activity was greatest beneath large trees and slowest in open areas for both seasons. Decomposition was slowest beneath large trees and quickest in open areas, suggesting photo-degradation played a major role in leaf decomposition. They conceded they expected decomposition to be higher beneath canopies than in open areas due to the microclimate favouring microbial activity, but they failed to anticipate the strong influence of solar radiation in leaf decomposition. It is also possible, however, that frass may be less affected by photo-degradation, and may decompose quicker than leaves beneath canopies during the moist conditions as I found that frass almost completely disappeared after a rainfall event.

Another factor not considered in this study when determining potential nutrient input was the effect of abscised leaf material. Like other lepidopteran larva, mopane worms are messy feeders (Risley, 1986). A study by Risley and Crossley Jr (1988) showed that significant quantities of green material was dropped to the ground due to the wasteful feeding often seen in lepidopterous larva. Similarly, mopane worm feeding observed during this study were also messy. Many trees withdraw nutrients from the leaves at the end of the growing season in preparation for senescent leaves to be dropped (Vitousek, 1982), which was noted in this study; nutrient levels fell by almost 50% from leaves on trees to leaf litter. The abscised leaf material dropped by feeding moths, however, may have the same levels of nutrients as actively photosynthesising leaves thus making them a richer source of nutrients than leaf litter. Furthermore the feeding method of lepidopteran larva involves shearing chunks of leaf material from the leaf, allowing leachate to escape from the damaged leaf. Seastedt et al. (1988) showed that this leachate is a potential source of nutrients, especially K. Further investigation is needed to determine the importance of damaged leaves in nutrient dynamics in mopane veld, but the results of this study suggest it could play an important role along with the frass.

5.3. SOIL NUTRIENTS

This section deals with the third and fourth objectives of evaluating nutrient differences between soil beneath trees compared to open areas and assessing the differences in soil nutrients beneath trees with and without mopane worms. There was a clear increase in soil nutrients beneath trees compared to open areas. This was expected as organic material accumulates beneath tree canopies. Fallen branches and herbaceous growth beneath trees trap fallen leaves; this accumulation of organic material slowly decomposes and adds nutrients to the soil. Organic carbon (C) compounds increase the cation exchange capacity (CEC) and thus allow better nutrient retention (Bot and Benites, 2005). Soil P and K were significantly higher beneath trees with worms. Carbon and N tended to be higher within the top layer of soil below trees with worms. Below 1 cm there were no significant difference across any treatments but there was a tendency in trees with worms to have higher levels of soil nutrients and C (except for N). Although not significantly so, results hint that the presence of mopane worms may have increased ammonia and nitrate.

The differences in soil nutrients were most pronounced in the top 1 cm of soil and declined with depth. These differences should be considered in context of a study by Mills and Fey (2004) who suggested that the top layer of soil, or pedoderm, has a disproportionate importance in soil processes as it influences the rate of infiltration and mineralisation of soil organic matter.

The soils of the study area are very sandy (> 88 %) with low levels of silt and clay (Chapter 2.3) and generally soils had low quantities of organic material. However, organic material was higher under tree canopies. The low rainfall levels in the study area reduces nutrient leaching from the profile, and the soils are considered to have a high base saturation with a low cation exchange capacity (O'Connor, 2014). The same study found that soils in the area had an almost neutral pH between 6 and 7. Under these conditions any added nutrients are readily available, not being bound by acids or cations (Brady and Weil, 2008). Therefore I suggest that added macro-nutrients may equate to increased soil fertility, and essentially benefit vegetative growth.

Previous work on lepidopterous outbreaks have shown an increase in soil N, and specifically the labile forms ammonium and nitrate (Fogal and Slansky Jr, 1985, Uselman et al., 2011, Chapman et al., 2003, Hollinger, 1986). However, my results were in agreement with those of Hunter (2001) who suggested that the lack of an increase in N could be because the study area is semi-arid thus preventing N leaching from frass. Another factor that needs to be considered when determining the fate of N in this study is the mopane worm itself. The worms grow rapidly and are considered rich in protein (Onigbinde and Adamolekun, 1998). Therefore the lack of N inputs from the worms may be because of the large amount of N worms require for such rapid growth, but this needs to be further investigated. Lovett et al. (2002) showed that worm faeces could sometimes immobilize N, due to huge inputs of labile C. The same study also showed that N from frass was retained in the organic matter in the soil. It is therefore possible that soils that are low in organic matter, such as those at Venetia, will have poor N retention. While some N could be lost to surface wash a more obvious dynamic relates to the mopane tree itself. As the mopane tree is a shallow rooted plant which goes through a growth period during and after a mopane worm outbreak, this may cause the N to be quickly reused by the tree and therefore not be resident in the soil for an extended period of time (Smit and Rethman, 1998).

Herbivory can increase soil C, mainly through an increase in root exudation (Holland et al., 1996, Hunter, 2001). A large proportion of a plant's assimilated C is allocated to root exudation and thus an increase in exudates will increase soil C. Sankaran and Augustine (2004) found the opposite; they showed that herbivory reduced soil C due to herbivores removing plant material destined for decomposition. It is possible that the difference between this study's results and those of Sankaran and Augustine (2004) are due to differences in the intensity of herbivory and the vast difference in the herbivore species involved in each case. This study concerns intense herbivory by the mopane worm and not only would this increase root exudates, but also worm excrement falling below the tree would be easily assimilated by it. Contrarily, tree defoliation by elephants may reduce soil C because elephant herbivory tends to be in relatively small amounts to a single tree and they usually defecate far away from their place of browse, thus removing the C from the area without triggering root exudation in the tree.

Phosphorus has been linked to stimulation of early fruiting, growth functions, root growth, seed development, and increasing winter hardiness in plants (Hodges, 2010). South African soils are generally poor in P (Laker, 2014) and thus any increases in P should have a beneficial effect on plant growth. Some studies on how insects affect nutrient dynamics have noticed an increase in P (Hollinger, 1986, Schowalter et al., 2011) while Seastedt and Crossley (1984) record an increase in P cycling with moderate levels of insect herbivory. Hollinger (1986) showed that the flow of P in a Californian oak forest more than doubled during a Californian oak moth outbreak. This result corresponds with the significant increase in soil P found in this study as a result of mopane herbivory. Phosphorous, unlike the other anions, has low mobility because of its strong reaction to both solid and solution soil phases (Hodges, 2010). Although the soils in the study area increases the mobility of P due to their high sand content and low CEC, P available to plants would still need to come from directly above the root zone to be near enough for the tree to use it (Hodges, 2010). Hence, P deposited directly from the canopy in the form of frass is an extremely important source.

Bond (2010) studied which nutrients limit the establishment of closed canopy woody vegetation and showed that K (and Ca) were most limiting in southern African systems. It is therefore interesting to note that potassium cycling through insect outbreaks seems to have received less attention than the other macro-nutrients, but Hunter

(2001) reported that increases in K can be because of K leaching from damaged leaves. The soil sampling showed mopane worms significantly increased K, but whether this was because of frass or abscised leaf material is not discernible by soil analysis alone.

5.4. SHORTFALLS AND FURTHER QUESTIONS

A limitation of the study is that the research for the decomposition experiment was carried out in the Southern Cape. While this region has a different climate to that of the study site weather information for both areas were included in the research design. This was done so that results could be interpreted as frass decomposition relative to leaf litter decomposition and not allow the reader to assume it would be relevant to the mopane veld. When interpreting this data and applying it to the data from the other experiments care was taken to be mindful of this limitation. It is suggested that an attempt be made to recreate the decomposition experiment in situ and compare the results with those form this study.

Due to the unpredictable nature of a mopane worm outbreak there are inherent challenges with collecting data from the beginning of an outbreak till the end. One could collect more valuable data were it possible to pre-empt an outbreaks location, size and extent. A method that could be employed to collect more rigorous data would be to conduct a long term study, monitoring the parameters laid out in this study, in an area where mopane worm outbreaks are prevalent. If this were to be done more varied testing could be done on factors such as topography, micro-climate and soil type.

A possible explanation for not detecting a change in soil N in this study was the methodology used. Soil N mineralisation can be detected by ion-exchange resin bags (Belovsky and Slade, 2000). Here, soil samples were taken to test for total soil N, ammonium and nitrate towards the end of each outbreak (Chapter 3). The lapse in time from deposition to sampling could have been long enough for all forms of N to be mobilized (i.e. assimilated by plants). An alternative method would have been to take inorganic N samples repeatedly during the outbreak, which would have given an indication of N mineralisation. These results differ from Frost's (2005) study in Zimbabwe where he found that frass increased N, ammonium and nitrate.

5.5. CONCLUSION

Substantial evidence exists that, similar to the findings of this study, large outbreaks of Lepidoptera can effect nutrient cycling. It is also evident that the degree of this effect can be influenced by a number of variables which include: the composition of the soil, climatic conditions, the size of the outbreak, the species composition of the vegetation and the species of the Lepidopteran involved (Lovett and Ruesink, 1995, Christenson et al., 2002, Hillstrom et al., 2010, Frost and Hunter, 2008, Hunter, 2001). Mopane veld largely occurs on nutrient poor soils, so any increase in nutrients could have positive impacts on vegetative growth. It is possible that the increase in nutrients would benefit the grass component but as the mopane is a shallow rooted plant, and possibly compete with grass for nutrients, this needs further investigation.

The significant increases of soil P and K is very interesting as they are generally limited nutrients in South African soils. These nutrients are both linked to increased winter hardiness, drought tolerance, seed production and growth. Future work should investigate whether trees that experience frequent mopane worm outbreaks grow faster than those that don't and whether those trees exposed to outbreaks benefit in other ways such as an increase in drought tolerance. Styles (1994) mentioned that mopane veld with frequent occurrences of mopane worms appeared healthier than mopane veld without worms. Recent localised extinctions of mopane worms have not yet been linked to mopane tree declines, or reduced vigour, but mopane veld is such an important local natural resource that such losses would have a negative impact. If such a link does exist, healthy mopane worm populations could offer additional possible benefits (apart from a nutritional one) to local communities in the form of increased production of an important resource.

Mopane trees lose their leaves at the end of the summer growing season and benefit from both the frass dropped and the leaves dropped after the mopane outbreak. Although frass was a poorer nutrient source, per hectare, than leaf litter for most nutrients, nutrients measured by weight were equal or higher in frass than in leaf litter. At the individual tree level, frass may thus provide a richer source of nutrients, an aspect worth further investigation.

What is apparent in this study is that frass decomposes quicker than leaf litter. McNaughton et al. (1988) suggested that litter that decomposes faster speeds up the

nutrient cycle. Additionally, this pulse of nutrients comes at a time when nutrient input is at its lowest. Trees do not usually drop leaves during summer, when outbreaks occur. Summer is also growing season for trees, and nutrient uptake is vital. This pulse of frass is accompanied by a pulse of abscised leaf material, still green and presumably rich in the nutrients normally reabsorbed before leaves are shed in winter. Both green leaves and frass have a lower C:N ratio which again may lead to faster decomposition. Chapman et al. (2003) linked frass to increases in the rate of decomposition in leaf litter. This presumably relates to the increased microbial activity normally associated with frass deposition. Mlambo and Mwenje (2010) found that leaves beneath trees decomposed slower than in the open, where it is more exposed to photo-degradation. Defoliation by mopane worms exposes leaf litter beneath the canopy to sun light, frass increases nutrients and presumably microbial activity, both of which contribute to existing leaf litter decomposition and, ultimately, nutrient availability for trees. Not only are worms depositing nutrients they are potentially mobilizing previously immobile nutrients as well.

Defoliation by mopane worms leads to a green flush when trees re-sprout. Regrowth contains fewer secondary compounds, and is higher in N and thus more palatable to herbivores (Hrabar, 2006). In this state, mopane tree leaves are of relatively high quality and thus have the potential to attract other herbivores that deposit dung and urine. This dynamic was briefly explored by Hrabar (2006) but deserves further investigation to understand its influence on nutrient cycling.

This study has shown the impact of mopane worms on nutrient cycling, and raised questions about the possible implications of such nutrient shifts in the mopane veld. The findings of this dissertation show that further long-term studies are required to fully understand the effects of these nutrient pulses and fluxes. For now, custodians of areas where mopane worms still occur would be wise to ensure the sustainable use of both worm and tree to maintain this relationship.

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