Ant community responses to fertilizer application and disturbance in a Mistbelt grassland, KwaZulu-Natal

by

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Ecological Science, School of Life Sciences of the College of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by National Research Foundation (NRF).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.
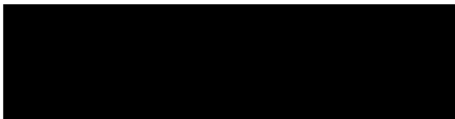
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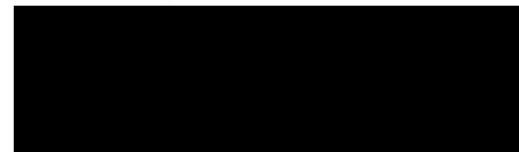
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DECLARATION OF PLAGIARISM

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b)   where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

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(vi)   this thesis is primarily a collection of material, prepared by myself, published as journal articles, or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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ABSTRACT

Land transformation and land-use intensification are major threats to biodiversity and ecosystem functions. Most of the land transformations are linked to habitat loss and disturbance. Grasslands are one of the most threatened biomes in South Africa due to disturbance. Ants are a dominant taxon in terrestrial ecosystems. They are frequently used to understand faunal responses to disturbance because they are sensitive to environmental change. Fire, mowing disturbances, and fertilization are important management practices for maintenance and grassland management in Southern African. These grass management practices are also known to influence ant community responses, but responses vary with habitat type and vegetation structure. However, the factors that drive these responses are not well understood. This study aimed to investigate ant community responses to burning/mowing disturbances and fertilizer application using the long-term (>60years) veld fertilizer and burning/mowing grassland experimental trials at Ukulinga research farm, South Africa. Therefore, the objectives of the current study were to (1) determine the effects of burning frequency and burning/mowing season on ant species richness and composition, and to determine soil correlates of these effects, and (2) to determine how ant species richness and composition vary with two different types of nitrogen fertilization; and (3) to assess whether these responses are related to grassland structure and composition. Ant sampling was conducted during wet (March 2019) and dry (July 2019) seasons using standardized pitfall traps in a long-term veld fertilizer trial (VFT) and burning and mowing trial (BMT) and grassland experiments, each replicated into three blocks. The VFT treatments consisted of control and two forms of N, limestone ammonium nitrate (LAN, 28%N) and ammonium sulphate (AS, 21%N), each separately applied annually at two levels (high and low): LAN (0.21 and 63.2 g m^{-2}), and AS (33.6 and 100.8 g m^{-2}). The BMT included two fully crossed fire treatments: frequency (annual, biennial, and triennial) and season (August and after spring rains) along with annual mowing in August and after spring rains and an undisturbed control. In BMT trials, burning frequency and burning/mowing season did not affect ant species richness nor composition. None of the soil variables were correlated with ant assemblage composition. This suggests that ant assemblage composition in the BMT is resistant to the regimes of burning and mowing. The overall findings of this study suggest that ant communities in Ukulinga grassland trials are resistant and resilient to disturbance. For ant diversity management conservation should be done at a landscape rather than plot level. LAN_low treatments were dissimilar to other fertilizer treatments in composition, whereas all

the other treatments were similar in species composition. However, only one species (*Leptogenys* UKZN_03) significantly varied between control and fertilized plots. Ant community response to nitrogen fertilization was neither related to grass composition, aboveground biomass nor bare ground cover. This suggests that grassland structure and composition are not important factors in explaining the differences in ant assemblage composition.

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Lastly, I would like to thank my grandmother and aunt for their emotional support and prayers during difficult times.

DEDICATION

I dedicate this dissertation to my family

"oMlambo, omaPhisa, omaBhedla, bona abangaphiswa abaphiswa ulaka,

Mdineka, Sishange, Maphisa"

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CHAPTER 1: General introduction and overview

1.1 Background to the study

Increasing land-use intensification due to human population resource demands puts more pressure on the environment, which is a threat to biodiversity. Land-use intensification and the global population increase have led to a rapid increase in land transformation for commercial and agricultural development (Lessard and Buddle, 2005). The latter has resulted in biodiversity loss, which negatively impacts ecosystem functioning (Philpott et al., 2010). Most of these transformations are associated with disturbance, removing biomass, leading to different flora and fauna functional responses.

Disturbance directly impacts plants by removing biomass. In contrast, effects on animals are rather indirect through changes in habitat structure which causes environmental stress, thereby influencing resource availability and competitive interactions (Andersen, 1995). Disturbance is important for biodiversity and grassland management. Burning, mowing, and fertilization are the most common management practices used in South African grasslands (Uys et al., 2004; Kirkman et al., 2014). All these management practices have different effects on grasslands biodiversity due to varying management intensity (Joubert, 2014). These management practices are common disturbances influencing flora and fauna communities in terrestrial ecosystems (Underwood and Fisher, 2006).

Fire is the most influential disturbance in grassland management (Kirkman et al., 2014). It is commonly used by wildlife managers and commercial farmers on a small and large scale to control bush encroachment (Fynn et al., 2003). Fire is an important management tool for fireadapted ecosystems, particularly open-habitats (grassland) because it helps to maintain the diversity, composition, and ecosystem function, preventing loss of biodiversity (Parr et al., 2004; Arnan et al., 2013; Kral et al., 2017; Yekwayo et al., 2018). Burning helps promote the growth of native plant species (particularly fire-prone plants) and thus reducing the growth of exotic species that outcompete native and indigenous species (Uys, 2000). Burning frequency and season are important factors to consider when using fire as a management tool (Fynn et al., 2003) because extensive burning may have detrimental effects on the grassland ecosystem (Uys et al., 2004). Fire can cause changes in the overall species richness, abundance, and composition of both flora and fauna.

As is the case for fire, mowing has dramatic effects on grasslands, because it directly removes aboveground biomass. According to Harpole and Tilman (2007), high mowing intensity may lead to homogenous vegetation structure, which indirectly affects faunal abundance and species. An increase in the accumulation of litter after mowing changes soil microclimate (Kirkman et al., 2014) which influences the nutrient status of the soil and foraging of arthropods (Joubert, 2014). Mowing has a negative impact on arthropods because they are most likely killed by machinery during mowing (Humbert et al., 2009; Humbert et al., 2010). Moreover, mowing reduces food sources and shelters for some species that use plants for habitats (Humbert et al., 2009; Socher et al., 2012), leading to low arthropod species richness.

In addition to mowing and burning, grasslands across the world are also subjected to fertilizers. Fertilizer application in grasslands is used to increase grassland productivity for enhanced livestock production (Tsvuura and Kirkman, 2013). The application of fertilizers changes the nutrient status of the soil, which may also affect species composition and biodiversity (Morris and Tainton, 2002). An increase in nutrient levels in the soil may be harmful to invertebrates and microbial activity (Kirkman et al., 2014).

Invertebrates are the most dominant and abundant animals on earth, playing a key role in ecosystem services (Cardoso et al., 2011). They are also good ecological and environmental bioindicators because they are abundant, species-rich, and sensitive to environmental changes (Samways, 1994). Ants (Hymenoptera: Formicidae) are among the most diverse group of invertebrates in terrestrial ecosystems (Schmidt and Solar, 2010; Pacheco and Vasconcelos, 2012; Wong and Guenard, 2017). Ants play a crucial role as bio-indicators of the ecosystem because of their diversity, functional importance, and varying community dynamics (Andersen and Majer, 2004; Del Toro et al., 2012).

1.2 Ant diversity and functional importance

Ants represent approximately up to 30% of the total animal biomass in terrestrial ecosystems (Hölldobler and Wilson, 1990). Their diversity varies along environmental gradients (Wiescher et al., 2012) and tends to be much greater in tropical regions and decreases with increasing latitude and altitude (Guénard, 2013). Ants occupy many tropic levels ranging from herbivores and omnivores to predators and scavengers (Del Toro et al., 2012). They are of importance to terrestrial ecosystems because they offer various ecosystem services that include nutrient cycling and soil aeration (Graham et al., 2009), seed dispersal (Christianini and Oliveira, 2010),

and improving soil structure (Underwood and Fisher, 2006; Andersen and Brault, 2010; Cardoso et al., 2011). Ants are good indicators of ecological disturbance (Andersen et al., 2002; Hoffmann, 2010) because they are easy to sample and sensitive to changes in the environment (Andersen and Majer, 2004; Schmidt and Solar, 2010).

1.3 Ant responses to disturbance and use as bioindicators

Ants are a good indicator group to use in understanding the responses of faunal communities to disturbance (Hoffmann, 2010). This is because of the ecological dominance and abundance of ants in terrestrial ecosystems. They have varying community dynamics, which makes them good indicators of ecological disturbance (Underwood and Fisher, 2006; Arcoverde et al., 2017). Over the past decades, ants have been globally used as bioindicators in monitoring programs, for example, studies on the mining industry (Andersen and Majer, 2004), soil quality (Frouz and Jilková, 2008). In South Africa, ants have been used as bioindicators for land rehabilitation (Van Hamburg et al., 2004; Jamison et al., 2016), restoration success by the mining industry (Majer and De Kock, 1992), the ongoing ecosystem monitoring programs in western Soutpansberg (Munyai and Foord, 2015a), Rietvlei Nature Reserve (Jamison et al., 2016) and the Iimbovane Outreach Project (Braschler, 2009).

Ants are sensitive to ecological change, therefore habitat disturbance has a significant effect on them because most of them live and forage in the ground (Wang et al., 2000; Schoeman and Foord, 2012). Changes in vegetation structure due to disturbance are likely to influence ant diversity and community composition (Bestelmeyer and Wiens, 1996). According to Schoeman and Foord (2012) vegetation type, structure, and complexity influences ant activities. The impact of disturbance on ant communities differs with habitat structure. Disturbance has a greater impact on vegetation structure in closed habitats compared to open habitats (Andersen, 2019). For example, Vasconcelos et al. (2017) found that fire had no effect on ant diversity in the grassland, savanna, and desert but effects were observed in forest habitats. Effects of disturbance on ant communities include loss of species diversity, changes in species composition, and interspecific interaction (Philpott et al., 2010).

The response of ant communities to disturbance, particularly fire has been well studied worldwide (Andersen and Muller, 2000; Andersen et al., 2006; Arnan et al., 2013). Long-term exposure to burning regimes, rainfall patterns, and vegetation structure have been identified as key factors that may influence the response of ant communities to disturbance. Previous ant

studies looking at the long-term fire regimes in Kruger National Park (Parr et al., 2004), fire frequency, and post-burn in Pilanesberg National Park (Parr et al., 2002) have been conducted in South African savannas. These studies have shown that ant communities in South Africa may be resilient and resistant to burning regimes. Evidence suggests that the effects of fire on ant assemblages depend on habitat type in which ants occupy and the extent of changes induced by the fire (Farji-Brener et al., 2002; Barrow et al., 2007; Pihlgren et al., 2010). Whereas, studies on the effects of mowing on ant communities in South Africa are rare, and they mostly focus on mowing intensity. For example, Dahms et al. (2005) found that low mowing intensity did not affect ant communities. While, Pech et al. (2015) reported shifts in species composition between mown and unmown plots, with a slight increase in the number of species in plots mown once or twice a year compared to unmown plots.

Previous studies on grassland fertilization have shown that long-term fertilization reduces ant species richness (Dauber and Wolters, 2004, 2005) and decreases nest density (Petal, 1976). Responses of ant communities were found to be strongly influenced by nitrogen and moisture in managed grasslands of Germany (Dahms et al., 2005). Reduced ant species richness is mostly linked to vegetation growth and soil humidity (Petal, 1976; Dahms et al., 2005). Most ants are thermophilic; therefore ant abundance decreases with decreasing temperature as a result of increasing plant growth following fertilization (Seifert, 2017; Heuss et al., 2019).

1.4 Study site description

For this study, I used the long-term burning and mowing trial (BMT) and the veld fertilizer trial (VFT) at Ukulinga, the University KwaZulu-Natal research farm outside Pietermaritzburg in KwaZulu-Natal, which are among the longest-running grassland experiments in Africa (Swift et al., 1994). The long-term BMT and VFT grassland trials were initiated by J.D Scott in 1950 and are still running uninterrupted. The original objectives of BMT and VFT were primarily for agricultural purposes, focusing on production. The treatments in the BMT were designed to examine the influence of mowing Tall Grassveld at different times in summer and removing the aftermath in winter by burning or mowing. Whereas the VFT was designed to find possible ways of increasing yield by fertilizing the veld with various elements (Morris and Fynn, 2001).

Research conducted on the Ukulinga long-term trials has contributed critically to the core knowledge of sustainable management of humid grasslands in South Africa (Morris and Fynn,

2001). Effects of burning, mowing and fertilization have been well studied in the mesic grassland of KwaZulu-Natal, and research outcomes are well documented. These include, a study by Fynn et al. (2003) which documented the effects of veld burning on total soil organic matter and microbial biomass. Kirkman et al. (2014) studied the response of plant community structure to fire frequency, while Chambers and Samways (1998) studied the response of grasshoppers to burning and mowing. Lastly, Tsvuura and Kirkman (2013) studied the influence of nitrogen addition on plant yield and species composition. Fynn and O'Connor (2005) reported on the influence of nutrient availability and soil pH on grassland vegetation. However, there is limited research on the effects of burning/mowing disturbance and nutrient addition on faunal communities of this grassland.

1.5 Research aims

This dissertation investigates ant (a dominant terrestrial faunal group) community responses to, fire, and mowing disturbances, and fertilizer application. The specific objectives of this dissertation were to: (1) determine ant community responses fire and mowing in long-term grassland experiments. And (2) assess the long-term effect of nitrogen addition on ant communities. Such studies are important because they help improve our understanding of the responses of a dominant faunal group as a basis for improved grassland management responses.

1.6 Dissertation structure

This dissertation comprises four chapters. This first chapter provides a general introduction and overview with aims and objectives of the study and background information about the study site, Midlands Mistbelt grassland in KwaZulu-Natal.

Chapter 2 assesses (1) the effects of burning frequency and burning/mowing season on ant species richness and composition. It also (2) determines soil correlations related to these effects in a long-term burning and mowing experiment in a Mistbelt Grassland of KwaZulu-Natal.

Chapter 3 determines (1) how ant species richness and composition vary with two different types of nitrogen fertilizers, and (2) lastly, assesses whether these responses are related to grassland structure and composition in a long-term fertilization grassland experiment in the Mistbelt Grassland of KwaZulu-Natal.

Chapter 4 provides an overall general discussion, conclusions of the study, and recommendations for future studies where appropriate.

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CHAPTER 2: Effect of long-term burning and mowing regimes on ant communities in a mesic grassland

2.1 Abstract

Ecological disturbance is fundamental for grassland management and the maintenance of biodiversity. Fire and grazing are the primary habitat disturbances influencing the structure and composition of grassland ecosystems. Ants are an ecologically dominant faunal group, and little is known about the effects of such grass biomass removal on them. Therefore, this study used a >60-year fire and mowing experiment at Ukulinga Research Farm, South Africa to assess the response of ant communities to long-term burning and mowing regimes in a mesic grassland. The experiment included two fully crossed fire treatments: frequency (annual, biennial, and triennial) and season (late winter and after spring rains) along with annual mowing in the late winter and after spring rains and an undisturbed control. Each of the nine treatments had three replicates, with sampling conducted using pitfall traps during wet (March 2019) and dry (July 2019) seasons. A total of 67 ant species from 29 genera were recorded, with *Tetramorium* (14 species) being the richest genus. Mean species richness in burnt plots (37 ± 4.24) was significantly higher than in unburnt plots (23 ± 20). Effects of burning frequency and burning/mowing season of treatments on species richness and ant assemblage composition were not significant. Overall, our findings demonstrate that ant assemblages in this grassland system appear to be highly resistant to the effects of burning and mowing regimes. The effects of burning/mowing on ant species richness at Ukulinga Research farm were based on whether the plot was burnt or not rather than the specifics of burning frequency. Our results suggest that for ants, plots should not be left unburnt/mown for a long time because this reduced ant diversity. However, caution should be considered when burning/mowing because other invertebrate or vertebrate taxa may respond differently than ants.

Keywords: assemblage composition, grassland, prescribed burning, mowing, frequency, season

2.2 Introduction

Ecological disturbance, defined as any factor that removes biomass (Grime, 1979; Andersen, 2000), is fundamental for the maintenance of grassland biodiversity and management (Kirkman et al., 2014). Ecological disturbance can be either natural or anthropogenic (Joubert, 2014).

Fire, herbivory, human-driven land-use change, are the most common forms of disturbance in grasslands (Lessard, 2019). Anthropogenic disturbance is one of the main drivers influencing ecosystem dynamics and vegetation structures (Arcoverde et al., 2018; Andersen, 2019).

In grassland ecosystems, fire and grazing are considered important tools used by land managers and conservation practitioners to maintain species diversity and communities (Parr et al., 2002; Uys and Hamer, 2007). Fire and grazing are dominant agents of biomass removal, affecting community dynamics through changes in vegetation structure, food supply, and competitive interactions (Philpott et al., 2010; Arcoverde et al., 2018). Fire and grazing both have a direct effect on vegetation, through the removal of plant biomass, change in species composition, trampling, and defecation by livestock (Abensperg-Traun et al., 1996; Arcoverde et al., 2017). While on the other hand, trampling by livestock leads to soil compaction and defecation leads to changes in terms of the nutrient status of the soil (Arcoverde et al., 2017).

Impacts of disturbance on biological communities depend on the frequency and intensity of disturbance (Kral et al., 2017; Arcoverde et al., 2018). Frequency, season (time in which burning takes place), intensity, and patch size (size of the burnt area) are relevant to disturbance regimes that contribute to the maintenance of savanna and grassland ecosystems (Uys and Hamer, 2007). However, incorrect use of these regimes can have significant effects on the composition and structure of grassland ecosystems globally (Kirkman et al., 2014). For example, overly frequent burning decreases plant diversity, thus leading to reduced stability in the ecosystem (Kirkman and Morris, 1999). Whereas, infrequent burning or fire exclusion in grasslands cause significant shifts in species composition of tall grassland species (Ghebrehiwot, 2010), which leads to palatable grass species such as *Themeda triandra* being replaced by unpalatable grass species such as *Aristida junciformis* and *Eragrostis curvula* (Uys, 2000; Kirkman et al., 2014). Shifts in species composition are mainly related to the effects of habitat disturbance on habitat structure.

Based on the five principles of disturbance dynamics of ant communities reviewed by Andersen (2019), disturbance has direct effects on habitat structure, which in turn indirectly affects micro-climate and resource availability. This makes habitat disturbance a key factor in structuring ant communities (Vasconcelos et al., 2017). The effect of disturbance on habitat structure is mainly a reduction in vegetation cover, which leads to increased habitat openness (Andersen, 2019). Ant species (thermophilic species) adapted to open habitats are often favored by disturbance compared to species that prefer closed habitats such as cryptic ants of

the litter layer (Hoffmann and Andersen, 2003). Disturbance is most likely to influence litter levels, a major determinant of species diversity (Fynn et al., 2004).

In managed grasslands, mowing (hay cutting) is another source of biomass removal influencing the composition and community structure. Mowing increases the accumulation of litter, which is beneficial to faunal species (e.g. arthropods) that use litter as habitat and nesting sites (Joubert, 2014). An increase in the accumulation of litter in the absence of fire or grazing changes soil microclimate (Kirkman et al., 2014), and species composition, hence change in microclimate influences the nutrient status of the soil and foraging of arthropods (Joubert, 2014). Like any other disturbance, mowing has both direct and indirect effects on the ecosystem which mainly affects vegetation structure and species composition (Heuss et al., 2019). Mowing directly affects arthropods because most individuals especially insects on the ground are likely to be killed during mowing and it also reduces the amount of food supply and destroys the nesting sites (Humbert et al., 2009; Heuss et al., 2019).

There is extensive literature on the effect of fire on plant diversity in South African grassland and savanna ecosystems (Fynn et al., 2004; Kirkman et al., 2014). This is because most studies (Fynn et al., 2004; Uys et al., 2004; Ghebrehiwot, 2010) conducted throughout South African have focused on grassland production and biomass (Trollope et al., 2002; Tsvuura and Kirkman, 2013). However, there are few studies (Chambers and Samways, 1998; Parr and Chown, 2003; Uys and Hamer, 2007) investigating the effect of fire and mowing on invertebrates in southern Africa.

In this study, we investigate how ants respond to long-term burning and mowing regimes because ants are likely to reflect responses of other ground-active invertebrate groups. Ants are a dominant group of invertebrates that contribute a large proportion of faunal biomass (Andersen et al., 2006; Arnan et al., 2013; Andersen, 2019) and play a significant role in many ecosystem services such as, decomposition, and pollination (Schmidt and Solar, 2010). As the indicators of ecosystem function, and communities often reflect the impact of habitat disturbance in a community (Parr et al., 2002). The effects of prescribed burning or mowing regimes on ant assemblages are considered indirect through changes in vegetation cover, and habitat composition (Parr et al., 2004; Philpott et al., 2010). Changes in habitat structure following disturbance influence microclimate, resource availability, soil variables, and nesting sites (Philpott et al., 2010; Heuss et al., 2019), thus leading to negative effects on ant assemblages.

Soil variables play a significant role in explaining ant diversity because most ants nest in the soil (Wang et al., 2001). Clay soils have been reported to reduce ant species richness due to difficulties in nesting in these soils (Boulton et al., 2005). Clay soils tend to support different ant communities because of poor drainage and waterlogging (Andersen et al., 2015). For example, Oliveira et al. (2017) found a high ant diversity and abundance of subordinate Camponotini and the generalized Myrmicinae on clay soils compared to sandy soils.

There is substantial literature on the effect of disturbance on ant and invertebrate communities, including Andersen (2019) latest review of principles of disturbance focusing on fire in grassy ecosystems of South Africa (Parr et al., 2004), Australia, and Brazil (Andersen et al., 2006; Andersen et al., 2007). For instance, in Kruger National Park, Parr et al. (2004) reported that burning had no major effect on the abundance and species richness of savanna ants. On the other hand, Uys and Hamer (2007) revealed that the frequency and season of burn significantly influenced species richness and abundance of epigaeic invertebrates, including ants. Contrary to Parr et al. (2002), they found that fire frequency did not affect ant communities in Pilanesberg National Park.

We used a long-term (established in 1950) experiment in Ukulinga Research Farm (Fynn et al., 2004) to assess the effect of disturbance on ant communities. The objectives of the study were to assess (i) the effect of frequency and season of burning and mowing on ant species richness and composition, (ii) to identify soil correlates of these effects.

2.3 Materials and Methods

2.3.1 Study area

This study was conducted at Ukulinga research farm (29° 40′ S; 30° 24′ E), of the University of KwaZulu-Natal in Pietermaritzburg, South Africa. Ukulinga Research Farm is eight km south of the University of KwaZulu-Natal, Pietermaritzburg with an altitude of 760- 1400 m (Mucina and Rutherford, 2006). Mean annual precipitation in this are is approximately 790 mm, occurring mostly during summer (September -April) as convective storms. Summers are warm to hot with a mean maximum temperature of 26.4°C in February, and winters are mild with occasional frost and a mean maximum temperature of 13.2°C in July. Ukulinga Research Farm grassland is classified as the Southern Tall Grassveld, which falls under the Midlands Mistbelt Grassland Vegetation unit (Mucina and Rutherford, 2006). This grassland occurs on a hilly and rolling landscape dominated by forb species, tall and sour grasses such as *Themeda*

triandra, *Hyparrhenia hirta*, *Panicum maximum,* and *Tristachya leucothrix,* and woody tree species such as *Senegalia karoo*, *S. nilotica*, *S. mearnsii,* and *Celtis africana*.

2.3.2 Experimental design

The long-term grassland experimental trials at Ukulinga were established in 1950. This experiment is a randomized block (three replicates), consisting of two experiments with different experimental units of burning and mowing. Burning and mowing were separately applied in two seasons (late winter and spring). However, burning was applied at different frequencies (annual, biennial and triennial). There has been no grazing for >60 years.

Sampling occurred in a subset of plots (13.76 m x 18.35 m) replicated in a three-block design. Sampling in this study was limited to a total of 27 plots (18 burnt, six mown, and three undisturbed control plots, Fig. 2.1). Burning treatments included: annual, biennial, and triennial burns applied in the late winter; annual, biennial, and triennial burns applied after the first spring rain. Mowing treatments included: annual mow applied in late winter, annual mow applied after the first spring rain. In addition to the eight treatments, each block has one control plot that has never been burnt/mown since the trial commenced in 1950.

(a)

(b)
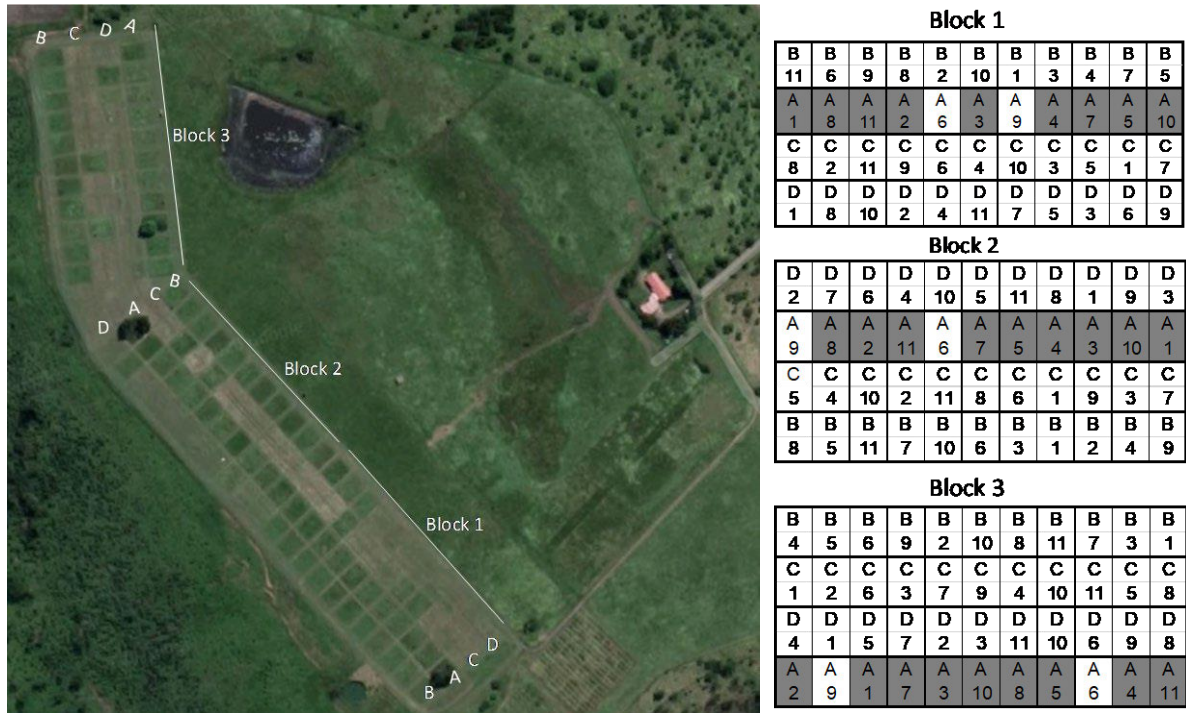
Figure 2.1: (a) South African map showing provinces and the location of the study area at Ukulinga University of KwaZulu-Natal research farm, Pietermaritzburg, (b) shows study design and experimental setup (treatments indicated by numbers: A1 = control (unburnt); A2 = annual burn in August; A3 = annual burn after the first spring rain; A4 = biennial burn in August; A5 = biennial burn after the first spring rain; A7 = triennial burn in August; A8 = triennial burn after the first spring rain; A10 = annual mow in August; A11 = annual mow after the first spring rain), sampled plots highlighted in grey (Image source: Google Earth).

2.3.3 Ant sampling

Standardized pitfall trapping following Munyai and Foord (2015b) was used to sample ants during both wet (March 2019) and dry (July 2019) seasons. Each of the 27 plots had 12 pitfall traps (100 ml in size) laid out in a 3 x 4 grid with 4 m spacing between traps. To limit edge effects, traps were set up 6 m into the plot. Pitfall traps contained 50% propylene glycol solution, which does not repel nor attract ants (Munyai and Foord, 2015b), and were left open for five days. Traps were then collected, and samples were removed in the laboratory and stored in 70% ethanol. Ants were then pinned and identified to species level where possible; otherwise, specimens were identified to the genus level and assigned to morphospecies. Voucher specimens will be deposited at the Iziko Museum of Cape Town, South Africa.

2.3.4 Soil variables

The soil variables soil depth, organic matter, pH, clay content, and acid saturation (Appendix A) were selected because they potentially influence ant nest distribution. Soil data from the plots were provided by Prof K.P Kirkman from the School of Life Sciences, University of KwaZulu-Natal, South Africa.

2.3.5 Data Analysis

Unless stated otherwise, data analysis was done using R Statistical Environment program v.3.5.1 (Team, 2017). For all analyses, data were analyzed based on pooled data across the 12 putfall traps within each seasons.

Individual-based rarefaction curves were computed in iNEXT online to estimate species richness and inverntory completeness using coverage estimator. The 95% confidence intervals were obtained based on bootstrap method with 120 replication (Chao et al., 2016).

Total species richness and abundance were compared between frequency and season of treatments using Analysis of variance (ANOVA). ANOVA was also used to determine significant differences in species richness and abundance between burnt/mown plot treatments and unburnt plots for each season of treatment.

Generalized Linear Models (GLMM) using a loglink function and Poisson error distribution were used to test the effects of burning frequency and burning/mowing season on ant species richness, abundance and the abundances of each of the most common species. Burning frequency and burning/mowing season treatments were included in the model as fixed factors while replicates were included as random factors in the model to account for pseudoreplication. The GLMM analysis was performed using the vegan and multcomp package in R studio version 3.5.1 program (Team, 2017). A generalized Linear Model (GLM) was used to fit individual species common enough for analysis. Only species with >50 abundance occurring across burning/mowing treatments were considered for analysis.

Multivariate analyses were undertaken using the PRIMER v.6.0 software program (Clark and Gorley, 2006) to assess the overall changes in ant assemblage composition between treatments at the plot level. The Bray-Curtis similarity index was used to determine differences assemblage structure between burning and mowing treatments. Data was square-root transformed prior analyses to reduce the weight of common species. Analysis of Similarity (ANOSIM) with 999 permutations was used to test for any significant differences in ant

assemblage composition between burning frequency (annual, biennial, triennial burn), and burning/mowing season (spring, august), and unburnt (control) plots. ANOSIM generates the Global R-statistic, which is an absolute measure of the distance between measured groups used to quantify similarity of assemblages being compred. The closer the significant Global R is to one, the more distinct differences are between treatments being compared, whereas an R-value close to zero indicates that assemblages are barely separable (Parr et al., 2004). A nonMultidimensional Scaling (nMDS) ordination was used to visualize the patterns of assemblage composition between fertilizer treatments.

Indicator Value analysis in R program v.3.5.1 (Team, 2017) was used to identify ant species characteristic of the burning and mowing regimes treatments and of unburnt plots. The indicator value method assesses the frequency of occurrence and uniqueness of a species to a treatment type. A high indicator value (IndVal) expressed as a percentage, indicates that a species can be considered an indicator for that treatment type because it has a higher probability of being sampled. For this study, indicator values for each species were calculated based on abundance data (data pooled across the season of treatments). Species with significant IndVal >70% were considered indicator species for that treatment type, and species with significant IndVal from 50-70% were regarded as detector species (McGeoch et al., 2002).

A Canonical Correspondence Analysis (CCA) from the CANOCO version 4.5 program (Ter Braak and Smilauer, 2002) was used to correlate ant community composition with soil variables. Based on direct gradient analysis, CCA extracts the major patterns in the data that are accounted for by the measured variables only. Species abundance data was not transformed, but an option of 'down-weighting of rare species' was selected. No soil variables significantly explained variation in ant species composition. Hence, no forward selection of soil variables was used. The results were shown in a tri-plot output, and only species with variability of more than 15% were included in the ordination tri-plot.

2.4 Results

2.4.1 The ant fauna

A total of 2 147 individual ants, comprising 67 ant species in 29 genera were recorded during the study. The most specious genera were *Tetramorium* (14 species), *Lepisiota* (six), *Monomorium,* and S*olenopsis* (5 each). The most abundant species were *Pheidole* sp. 2

(*megacephala* gp.), representing 24.7% of total ant abundance, *Crematogaster rectinota* (8.8%), *Acropyga* sp. 1 (6.9%), *Tetramorium* sp. 3 (*setigerum* gp.) (6.5%), and *Lepisiota capensis* (4.8%). Species rarefaction curves (Fig. 2.2a&b), based on individuals approximated an asymptote, with a sample coverage > 0.95 for all treatments and season of treatments. However, there was no difference in total species richness between treatments as the confidence intervals of the curves were overlapping (Fig. 2.2 a&b).
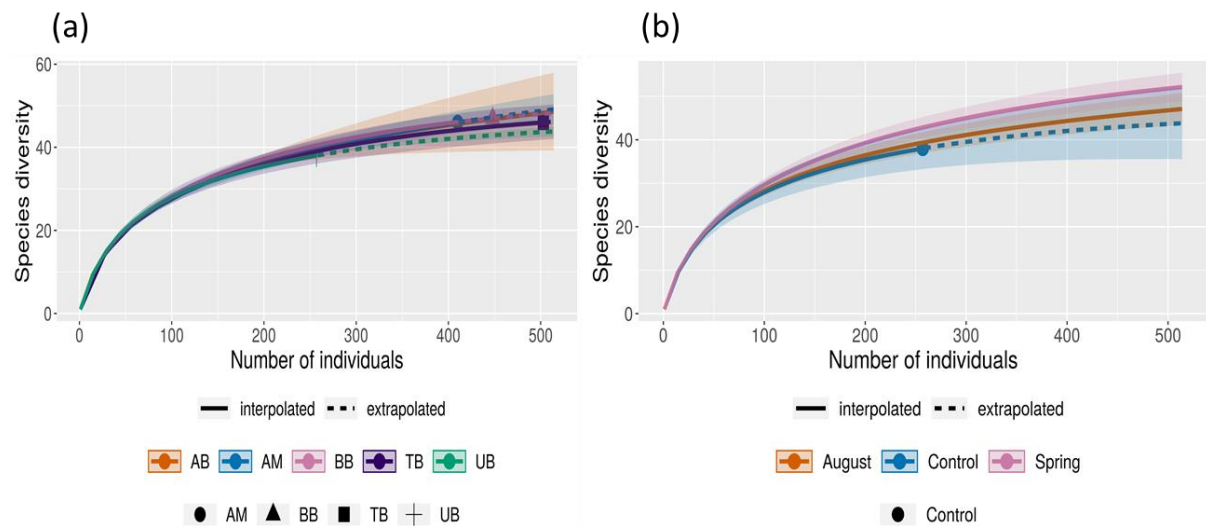
Figure 2.2: Individual-based rarefaction curves showing (a) burning/mowing treatments and (b) season of treatments. Annual burnt (AB), annual mown (AM), biennial burnt (BB), triennial burnt (TB) burn, and unburnt (UB) plots at Ukulinga Research Farm.

2.4.2 Species richness and abundance

Mean species richness was greatest in burnt (37 ± 4.24) intermediate in unburnt (23 ± 2) and lowest in mown treatments (20.8 ± 2.13), with a significant difference (F = 8.978; P = 0.003) between burnt and unburnt treatments. However, there was no significant difference (F = 0.153, df = 3, P > 0.05; Fig. 2.3) in species richness between burnt plots subjected to different season of burning (F=0.207, df = 2, p > 0.05; Fig. 2.3). Effect of mowing season on ant species richness was also not significant (F = 1.2, p > 0.05; Fig. 2.4).

There was a significant difference in species richness between burnt and unburnt plot treatments (F = 8.978; p = 0.003), with a total of 27 species exclusively occurring in the burnt treatments and only two species; *Lepisiota* UKZN_08 (*spinosior* gp.) and *Tetramorium* UKZN_19 (*squaminod*e gp.) exclusively occurring in the unburnt plots (See Appendix B)
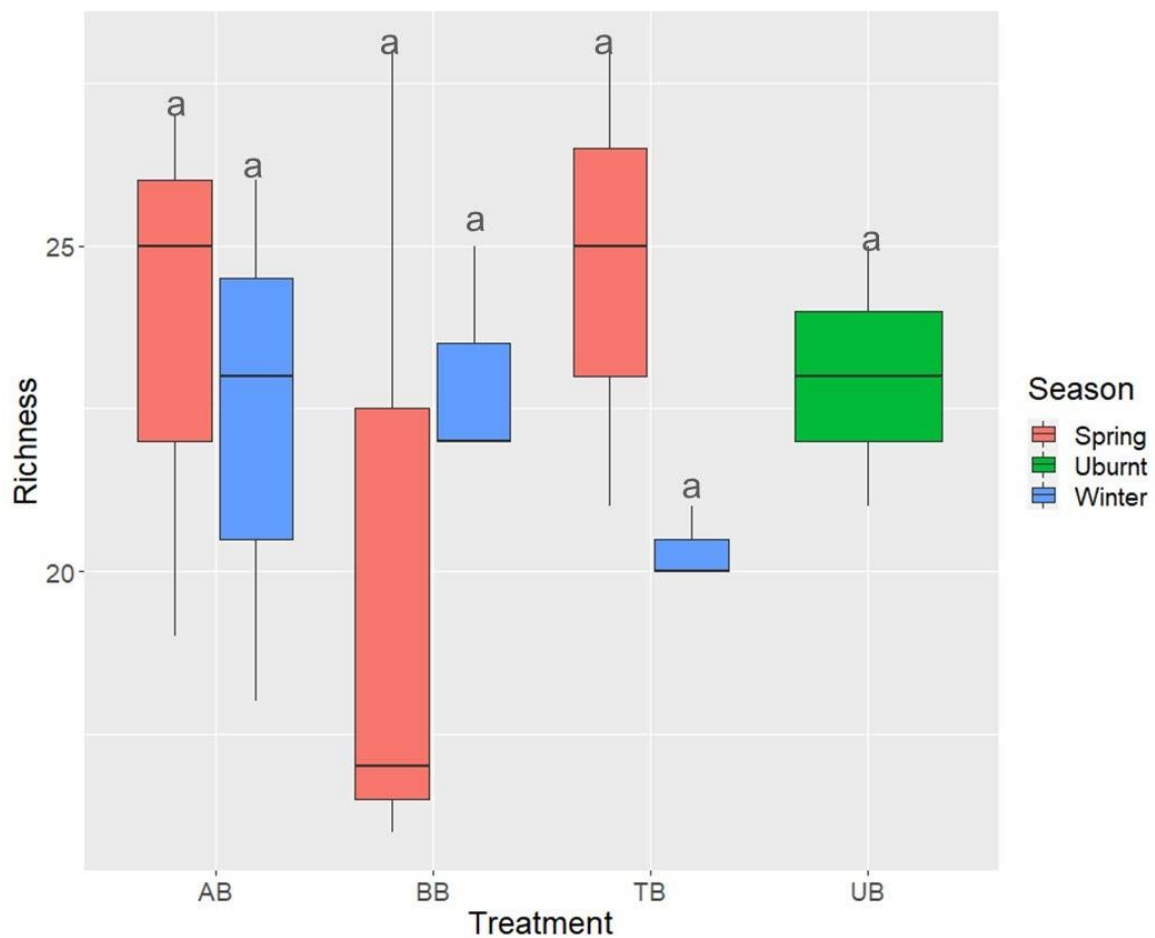
Figure 2.3: Box and whisker plots showing the effect of burning treatments and season on ant species richness in the Ukulinga research farm, South Africa. Coloured box plots indicate season of treatments: AB = Annual burnt; AM = Annual mown; BB = Biennial burnt; TB = Triennial burnt, and UB = Unburnt plots. The solid bar denotes median values.
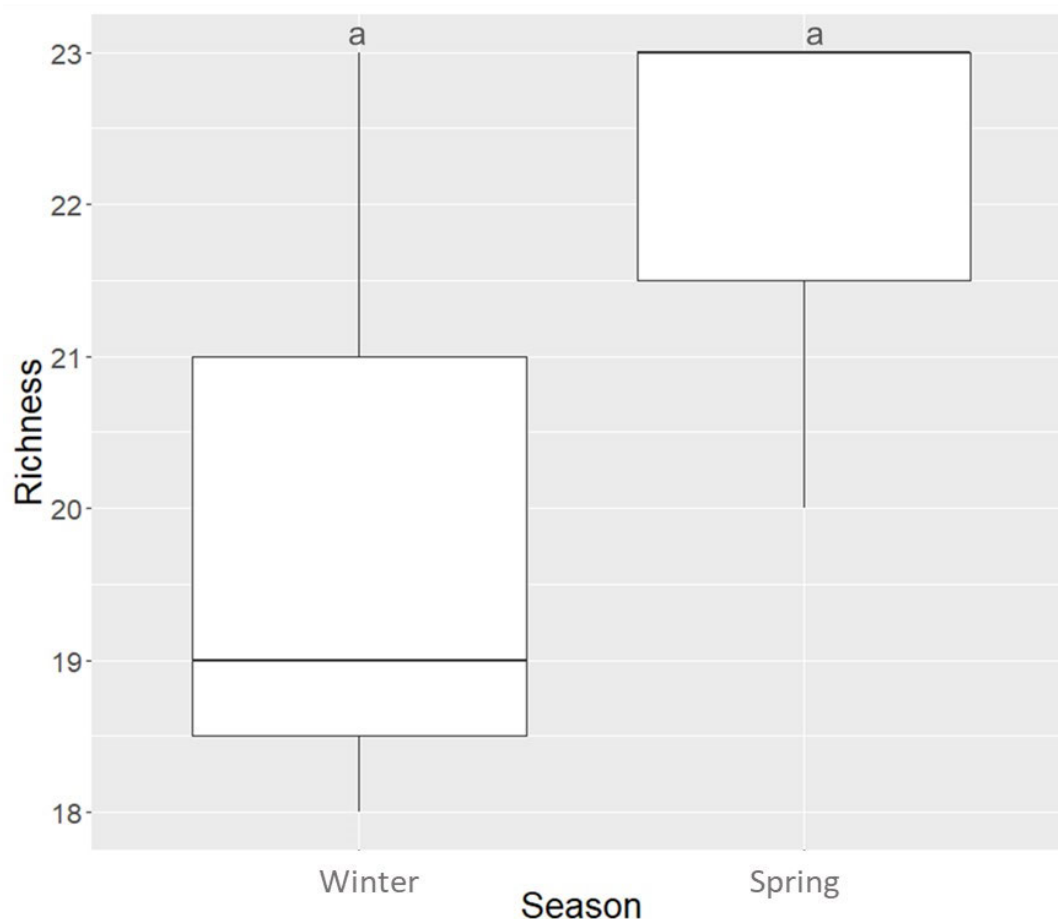
Figure 2.4: Box and whisker plots showing the effect of mowing season on ant species richness in the Ukulinga research farm. The solid bar denotes median values. Significant difference denoted by the letter "a"

Four of the nine most common species showed a statistically significant ($p < 0.05$) response to burning and mowing (Table 2.1). The most marked was for *Crematogaster rectinota*, which occurred primarily in annually burnt plots (12.7 ± 2.5) and was not recorded at all in the unburnt plots. Although occurring across all plots *Pheidole* UKZN_02 was most common in unburnt plots (24.7 ± 1.15). *Acropyga* UKZN_01 abundance was highest in annual burnt and lowest in the unburnt plots, whereas T*etramorium* UKZN_03 (*setigerum* gp.) preferred triennial burnt plots compared to annual burnt plots (Fig. 2.5; Appendix C).

Table 2.1: Results of GLM tests of the effect of burning and mowing treatments on the most common species (>50 abundance).

Species	P-value
Acropyga UKZN_01	**0.001**
Crematogaster rectinota	**0.000**
Lepisiota capensis	0.119
Monomorium UKZN_01	0.482
Pheidole UKZN_02 (*megacephala* gp.)	**0.000**
Pheidole UKZN_03	0.907
Solenopsis UKZN_01	0.969
Tetramorium UKZN_03 (*setigerum* gp.)	**0.003**
Tetramorium UKZN_09 (*similimum* gp.)	0.876
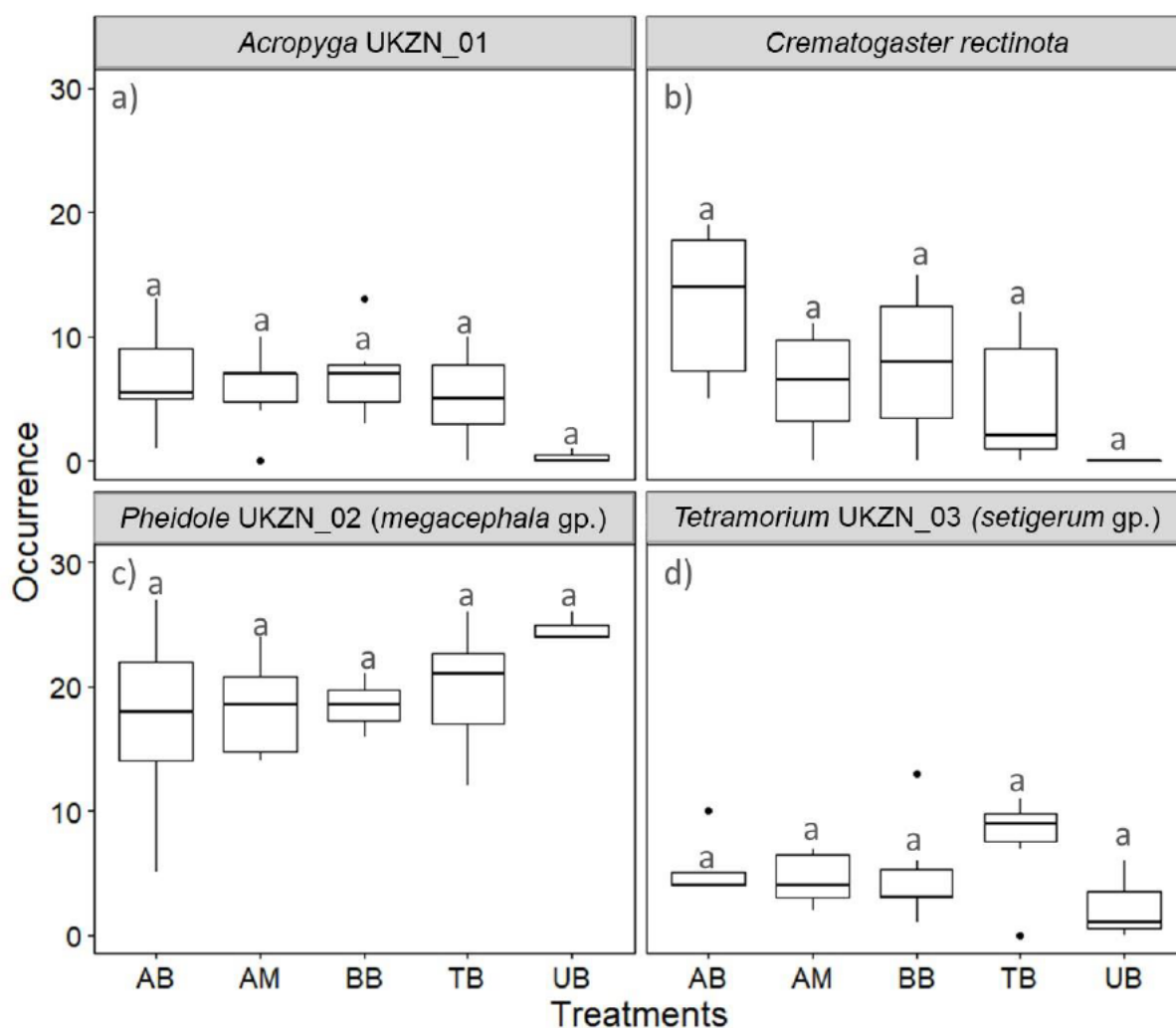
2.4.3 Ant assemblage composition

ANOSIM tests revealed that ant assemblage composition did not vary significantly with frequency of burning or mowing (Global R = 0.07, p = 0.145; Fig. 2.6), or with season of treatment (Global R = 0.015, p > 0.05; Fig. 2.7).
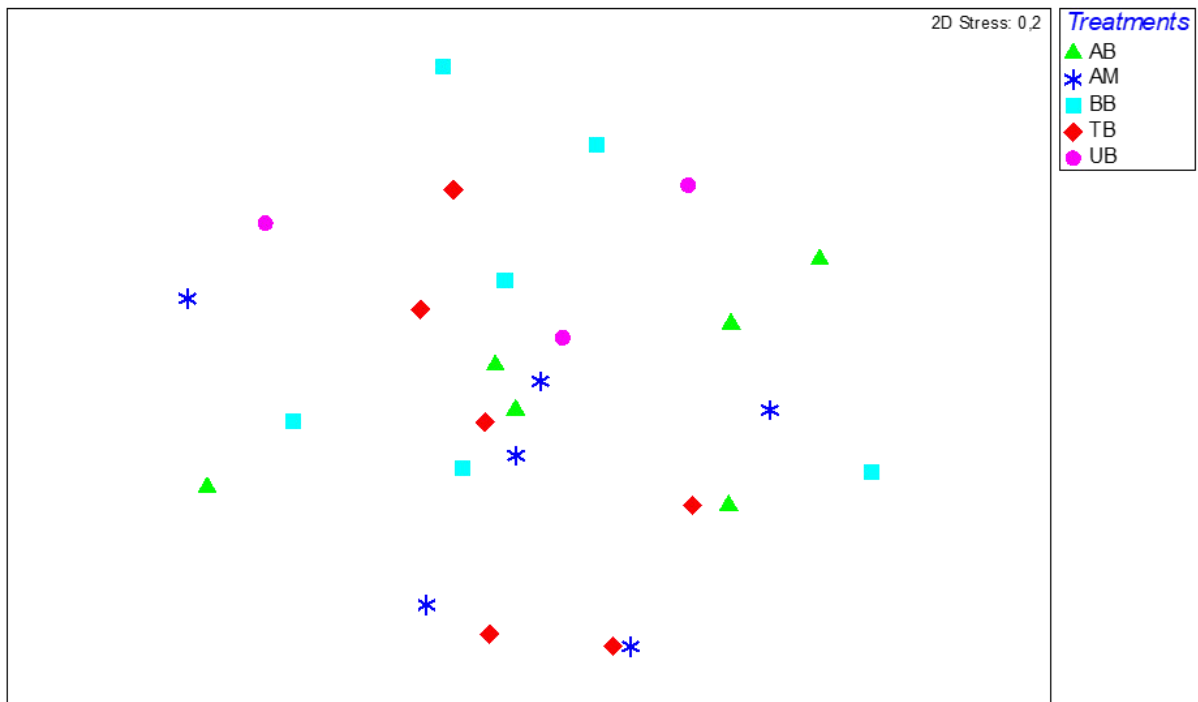
Figure 2.6: Non-metric Multidimensional Scaling (nMDS) ordination of plots based on presence-absence data, showing differences between the frequency of treatments: Annual burnt (AB), annual mown (AM), biennial burnt (BB), triennial burnt (TB) burnt, and unburnt (UB) treatments on ant assemblage sampled at Ukulinga research farm, Pietermaritzburg.
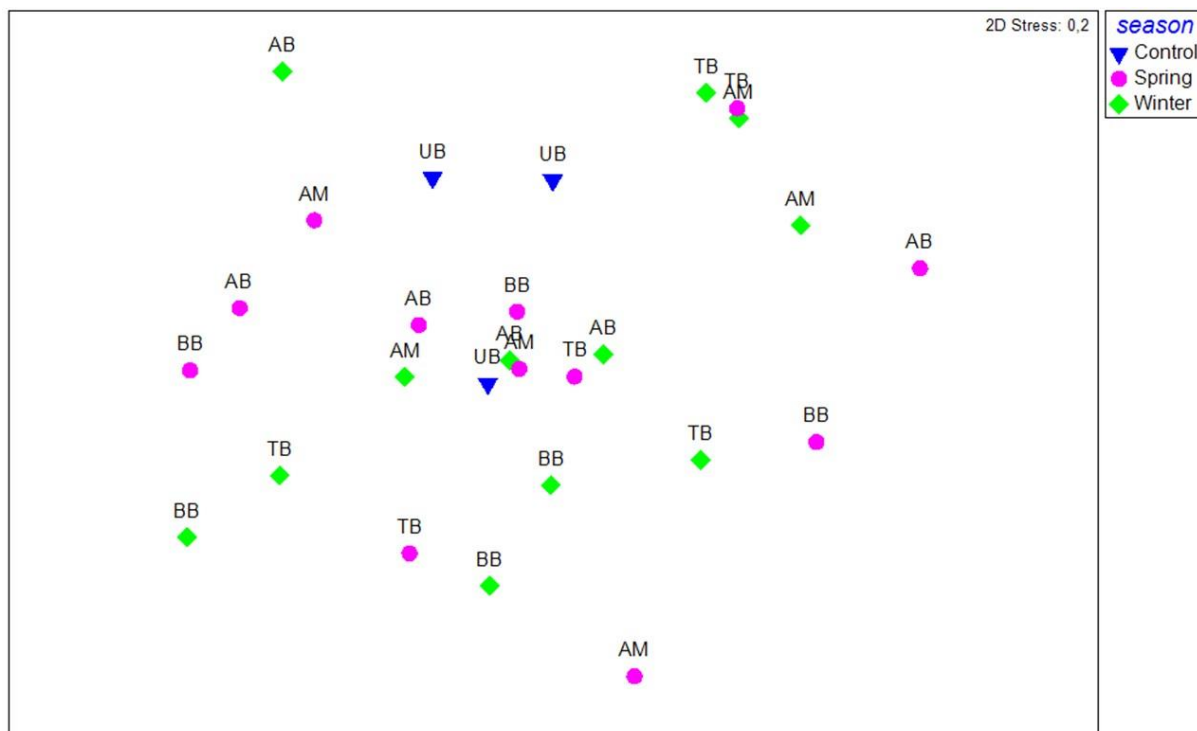
Figure 2.7: Non-metric Multidimensional Scaling (nMDS) ordination plots based on (data pooled across sampling season) showing differences between the season of treatments. The two-dimensional plot with stress-level = 0.2 was based on presence/absence. Annual burnt (AB), annual mown (AM), biennial burnt (BB), triennial burnt (TB) burnt, and unburnt (UB) plots.

2.4.4 Indicator species

IndVal analysis revealed that unburnt plot treatment had three indicator species, *Tetramorium* UKZN_19 (*squaminode* gp.), *Camponotus* UKZN_05 *(maculatus gp.),* and *Tetramorium nr grassi*, and four detector species, *Tapinolepis* UKZN_05, *Plagiolepis* UKZN_01, *Camponotus* UKZN_02 (*cinctellus* gp.), and *Crematogaster rufigena.* Biennial and triennial burn treatments each had one detector species (*Tetramorium* UKZN_07 (*setigerum gp.*) *and Tetramorium* UKZN_09 (*simillimum* gp.)) respectively. No species could be regarded as indicators for biennial burn and triennial burnt plot treatments. While no indicator nor detector species were found for annual burn and annual mown treatments (Table 2.2).

Table 2.2: Percentage indicator values for ant species with significant (p <0.05) IndVals for burning, mowing, and unburnt plot treatments (data pooled across the season of treatments) in the Ukulinga research farm. Species identified as indicators (IndVal >70%) are highlighted in bold, with others being detector (IndVal <70%) species. Significant level denoted by *p <0.05,

** p <0.01, *** p <0.001

Treatment type and Species	%IndVal	*p*-value
Unburnt		
Tetramorium UKZN_19 (*squaminode* gp.)	**78**	**0.005****
Camponotus UKZN_05 (*maculatus* gp.)	**77**	**0.005****
Tetramorium nr grassi	**73**	**0.008****
Tapinolepis UKZN_05	69	0.016*
Plagiolepis UKZN_01	64	0.031*
Camponotus UKZN_02 (*cintellus* gp.)	61	0.017*
Crematogaster rufigena	53	0.048*
Annual burn		
Crematogaster rectinota	40	0.009**
	-	-
Annual mow		
Biennial burn		
Tetramorium UKZN_07 (*setigerum gp.*)	59	0.037*
Triennial burn		
Tetramorium UKZN_09 (*similimum* gp.)	59	0.023*

2.4.5 Ant assemblage composition

The Canonical Correspondence Analysis (CCA) model was non-significant ($\chi^2 = 1.163$, F= 0.838, *P* = 0.812; Fig. 2.8).
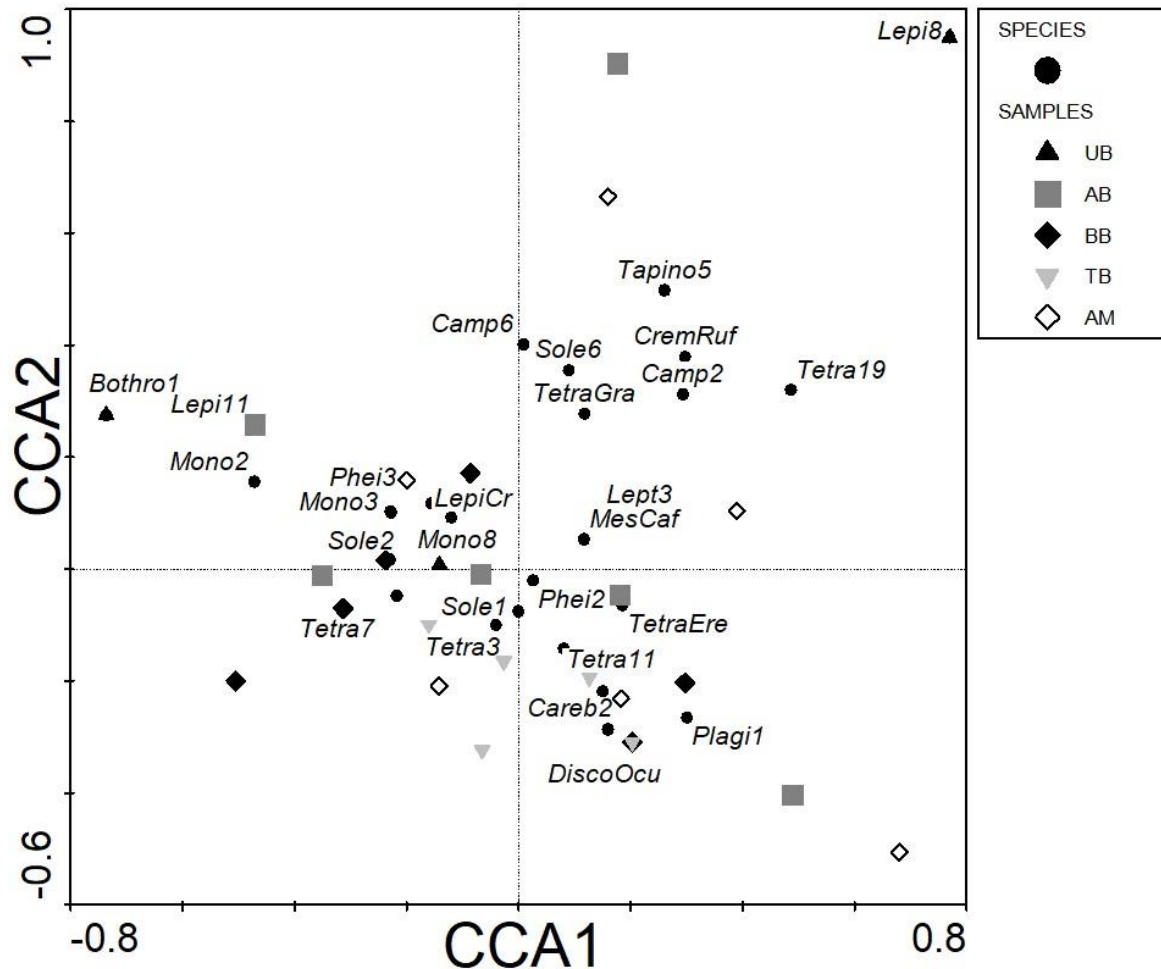
Figure 2.8: Canonical Correspondence Analysis (CCA) ordination plot of species and replicates for each of the sampled plots in the Ukulinga research farm. Ant species are represented as black circles and the corresponding labels are in italics. Treatments are represented by samples: UB - unburnt, AB - annual burn, BB - biennial burn, TB - triennial burn, and AM - annual mow. See Appendix D for the list of species abbreviations and used.

2.5 Discussion

Mean ant species richness was significantly higher in burnt treatments than in mown and unburnt plots. Contrary to the study presented here, Parr et al. (2004) found that burning did not affect mean ant species richness and abundance in savannas of Kruger Nation Park, South Africa. Dahms et al. (2005) reported that management type (mowing or grazing) did not affect species richness in the grasslands of Central Europe.

The findings in this study follow a consistent trend that has been reported in the tropical savanna in Australia (Andersen et al., 2006) where the number of ant species was constantly higher in burnt compared to unburnt habitats. Fire suppression in the unburnt plots had a greater impact on ant communities compared to burnt plots, this is because disturbance generally has

little effect on open habitat compared to closed habitats (Andersen, 2019). Low ant species richness in the unburnt plots is most likely due to tree canopy cover which influences ant foraging activities due to low temperatures. Lassau and Hochuli (2004) found that leaf litter, ground cover, and tree canopy cover were variables that negatively influenced ant species richness. Most ants are thermophilic (Hölldobler and Wilson, 1990), therefore open habitats such as burnt and mown plots are likely to have higher temperatures compared to closed and complex habitats with low temperatures which limit ant foraging activity.

The frequency of burning/mowing treatments did not affect mean species richness. These findings are consistent with those from other studies where fire frequency was not a critical factor influencing ant species richness and abundance (Parr et al., 2002; Parr et al., 2004). Furthermore, studies on the effects of mowing on ants have found varying results. For example, Heuss et al. (2019) found that mean species richness decreased with increasing mowing intensity, whereas, Dahms et al. (2005) found no effect of mowing intensity (mown once or twice per year). Studies have shown that burning in autumn supports greater ant species richness (Uys and Hamer, 2007). However, this was not the case in this study as the season of treatments did not affect ant species richness. Similar findings were also reported by Chambers and Samways (1998), whereby the season of burn had no difference in mean species richness and abundance of grasshoppers.

Based on the principles of disturbance proposed by Andersen (2019), canopy openness is a key driver of variation structuring ant communities. Canopy openness regulates the amount of light reaching the ground which mediates the impact on ant communities (Lessard, 2019). Closed canopy cover influences surface temperature and ant foraging activities due to the accumulation of litter which changes microclimate (Arnan et al., 2013). Change in microclimate is one of the indirect effects of habitat disturbance (Andersen, 2019) that affects habitat suitability of ants, whereby ant species adapted to open habitats are reduced because such ant species forage at a certain optimum temperature (Cerda et al., 2013).

Ant assemblage composition was not correlated with any of the measured soil variables. These findings suggest that soil pH, organic matter, clay content, acid saturation, and soil depth were not important factors for structuring ant assemblages in this study. This is surprising because these variables have been reported to influence ant assemblage composition in southern Africa. Munyai and Foord (2015b) found that percentage clay was significantly associated with high species density in Soutpansberg mountain. Similarly, Parr (2008) also reported that percentage

clay had a negative effect on the dominance level of dominant ant species in Kruger National Park. Other studies have found varying results, for example, Van Gils et al. (2010) found that the presence of leafcutter ant (*Atta sexdens*) nests in the Colombian Amazon were negatively correlated with pH, canopy cover, and soil resistance. In the savanna of northern territory in Australia, variation in species composition in burnt and unburnt plots was linked with sampling time and soil type than fire treatments (Hoffmann, 2003). These findings suggest that underlying factors that were not considered in this study influence ant assemblage composition. Temperature and ground cover are some of the environmental factors that have been hypothesized to affect ant distributions (Wiescher et al., 2012).

All burnt plots had detector species and no indicator species. Only three species (*Tetramorium* UKZN_19 (*squaminode* gp.), *Camponotus* UKZN_05 (*maculatus* gp.), and *Tetramorium nr grassi*) were identified as indicators for unburnt plots, which can also be used to monitor the response of ants to burning. *Camponotus maculatus* has nocturnal foragers (Andersen et al., 2006), which may be a strategy to avoid interactions with diurnal dominant ants. However, the occurrence of *C. maculatus* in unburnt plots is most likely related to thermal temperature and ant dominance. The presence of *Tetramorium* species in unburnt plots as indicator species is most likely associated with their habitat preference because most species in this genus are known as generalized foragers that build their nest in leaf-litter, decaying wood, and into the soil (Brown Jr, 2000). However, this varies among *Tetramorium* species because not all of them prefer shady habitats since they have wide habitat distributions and are mainly associated with areas that support low ant diversity (Andersen, 1997; Andersen et al., 2006).

2.6 Conclusion

Ant assemblages have been documented to be resilient and resistant to fire (Andersen and Muller, 2000; Parr et al., 2002; Hoffmann, 2003). Keeping with other studies, and assemblages in this study were highly resistant to burning and mowing, this was evident for both frequency and season of treatments. Similar responses by ants have been documented elsewhere. For example, Parr and Andersen (2008) reported no significant difference in the overall ant assemblage composition. Ant assemblages in South African savanna (Parr et al., 2002), and rangelands of Australia (Hoffmann, 2003) were also found to be resistant to fire with relatively few species showing clear and significant responses to fire. Findings in this study have clearly shown that there are other underlying factors responsible for structuring ant assemblage composition at Ukulinga. Parr et al. (2004) suggested that high resilience and resistance of ant assemblages were responses most likely to changes in vegetation habitat structure, and the

long-term history of burning. Given the long-term history of burning and mowing in this grassland, ant species present in these plots might have evolved various ways to cope with burning and mowing, which may explain the high resilience of ant assemblages.

Other invertebrate or vertebrate taxa are most likely to respond differently to burning or mowing. Therefore, caution when burning or mowing should be considered because it may affect other invertebrate or vertebrate taxa differently compared to the effects on ants. Removal of biomass through burning and mowing exposes ground cover to high temperatures compared to unburnt plots with canopy cover which may influence ant foraging activity due to temperature differences. Therefore, for future studies, it is important to assess the amount of temperature that reaches the ground between burnt and mowing treatments because this may have an impact on ant foraging activity and structuring ant assemblage composition. There is also a need to assess ant functional traits that may be responsible for structuring ant assemblage composition in relation to their adaptive traits to burning and mowing treatment.

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CHAPTER 3: Responses of a grassland ant community to longterm nitrogen fertilization

3.1 Abstract

Land-use intensification is one of the main drivers of species diversity loss. Grasslands harbour a high species diversity but are threatened by forever increasing grassland management practices, including nutrient addition. However, the effects of nutrient addition on faunal assemblages are not well understood. This study made use of a long-term grassland fertilization experiment to (i) determine how ant species richness and composition vary with two different types of fertilizer, (ii) identify ant species contributing to the differences between treatments, and (iii) assess whether the response of ant assemblage composition is related to grassland structure and composition. Fertilization can affect ants through disturbance of nesting site with machinery, and indirectly through effects on plants, leading to changes in ground temperature due to increased plant biomass. We sampled 15 plots, each 9 m x 2.7 m in size and with 1 m walkways between them. The treatments consisted of control and two forms of N, limestone ammonium nitrate (LAN, 28%N) and ammonium sulphate (AS, 21%N), each separately applied annually at two levels (high and low): LAN (0.21 and 63.2 g m^{-2}), and AS (33.6 and 100.8 g m^{-2}) for more than 60 years. Each of the five treatments had three replicates, with sampling conducted using pitfall traps during wet (March 2019) and dry (July 2019) seasons. A total of 35 ant species from 18 genera were recorded, with *Tetramorium* (8 species) being the richest genus. Nitrogen fertilization had a significant effect on ant abundance with little effects on species richness. Mean ant species richness significantly increased (from 17.3 in AS_low to 18.2 in AS_high) with an increasing level of N fertilization in ammonium sulphate treatments. High levels of fertilization had a significant effect on ant assemblage composition. One of the five common species responded to nitrogen fertilizer. Differences in ant species richness and assemblage composition were neither related to bare ground cover nor aboveground biomass. When considering grassland management, low levels of N fertilization are suitable fertilizers to increase habitat heterogeneity, thereby increasing resource availability. We suggest that biodiversity conservation be considered at a landscape scale rather than plot level for greater ant diversity.

Keywords: Anthropogenic impacts; fertilization; nitrogen; nutrient addition; species richness.

3.2 Introduction

Grasslands are complex ecosystems that are beneficial to society, through the provision of various ecosystem services (i.e., provision, regulating, cultural, and support), and they play an important role in carbon storage, and climate regulation (Fornara and Tilman, 2012; Rumpel et al., 2015). Grasslands were traditionally used for biomass production for livestock and food security, but the increase in population resulted in an increasing demand for food supply, thus making the use of fertilizers essential for global food security (Powlson et al., 2011; Vargová et al., 2020). However, increased land-use intensity has led to further damage to grassland systems. Nutrient addition is an important component of land-use change globally, which affects both plant and animal communities (Bujan et al., 2019).

Nutrient availability and production are two important determinants of plant community structure (Grime, 1979). Nitrogen (N), phosphorus (P), and potassium (K) are important soil nutrients that generally occur in low quantities in plant tissues (Sterner and Elser, 2002). These nutrients are incorporated with inorganic fertilizers as a grassland management practice (Sibanda et al., 2017). This includes applying organic and inorganic fertilizers such as ammonium nitrate and ammonium sulphate, in combination with phosphorus and lime fertilizers (Sibanda et al., 2017). The use of inorganic fertilizers in grasslands (like fooder crops) is a common agricultural practice used to increase the fertility of the soil for vigorous plant growth, improved nutritional quality, and enhance grass biomass and overcome nutrientlimited production (Tsvuura and Kirkman, 2013).

Experiments on fertilizer application in grasslands have shown that plant diversity decreases with nutrient addition, particularly nitrogen (Tsvuura and Kirkman, 2013). Effects of fertilization on grassland vegetation generally result in increased biomass production, which favours fast-growing plant species (Socher et al., 2013), and compositional change, which is more pronounced in forbs than grasses (Grime et al., 2000). Changes in species composition favours highly productive and competitive plants (Clark et al., 2007; Cleland and Harpole, 2010), thereby reducing overall species richness due to competitive exclusion. For example, Fynn and O'Connor (2005) had previously found that shorter grasses such as *Themeda triandra* were replaced by taller grasses such as *Panicum maximum* due to fertilization. Similar findings were also reported by Ward et al. (2017b), whereby *Themeda triandra* and *Tristachya leucothrix* declined in response to nitrogen fertilization. A lot has been done on how plants

respond to nutrient addition (Fynn and O'Connor, 2005; Craine et al., 2008; Tsvuura and Kirkman, 2013; Ward et al., 2017b), with very few studies (Van der Wal et al., 2009) investigating the response of nutrient addition on faunal communities.

Just like application of nutrients affects plant species richness, plant productivity, plant composition, and plant tissue quality, faunal communities are most likely to show a similar response. For example, nitrogen addition decreased insect species richness and increased insect abundance in Cedar Creek in the United States of America (Haddad et al., 2000). Belowground soil biota (collembolans and enchytraeids) were reported to increase with increasing plant biomass following nutrient supply in a long-term fertilizer experiment in the Netherlands (Van der Wal et al., 2009). Nitrogen addition has been identified as a contributing factor that decreases soil microbial respiration and biomass, resulting in shifts in soil microbial community composition (Ramirez et al., 2010; Pan et al., 2014). According to Kaspari et al. (2017) increased in N supply suppresses microbial activity and the abundance of invertebrate diversity.

Ants are some of the most crucial organisms that provide various ecosystem services on earth (Andersen et al., 2002). They are highly diverse and abundant, making them important ecosystem engineers (Jouquet et al., 2006; Del Toro et al., 2012). Ants alter plant communities through seed dispersal (Handel and Beattie, 1990), seed consumption (Dauber et al., 2006), and predation of herbivorous insects (Sanders and van Veen, 2011). Additionally, ants significantly impact the physical, chemical, and biological properties of the soil by altering nutrients, organic matter, and carbon within their nests (Cammeraat and Risch, 2008). Ants build subterranean nests that generally improve/modify soil structure, thereby increasing plant diversity (Del Toro et al., 2012).

Fertilization directly affects ants, through the destruction of nesting sites with machinery application of fertilizers (Heuss et al., 2019). Effects of nutrient addition on ant species richness, abundance, and assemblage composition are mostly indirect, through the effects on plants. These effects are complicated because changes in plant community may have different effects on ant species richness. High nitrogen fertilizer results in higher tissue quality, which changes the nutritional quality of plants eaten by ants such as leaf-cutting ants (Farji-Brener and Ghermandi, 2008) and seed harvesting ants. Nitrogen fertilizer may also indirectly affect ants through changes in habitat structure following increased plant biomass which affects the habitat suitability of ants (Arnan et al., 2013). However, an increase in biomass production can

also offer food resources to a greater number of species. For example, plants with high biomass are most likely to attract insect species such as honeydew-producing insects (e.g. aphids) and herbivore species thereby increasing the number of ant species feeding on honeydew (Wills and Landis, 2018). Effects of nutrient addition on ants are complex, making it difficult to predict ant community responses to nitrogen addition.

Among the few studies worldwide which investigated the response of ants to nutrients addition, this includes Dahms et al. (2005) in Germany the latter strudy foud that nitrogen had a strong influence on ant species richness; Jacquemin et al. (2012b) in Ecuado found that response of ants to nutrient sipply varied according to their trophic groups, whereby density of omnivores, fungus and nectar eaters did not change compared to predatory ants that showed a major decrease, and Bujan et al. (2019) found 48% lower nest densty in plots where nitrogen was added compared to those that did not receive nitrogen in the Republic of Panama. Therefore, this study used a long-term fertilizer trial experiment in Ukulinga Research Farm to assess the effect of long-term nitrogen fertilization on ant communities. The objectives of the study were to (i) determine how ant species richness and composition vary with two different types of fertilizer, and (ii) assess whether the response of ant assemblage composition is related to grassland structure and composition.

3.3 Materials and Methods

3.3.1 Study site

This study was carried out at Ukulinga Research Farm (29°40'10.70"S 30°24'6.51"E) of the University of KwaZulu-Natal in Pietermaritzburg, South Africa. The nutrient addition trial lies on top of a plateau with an altitude ranging from 838 m to 847 m a.s.l. Soils in this area consist of Ecca group shales of the Karoo sedimentary sequence (Mucina and Rutherford, 2006). The soil depth varies from 500 to > 1000 mm. The vegetation is classified as the Southern Tallgrass veld Sub-Escarpment Savanna (Mucina and Rutherford, 2006), dominated by *Themeda triandra*, *Hyparrhenia hirta*, *Tristachya leucothrix,* and *Panicum maximum*. The mean annual precipitation in this area is 790 mm, occurring mostly from October to April. Mean monthly minimum and maximum temperatures range from 8.8°C in July and 26.4°C in February.

3.3.2 Effect of N-fertilization on plant

To interpret the impact of fertilizers on ant assemblages, we used existing grass species composition, biomass, and forb species composition data (Appendix E) collected in

September-November 2019 available in the School of Life Sciences, University of KwaZuluNatal, South Africa. Effects of fertilizer treatments on vegetation show that the form of N has a strong effect on forb species richness (P<0.001) but not on grass species richness (P>0.05) (Appendix F). Forb and grass species decreased with increasing N concentration. Whereas, plant biomass was much lower in high levels of nitrogen fertilization. However, there was no variation in percentaege biomass cover and bare ground cover in relation to fertilizer treatments (Appendix G).

3.3.3 Experimental design

The experiment consisted of 15 plots, each 9 m x 2.7 m in size with 1 m walkways between plots. Treatments consisted of control and two forms of N, limestone ammonium nitrate (LAN, 28%N) and ammonium sulphate (AS, 21%N). They are effective nitrogen fertilizers used to increase yield and protein content in plants (Fynn and O'Connor, 2005). LAN is easily soluble and has the lowest acidifying effect on the soil due to the nitrogen content which is readily taken up by plants compared to ammonium sulphate which accelerates soil acidification (Eckard, 1990). These fertilizers are applied annually at two levels (high and low). Half of the application of N is applied in spring and the other half in mid-summer. The application rate for LAN (0.21 and 63.2 g m^{-2} for low and high respectively) and AS (33.6 and 100.8 g m^{-2}). Treatments are applied randomly in three blocks (Fig. 3.1). Abbreviations used for the two forms of N treatments were: AS_low = AS33.6; AS_high = AS100.8; LAN_low = LAN0.21; LAN_high = LAN63.2.
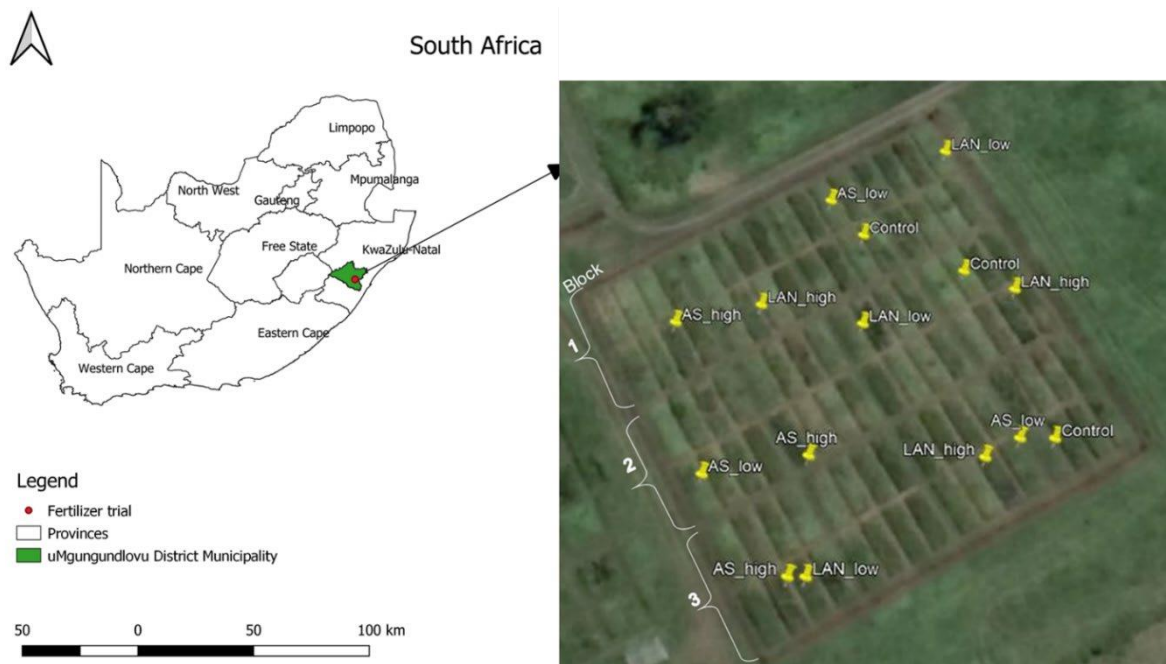
Figure 3.1: Google Earth image showing South African map and the layout of fertilizer treatments (AS_low =Ammonium sulphate_low; AS_high = Ammonium sulphate_high; LAN_low = Limestone ammonium nitrate_low; LAN_high =Limestone ammonium nitrate_high; and control plots) sampled at Ukulinga research farm in KwaZulu-Natal in uMgungundlovu District Municipality

3.3.4 Ant sampling

A standardized pitfall trapping (100 ml) method was used to sample ants during the wet (March 2019) and dry (July 2019) seasons. Each plot consisted of four traps with 2 m spacing between traps placed in the centre of each plot. Pitfall traps contained 50% propylene glycol solution (which does not repel nor attract ants (Munyai and Foord, 2015b)) as a preservative and were left open for five days. Samples were processed and washed in the laboratory and stored in 70% ethanol. Ants were then pinned and identified to species level where possible otherwise identified to the genus level and assigned to morphospecies. Pinned voucher specimens will be deposited in KwaZulu-Natal and Iziko Museum.

3.3.5 Data analysis

All analyses were conducted on combined data across the two sampling seasons. All analyses for species composition were analyzed based on presence and absence data.

Individual-based rarefaction curves were computed in iNEXT online to estimate species richness and inventory completeness using the coverage estimator. The 95% confidence intervals were obtained based on the bootstrap method with 120 replication. (Chao et al., 2016).

Generalized Linear Mixed Models (GLMM) using a loglink function and Poisson error distribution were used to test the effects of nutrient addition on ant species richness, abundance, and the abundances for each of the most common species. A generalized Linear Model (GLM) was also used to fit occurrence of individual species common enough for analysis. Only species with >50 abundance occurring across burning/mowing treatments were considered for analysis. Fertilizer treatments were included in the model as fixed factors while replicates were included as random factors in the model to account for temporal pseudoreplication. The GLMM analysis was performed using the vegan and multcomp package in R studio version 3.5.1 program (Team, 2017).

Multivariate analyses were undertaken using the PRIMER v.6.0 software program (Clark and Gorley, 2006) to assess overall changes in ant assemblage composition between treatments at the plot level. The Bray-Curtis similarity index was used to determine differences in assemblage structure between fertilizer treatments. Data were square-root transformed prior analyses to reduce the weight of common species. Analysis of Similarity (ANOSIM) with 999 permutations was used to test for any significant differences in ant assemblage composition between fertilizer treatments. ANOSIM generates the Global R statistic which can be used to quantify the similarity of assemblages being compared. The closer the significant Global R is to one, the more distinct the differences are between the assemblages that are being compared. A non-Multidimensional Scaling (nMDS) ordination was used to visualize the patterns of assemblage composition between fertilizer treatments.

A Canonical Correspondence Analysis (CCA) from the CANOCO version 4.5 program (Ter Braak and Smilauer, 2002) was used to correlate ant community composition with grass species composition, vegetation (biomass), and ground cover (bare ground). Based on direct gradient analysis, CCA extracts the major patterns in the data that are accounted for by the measured variables only. Species abundance data was not transformed, but an option of 'down-weighting of rare species' was selected. The variation in ant species composition explained by the measured variables was non-significant. Hence, no forward selection of variables was used. Only species with a variability of more than 10% were included in the ordination tri-plot.

3.4 Results

3.4.1 Species abundance and richness

A total of 2 191 individual ants comprising 35 species from 18 genera and 4 subfamilies were collected during the study. A total of 17 species were recorded in the dry season and 34 species in the wet season. The richest genera were *Tetramorium* (8 species) and *Monomorium* (4). The most abundant species were *Crematogaster rectinota* (51.8% total ants collected), *Pheidole* UKZN_02 (*megacephala* gp.) (20.5%), and *Leptogenys* UKZN_03 (8.4%). Species rarefaction curves based on individuals approximated an asymptote, with a sample coverage > 0.84 for all fertilizer treatments (Fig. 3.2). Overall ant sampling, species richness was greater in ammonium sulphate (AS_low and AS_high) treatments (25 and 22 species respectively) compared to LAN treatments and the control, which had the same number of species (19 species). However these differences were non-significnt as the confidence intervals (Cis) of the curves are overlapping (Fig. 3.2). Species evenness ranged between 0.1 to 0.7, with little variation between treatments, with no significant (P > 0.05) differences between (Table 3.1).
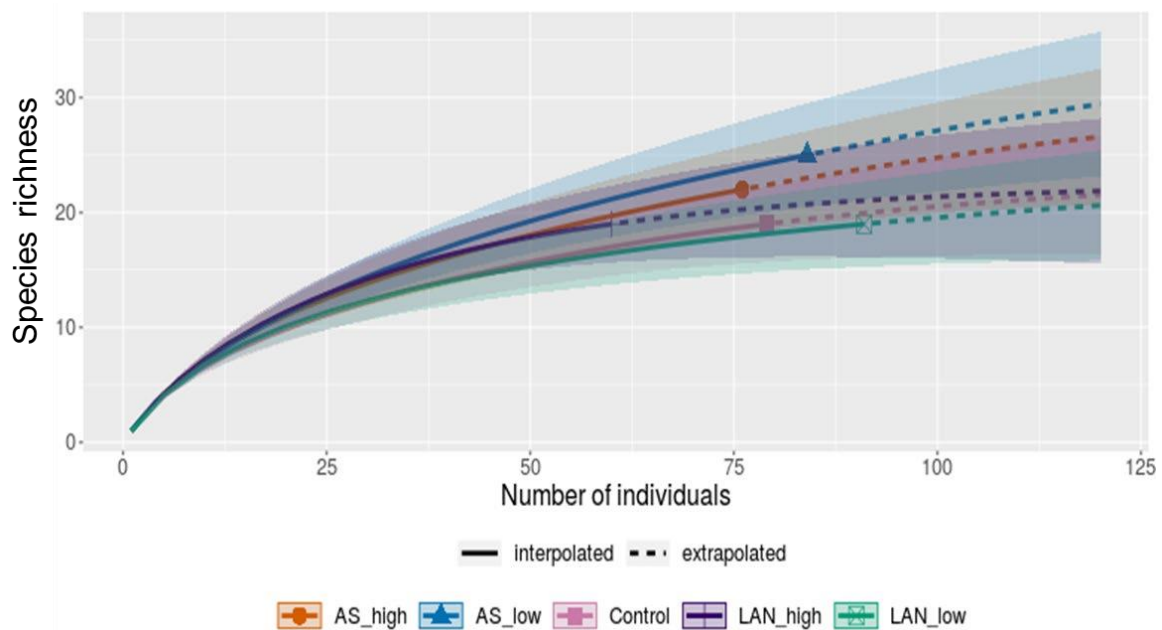
Figure 3.2: Cumulative species richness curves depicting how the sample completeness (measured by sample coverage) increases with sample size in each of the fertilizer treatments. accumulation curve with a 95% confidence interval. The solid lines represent interpolated samples and dashed lines are extrapolated up to a maximum sample size of 120 based on bootstrap estimation.

Table 3.1: Abundance, species richness, and diversity measure indexes (Dominance_D, Simpson_1-D, Shannon_H, Evenness_e^H/S) calculated for fertilizer treatments

Treatments	Abundance	Richness	Dominance_D	Simpson_1_D	Shannon_H	Evennes_e^H/S
Control 1	169	11	0.465	0.535	1.178	0.295
Control 2	276	12	0.419	0.580	1.187	0.273
Control 3	104	9	0.410	0.589	1.193	0.366
AS_low 1	232	17	0.479	0.520	1.257	0.206
AS_low 2	62	9	0.426	0.573	1.308	0.410
AS_low 3	57	14	0.201	0.798	2.001	0.528
AS_high 1	189	10	0.342	0.657	1.417	0.412
AS_high 2	186	19	0.572	0.427	1.208	0.176
AS_high 3	71	9	0.504	0.495	1.101	0.334
LAN_low 1	246	12	0.679	0.320	0.841	0.193
LAN_low 2	189	12	0.291	0.708	1.653	0.435
LAN_low 3	141	8	0.484	0.515	1.069	0.364
LAN_high 1	135	13	0.402	0.597	1.377	0.304
LAN_high 2	36	12	0.137	0.862	2.213	0.761
LAN_high 3	98	9	0.773	0.226	0.593	0.201

Mean ant abundance was significantly lower in LAN_high fertilizer treatments than in other treatments, which varied significantly from each other, with the highest abundance recorded in LAN_low (30.333 ± 5.686), and control plot (26.333 ± 7.571) treatments (Fig. 3.3a, Table 3.2). Mean species richness was significantly high in AS_low than other treatments which showed no variation in relation to fertilization (Fig. 3.3b, Table 3.2).
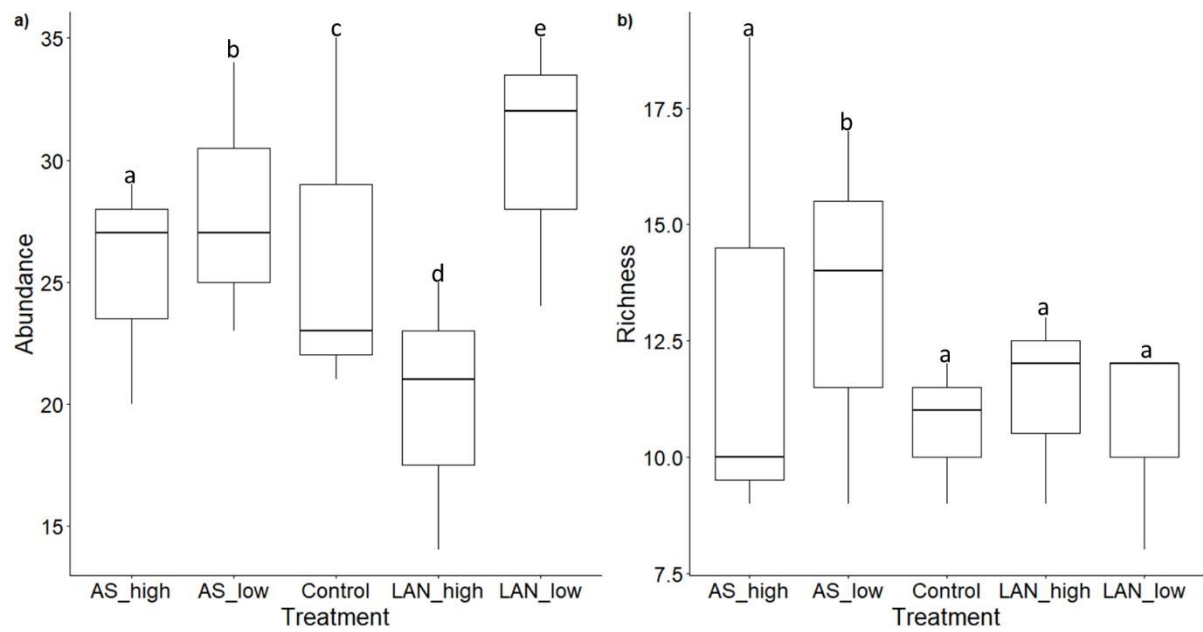
Figure 3.3: Boxplot representing mean, standard deviation (SD) and standard error (SE) of ant abundance (a) and species richness (b) across the fertilizer treatments: AS_high = ammonium sulphate; AS_low = ammonium sulphate; LAN_high = limestone ammonium nitrate; LAN_low, sampled at Ukulinga research farm, Pietermaritzburg, South Africa. Treatments with identical letters are not significantly different at the level $P < 0.05$ (Tukey HSD-Test).

Table 3.2: Summary results of generalized linear mixed models for total ant species richness and abundance between fertilizer treatments. *Indicates significant level for P < 0.05

Factors	Estimate std.	Error	z value	Pr(>|z|)
Abundance				
Intercept	5.001	0.04	105.63	< 2e-16 ***
AS_low	-0.24	0.07	-3.36	0.000 ***
Control	0.21	0.06	3.26	0.001**
LAN_high	-0.25	0.06	4.05	5.00e-05***
LAN_low	0.51	0.07	-6.55	5.78e-11***
Richness				
Intercept	0.78	0.17	4.49	7.04e-06 ***
AS_low	-0.47	0.07	-6.31	2.73e-10 ***
Control	0.003	0.06	0.05	0.958
LAN_high	-0.05	0.08	-0.74	0.46
LAN_low	0.09	0.06	1.44	0.15

Of the five species common enough for statistical analysis, *Leptogenys* UKZN_03 was the only species that varied significantly with fertilizer treatments. *Leptogenys* UKZN_03 showed a high preference for control and LAN_low treatments with no individuals recorded in LAN_high treatment plots (Fig. 3.4). The occurrence of the other four species did not vary with fertilizer treatments.

3.4.2 Ant species composition

LAN_low fertilizer treatments were dissimilar to other fertilizer treatments, whereas all the other treatments including control treatments were similar in composition . Pairwise ANOSIM test revealed differences in ant assemblage composition between LAN treatment plots rather than between AS treatments. Ant assemblages in LAN_high were significantly (R =0.245, P = 0.024; Fig. 3.5, Appendix G) different from that of LAN_low, and AS_high was also significantly different from LAN_low (R = 0.256, P = 0.04; Fig. 3.5, Appendix G). Ant assemblage composition in control plots was different from that of AS_high, but these differences were not significant (R = 0.219, P = 0.067; Fig. 3.5, Appendix H)
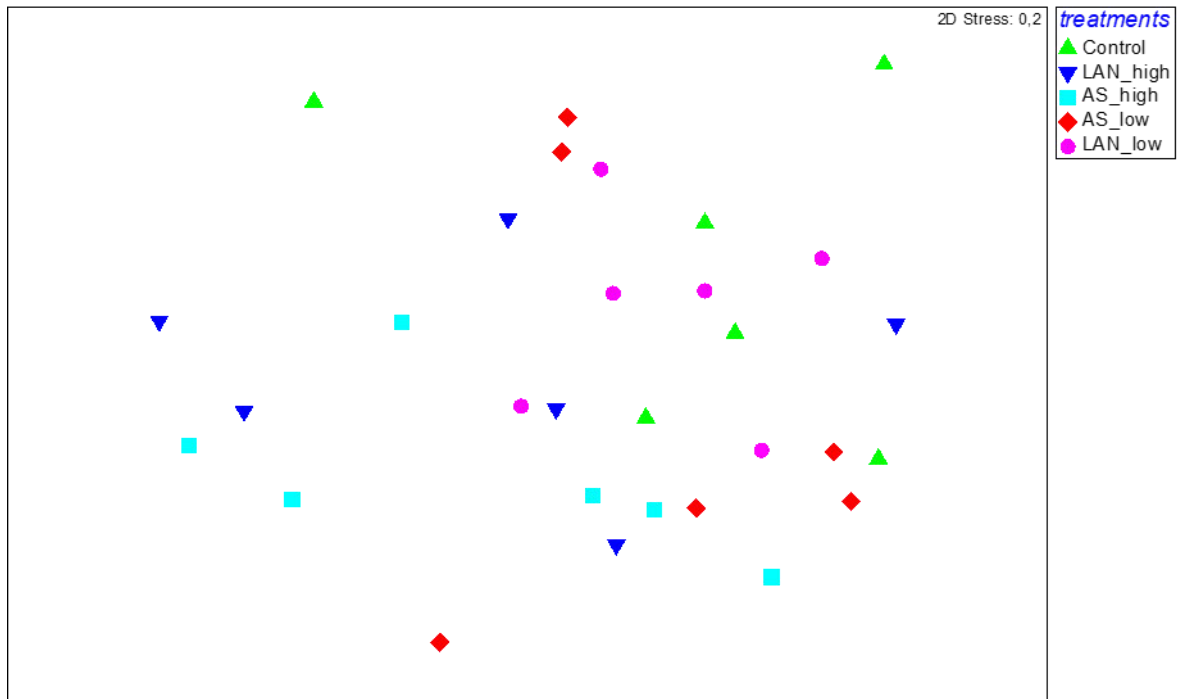
Figure 3.5: nMDS ordination of fertilizer treatments based on ant species composition (abundance data) sampled at Ukulinga research farm, Pietermaritzburg. Key to fertilizer treatments: LAN_low = limestone ammonium nitrate_low; LAN_high = limestone ammonium nitrate_high; AS_low = ammonium sulphate _low; AS_high = ammonium sulphate _high. Overall species composition did not vary with aboveground biomass and bare ground variables. There was no variation in ant species with grass species composition or with aboveground biomass and bare ground cover ($\chi^2 = 1.52$, F = 1.247, P = 0.24; Fig. 3.6).
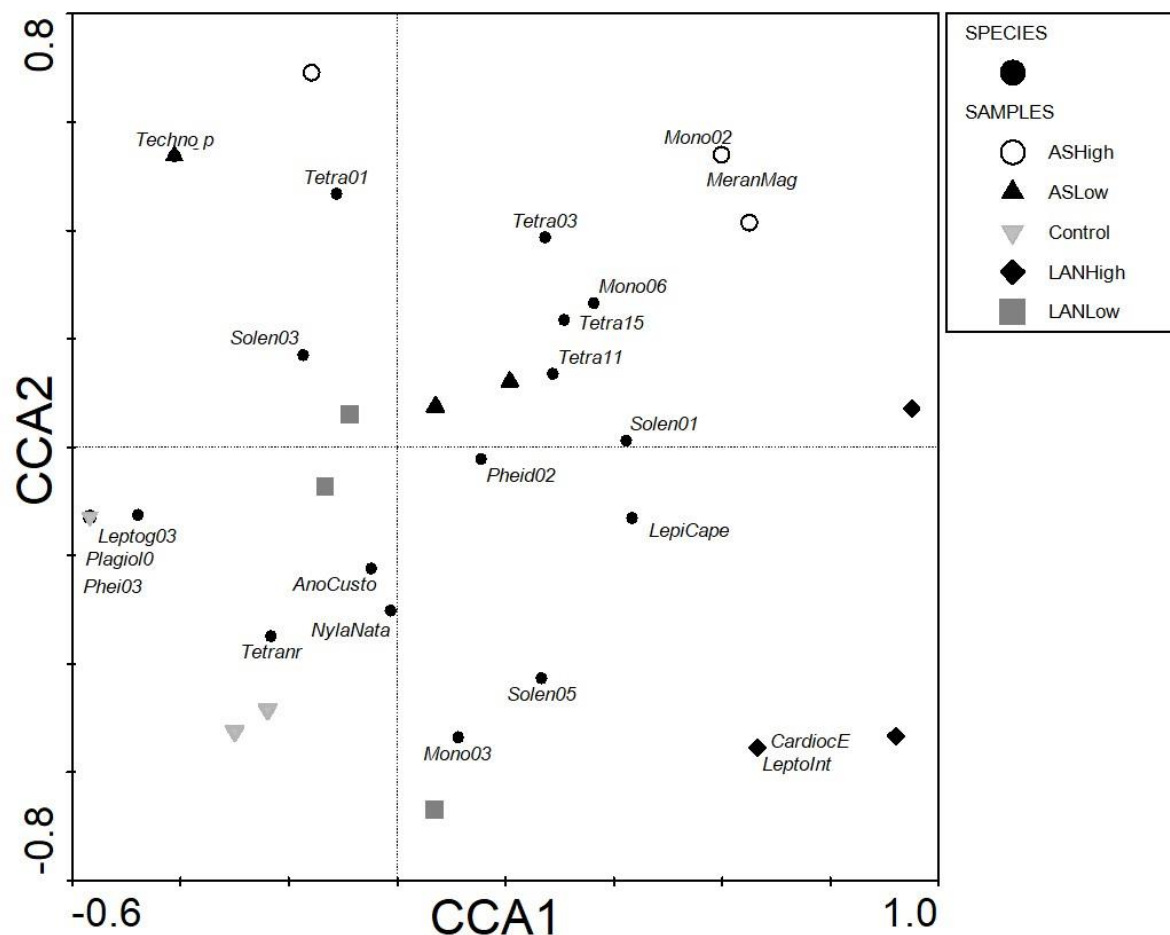
Figure 3.6: Canonical Correspondence Analysis of ant species composition and five nitrogen fertilization treatments (samples) in the Ukulinga research farm in KwaZulu-Natal, Pietermaritzburg, South Africa. Ant species are represented by black circles and the corresponding labels in italics. Full species names are given in Appendix I.

3.5 Discussion

This study investigated how ant species richness and composition vary with the two types of nitrogen fertilizer, identify ant species that contribute to these differences, and whether responses of ants to fertilization are related to grass species composition, aboveground biomass, and bare ground cover. We found strong effects on ant abundance, but very limited effects on ant species richness and assemblage composition. Five common species (Fig. 3.4) contributed to the differences in ant assemblage composition. Responses of ants to fertilization were neither related to aboveground biomass production nor bare ground cover.

Our results revealed that a low level of ammonium sulphate significantly had a positive effect on ant species richness. Contrary to other studies (Dahms et al., 2005; Pihlgren et al., 2010;

Heuss et al., 2019) that reported fewer ant species in fertilized treatments, linked to increased growth of taller plant species. In general, nitrogen increases plant growth, favouring fastgrowing plant species, decreasing ground exposure to the sun (Socher et al., 2013), thus reducing soil temperature, which is most likely to reduce the occurrence of ants species adapted to open habitats (Heuss et al., 2019). This had a strong effect on ant abundance, which varied significantly between treatments. Ant abundance responded positively to low levels of Nfertilization and control treatments, but high levels of N-fertilization significantly reduced ant abundance. An increase in above-ground biomass usually leads to changes in habitat structure and competition which generally leads to the dominance of competitive species and exclusion of non-competitive species (Andersen, 1992).

Out of the five common species, *Crematogaster rectinota*, *Pheidole* UKZN_02 (*megacephala* gp.), and *Leptogenys* UKN_03 were the most common species contributing to high ant abundance between treatments. *Crematogaster rectinota* and *Pheidole* UKZN_02 (*megacephala* gp.) occurred in high abundance and were not influenced by fertilization. These ant species are mostly found in highly disturbed habitats and are known to be disturbancetolerant (Seifert and Pannier, 2007). It is not surprising that these species were abundant in highly fertilized plots, because they are very common in temperate grassland and have a wide distribution range, and are known to be unaffected by grassland management. They tend to outcompete and control ant assemblage species composition in the habitats they occupy (Grill et al., 2008). Whereas species like *Leptogenys* UKZN_03 were significantly influenced by the level of N-fertilization and occurred in low abundance. The *Leptogenys* species are specialist predators with specialized diets which makes them less competitive and occurring in low abundance (Andersen, 1995).

Differences in ant assemblage composition were observed between control plot treatments and high levels of N-fertilization. The abundance of nesting sites (Abensperg-Traun et al., 1996), differences in microclimate and food resources (Armbrecht and Perfecto, 2003) are important factors that determine ant community responses (Andersen, 2000). The CCA (Fig. 6) showed that ant species adapted to open habitats were associated with control plots, indicating that open habitat species are dependent on unfertilized plots, corresponding with the findings obtained by Pihlgren et al. (2010). Vegetation cover, bare ground cover, and leaf litter percentage have been described as important factors influencing ant assemblage composition (Parr et al., 2004; Gibb and Parr, 2013). However, in this study responses of ant assemblage

composition were neither related to aboveground biomass and bare ground cover nor grass species composition.

Given the size (1 m apart) and proximity of the plots and the spacing between traps, there is a high possibility of a species community homogenization. Based on the long-term fertilization of these plots, there is a high possibility that ant species in this grassland are resistant to nutrient addition. This is because ant species living in constantly used grasslands can tolerate large climatic variations, such as changes in ground temperature and soil humidity (Seifert, 2017). Moreover, disturbance has little impact on habitat structure in open habitats compared to closed habitats (Andersen, 2019), thus leading to resilience in ant communities.

3.6 Conclusion

To our knowledge, this is the first study analyzing the effect of N-fertilization (from low to high level of N fertilization) on ant assemblage composition in Southern Africa. Increasing the concentration of nitrogen fertilization led to a slight reduction in ant species richness and abundance. Effects of N-fertilization were weaker on ant assemblage composition compared to plant communities, demonstrating that nitrogen addition was an important factor for plant community composition, and appears to be the main factor driving competitive interactions among grass species. Furthermore, this study has shown that ant assemblage composition in this grassland system is mostly likely resilient to nutrient addition, which is most common in open habitats due to recurring disturbance. In addition, it also led to species community homogenization. When considering grassland management, low levels of N-fertilization are the most suitable fertilizers to increase habitat heterogeneity (increase plant species) in grasslands, thereby increasing resource availability and reduce competitive exclusion between ant species. For maintenance of higher ant diversity, we suggest that biodiversity conservation be considered at a landscape scale, rather than plot level.

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CHAPTER 4: Conclusion and recommendation for further research

4.1 Introduction

Land-use intensification and disturbance are major threats to biodiversity, that may affect ecosystem functioning (Philpott et al., 2010). Ecologists and conservation managers have investigated the importance of fire, mowing, and nutrient application as important grassland management practices (Uys et al., 2004; Kirkman et al., 2014) and structuring and maintaining biodiversity. The most common management practices in grassland ecosystems, fire, mowing, grazing, and nutrient application have been documented mostly in southern Africa (Parr et al., 2002; Uys and Hamer, 2007). The impacts of these management practices on biological communities depend on the frequency, intensity, and time in which disturbance takes place (Kral et al., 2017; Arcoverde et al., 2018). The current study aimed to contribute to the knowledge of understanding disturbance dynamics of grassland ant communities by investigating the response of ant communities to fire, mowing, and fertilization.

The response of ant communities to disturbance is quite complex and differs with habitat type (Barrow et al., 2007). Responses of ant communities to fire are linked to changes in habitat type and structure (Barrow et al., 2007; Vasconcelos et al., 2017). For instance, species may increase in abundance following fire thereby increasing foraging activity in burnt than unburnt habitats. Some species may decline in abundance due to lack of resources and destruction of nesting sites during the application of fertilizers (Dahms et al., 2005) because most ants lay their nest above the ground (Hoffmann and Andersen, 2003). However, mowing disturbance is beneficial to other species because litter from mowing provides habitat resources like nesting sites and refuges amongst others (Humbert et al., 2009; Humbert et al., 2010). Therefore, understanding factors that drive responses of biological communities is of importance for maintaining biodiversity and conservation management strategies.

4.2 Revisiting the aims and objectives

The current study aimed to determine ant community responses to burning and mowing (Chapter 2). Results indicate that ant communities in this grassland are resistant to burning and mowing disturbances. These findings support the idea that ant communities that have a longterm history of burning and mowing are most likely to display high resilience and

resistance to these disturbances (Andersen and Muller, 2000). The latter suggest that ant communities in the study site are not affected by burning and mowing regimes. Findings in this study support Andersen (2019) principles of disturbance (principle 4 of habitat openness) that disturbance has little or no effect in highly disturbed environments. Since burning and mowing do not affect ants, this suggests that for grassland management and biodiversity conservation, caution should be taken into consideration when burning or mowing because other fauna taxa may be affected. The second aim of the study (Chapter 3) was to assess the effect of long-term nitrogen fertilization on ant communities. Findings show that ant species richness and abundance responded positively to nitrogen fertilization. Ant species richness increased with increasing levels of ammonium sulphate fertilizer treatments. High levels of both ammonium sulphate and limestone ammonium nitrate fertilizers influenced ant assemblage composition. Findings in the current study suggest that low levels of nitrogen fertilizers are ideal for increasing species diversity as they increase habitat heterogeneity and resource availability for species.

Habitat structure and soil variables are not important factors structuring ant assemblage composition in the current study site. This suggest that ants in the current study may be adapted to the current grassland management practices. It is, however, possible that other abiotic (e.g. temperature) and biotic (e.g. competition) factors not considered in this study may be responsible for structuring ant communities in this system. The findings of the study also suggest that the scale and size of the plots may be another factor to consider in order to determine the response of ant communities to these disturbances.

4.3 Contributions of the study

The current is one of many studies that have documented the responses of ant communities to disturbance in savanna and grasslands systems. Grassland biome is one the second most diverse in South Africa but is poorly managed and highly disturbed ecosystem (Mucina and Rutherford, 2006). Ants are highly responsive to environmental change and useful faunal groups to study the response of biodiversity to disturbance (Andersen and Majer, 2004; Underwood and Fisher, 2006). This study contributes to general knowledge about responses of ant communities to disturbance in grasslands and savanna ecosystems (Parr et al., 2002; Parr et al., 2004; Parr and Andersen, 2006, 2008; Philpott et al., 2010; Andersen, 2019). The findings in this study (Chapter 2) revealed that ant communities in this grassland system are resistant to burning and mowing regimes. The current findings concur with various studies in

South Africa, for example Parr and Chown (2003) and Parr et al. (2004). The latter studies reported that ant assemblages appear to be highly resilient and resistant to fire regimes and responses were linked to vegetation structure (Parr et al., 2002; Parr and Chown, 2003).

Although most studies in south Africa have investigated the response of invertebrate diversity to fire and mowing disturbances in grasslands, for example, Chambers and Samways (1998); Parr and Chown (2003); Parr et al. (2004), and Uys and Hamer (2007) the current study is the first to determine the effect of long-term fertilization on ant communities in managed grasslands (Chapter 3). To date, the only studies conducted on this topic so far are in Germany and Sweden grassland (Dahms et al., 2005; Pihlgren et al., 2010), Panamanian forested (Bujan et al., 2019) habitats. This study, therefore, contributes to the knowledge of how nutrient addition affects ants in managed grasslands. The current study has shown that ant species richness increases with an increasing level of ammonium sulphate fertilizer. The high species richness and distinct ant assemblage composition associated with ammonium sulphate suggest that there is a need to conserve this grassland. The findings of the study further suggests that for biodiversity conservation, low levels of nitrogen fertilization are suitable for greater ant diversity. The findings of the current study can be used to monitor the responses of ant communities in this long-term grassland experiment and broad grassland management as far as nitrogen fertilization is concerned.

4.4 Challenges and future possibilities

One of the challenges was to determine whether nutrient addition influenced soil pH and whether the temperature was a driving factor in structuring ant assemblage composition. Although this study did not measure temperature, its influence due to increasing biomass may influence ant communities. Because ants are highly responsive to temperature, it is important to investigate whether the temperature influences structuring ant communities. According to Arnan et al. (2014), ant communities vary in species composition in relation to functional diversity and the environment they occupy. Therefore, it is important to study how ant functional traits vary with disturbance regimes. In this study, only frequency and season of burning and mowing were considered. Future studies may consider the impact of intensity of these disturbances on ant communities to advance knowledge in the field. Because the abundance of nesting sites is one of the factors influencing ant communities (Abensperg-Traun et al., 1996). Therefore, future studies can use different sampling methods targeting hypogaeic ant species to determine whether the intensity of nitrogen fertilizers influences nesting sites,

which may impact species richness and abundance. There is a need to conduct studies at a landscape level using beta diversity to draw robust conclusions about the effects of disturbance and nutrient addition on ant communities. This will provide an insight for conservation mangers on how to maintain ant biodiversity.

4.5 Final comments and summary of the conclusion

Responses of ant communities to nutrient addition and disturbances depend on the impact which disturbance has on habitat structure. This study has shown that ant communities are highly resilient to nutrient application and disturbance. However, different taxon may respond differently to disturbance and nutrient addition.

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Appendices

Appendix A. Soil variables measured for each replicate of the five treatments. UB - unburnt, AB - annual burn, BB - biennial burn, TB - triennial burn, and AM - annual mow

Treatment	Depth	AcidSat%	pH	Organic content	Clay %
UB1	20	19	3.95	2.9	45
UB2	10	6	4.2	2.6	36
UB3	40	2	4.62	2.8	42
AB1	10	5	4.38	2.2	36
AB2	30	2	4.72	2.7	38
AB3	50	3	4.71	2.7	38
AB4	20	5	4.44	2.4	35
AB5	10	16	4.02	2.6	42

AB6	30	5	4.49	2.2	38
BB1	40	3	4.65	2.6	36
BB2	30	7	4.18	2.8	41
BB3	30	2	4.91	2.5	37
BB4	30	3	4.62	2.3	34
BB5	30	2	4.42	2.5	37
BB6	30	3	4.67	2.5	38
TB1	30	3	4.66	2.5	36
TB2	40	3	4.32	2.6	38
TB3	40	2	4.71	2.7	39
TB4	40	2	4.6	2.5	39
TB5	40	3	4.63	2.6	36
TB6	40	3	4.86	2.6	38
AM1	30	2	4.53	2.3	34
AM2	20	13	4.12	2.8	43
AM3	50	6	4.32	2.6	37
AM4	20	9	4.24	2.2	35
AM5	30	4	4.52	2.8	40
AM6	40	3	4.49	2.8	40

Appendix B: Records of ant species across burnt, mown, and unburnt plot treatments Ukulinga research farm, Pietermaritzburg, South Africa.

Species	Burnt	Mown	Unburnt	Grand Total
Acropyga UKZN_01	221	83	1	305
Acropyga UKZN_02	1			1
Aenictus UKZN_01	20	6		26
Anochetus ?UKZN_02	14			14
Anochetus faurei		4		4
Anoplolepis custodiens	106	93		199
Bothroponera ?UKZN_01	1			1
Camponotus ?UKZN_06 (*niveosetosus* gp.)	1	1		2
Camponotus UKZN_02 (*cinctellus* gp.)	17		18	35
Camponotus UKZN_04	1			1
Camponotus UKZN_05 (*maculatus* gp.)	6	4	10	20
Cardiocondyla ?emeryi	1			1
Carebara UKZN_02	3		1	4
Crematogaster ?*rufigena*	3		4	7
Crematogaster rectinota	1072	196		1268
Diplomorium UKZN_02	20	6	2	28
Discothyrea oculata	2	2	1	5
Dorylus UKZN_01	2		1	3

Hagensia havilandi	5	1		6
Hypoponera UKZN_04	2	1	4	7
Lepisiota ?capensis	121	26	22	169
Lepisiota ?crinita	38	12	2	52
Lepisiota ?UKZN_03 (*capensis* gp.)	4			4
Lepisiota ?UKZN_08 (*spinosior* gp.)			1	1
Lepisiota UKZN_05 (*spinosior* gp.)	3			3
Lepisiota UKZN_11	1			1
Leptogenys intermedia	33	5	22	60
Leptogenys UKZN_03	5	4	3	12
Mesoponera caffraria	42	14	8	64
Mesoponera nr sharpi	5			5
Monomorium ?junodi	32	1		33
Monomorium ?UKZN_02 (*salamonis* gp.)	37			37
Monomorium UKZN_01	136	17	27	180
Monomorium UKZN_03	96	12		108
Monomorium UKZN_08	104	37	8	149
Myrmicaria natalensis	59	6		65
Nylanderia natalensis	40	1	3	44
Parasyscia ?UKZN_04	1	1		2

Pheidole UKZN_02 (*megacephala* gp.)	6131	1826	2091	10048
Pheidole UKZN_03	362	40	12	414
Plagiolepis UKZN_01	4		7	11
Plectroctena mandibularis	6	2		8
Solenopsis UKZN_01	67	22	20	109
Solenopsis UKZN_02	4		1	5
Solenopsis UKZN_03	12	14	2	28
Solenopsis UKZN_05	4	1		5
Solenopsis UKZN_06	5			5
Strumigenys ?faurei	7			7
Strumigenys ?UKZN_01	9	8		17
Strumigenys ?UKZN_02	5	1		6
Tapinolepis ?UKZN_05	1		3	4
Tapinolepis UKZN_04		1		1
Technomyrmex cf. pallipes	36	7	3	46
Tetramorium ?UKZN_03 (*setigerum* gp.)	181	33	12	226
Tetramorium ?UKZN_07 (*setigerum* gp.)	17	2		19
Tetramorium ?UKZN_08 (*similimum* gp.)	12	2	4	18
Tetramorium ?UKZN_09 (*similimum* gp.)	63	23	15	101
Tetramorium ?UKZN_11 (*simillimum* gp.)	35	9	9	53
Tetramorium ?UKZN_15 (*sericeiventre* gp.)	153	1	91	245

Tetramorium ?UKZN_18 (*squaminode* gp.)	24	6	6	36
Tetramorium ?UKZN_19 (*squaminode* gp.)			9	9
Tetramorium ?UKZN_21 (*squaminode* gp.)	39	10	6	55
Tetramorium notiale	10	7	12	29
Tetramorium nr erectum	7	2	4	13
Tetramorium nr grassii	6	1	26	33
Tetramorium thoth	11	11	1	23
Tetramorium UKZN_42	1	1		2
Grand Total	9467	2563	2472	14502

Appendix C: Records of ant species across treatment type (AB, annual burn; AM, annual mow; BB, biennial burn; TB, triennial burn; UB, unburnt plots) and season (August, Spring, and Control) of treatment Ukulinga research farm, Pietermaritzburg, South Africa.

Season of treatments	August				Control	Spring				Total				
Treatments	AB	AM	BB	TB	UB	AB	AM	BB	TB	AB	AM	BB	UB	TB
No. of plots	3	3	3	3	3	3	3	3	3	6	6	6	6	3
Acropyga UKZN_01	16	18	28	18	1	24	17	14	13	40	35	42	1	31
Acropyga UKZN_02						1				1				
Aenictus UKZN_01	2				1	1	3		3	3	3		1	3
Anochetus ?UKZN_02				1		3		2		3		2		1
Anochetus faurei		2									2			
Anoplolepis custodiens	11	9		10		6	2	1	2	17	11	1		12
Bothroponera ?UKZN_01	1									1				
Camponotus ?UKZN_06 (*niveosetosus* gp.)		1							1		1			1
Camponotus UKZN_02 (*cinctellus* gp.)	2		1	1	12	2		1	5	4		2	12	6
Camponotus UKZN_04						1				1				
Camponotus UKZN_05 (*maculatus* gp.)	2			2	9	1	2			3	2		9	2
Cardiocondyla ?*emeryi*						1				1				
Carebara UKZN_02			1	1	1				1			1	1	2
Crematogaster ?*rufigena*					4				3				4	3
Crematogaster rectinota	35	14	29	5		41	23	18	23	76	37	47		28

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diplomorium UKZN_02	1	2			1	1		2		2	2	2	1	
Discothyrea oculata	1	1	1		1					1	1	1	1	
Dorylus UKZN_01					1	1		1		1		1	1	
Hagensia havilandi						1	1	1	2	1	1	1		2
Hypoponera UKZN_04		1	2		3						1	2	3	
Lepisiota ?*capensis*	4	7	8	14	16	10	10	10	25	14	17	18	16	39
Lepisiota ?*crinita*	9		5	3	2	1	5		5	10	5	5	2	8
Lepisiota ?UKZN_03 (*capensis* gp.)						1				1				
Lepisiota ?UKZN_08 (*spinosior* gp.)					1							1		
Lepisiota UKZN_05 (*spinosior* gp.)				1					2					3
Lepisiota UKZN_11			1									1		
Leptogenys intermedia	3		8	1	13	1	5	11	4	4	5	19	13	5
Leptogenys UKZN_03		3	2	1	3		1	1	1		4	3	3	2
Mesoponera caffraria	6	3	4	3	5	2	9	8	3	8	12	12	5	6
Mesoponera nr sharpi						2				2				
Monomorium ?*junodi*	1					2	1			3	1			
Monomorium ?UKZN_02 (*salamonis* gp.)	5					4				9				
Monomorium UKZN_01	4	9	7	10	17	8	6	8	13	12	15	15	17	23
Monomorium UKZN_03	9	3	1	3		3				2	12	3	1	5
Monomorium UKZN_08	6	7	3	4	1	11	3	5	4	17	10	8	1	8

Species														
Myrmicaria natalensis	4		1	1		1	1		2	5	1	1		3
Nylanderia natalensis		2	6	2	10	1	6	1	10	1	8	2		7
Parasyscia ?UKZN_04						1	1			1	1			
Pheidole UKZN_02 (*megacephala* gp.)	52	55	55	65	74	65	55	56	54	117	110	111	74	119
Pheidole UKZN_03	20	3	3	2	2	11	4	10	6	31	7	13	2	8
Plagiolepis UKZN_01				2	4				1				4	3
Plectroctena mandibularis	3	1				1	1	2		4	2	2		
Solenopsis UKZN_01	8	8	8	9	12	6	5	6	3	14	13	14	12	12
Solenopsis UKZN_02			1	1	1			1	1			2	1	2
Solenopsis UKZN_03	3	6		1	1		2	4	1	3	8	4	1	2
Solenopsis UKZN_05			1					1	1	1		1	2	1
Solenopsis UKZN_06									3	1			3	1
Strumigenys ?*faurei*	1			1					2	3	1		2	4
Strumigenys ?UKZN_01		4	2	1		1	1	2	1	1		5	4	2
Strumigenys ?UKZN_02								1		1		1		1
Tapinolepis ?UKZN_05			1		3								1	3
Tapinolepis UKZN_04								1				1		
Technomyrmex cf. pallipes	5	4		5	2	4	2	6	5	9	6	6	2	10
Tetramorium ?UKZN_03 (setigerum gp.)	13	10	9	28	7	19	17	20	18	32	27	29	7	46
Tetramorium ?UKZN_07 (*setigerum* gp.)		2	6	2		3		5		3	2	11		2

Tetramorium ?UKZN_08 (*similimum* gp.)		1			3	1	1	2	3	1	2	2	3	3
Tetramorium ?UKZN_09 (*similimum* gp.)	4	3	6	11	9	1	11	1	14	5	14	7	9	25
Tetramorium ?UKZN_11 (*simillimum* gp.)	4	3	6	10	7	2	4		6	6	7	6	7	16
Tetramorium ?UKZN_15 (*sericeiventre* gp.)	8		2	10	13	10	1	1	19	18	1	3	13	29
Tetramorium ?UKZN_18 (*squaminode* gp.)	3	2	3		5		3	5	5	3	5	8	5	5
Tetramorium ?UKZN_19 (*squaminode* gp.)					2								2	
Tetramorium ?UKZN_21 (*squaminode* gp.)	1	4	4	4	4	5	5	6	3	6	9	10	4	7
Tetramorium notiale	2	2	4		2	2	4		1	4	6	4	2	1
Tetramorium nr erectum		1	3	1	4		1	2	1		2	5	4	2
Tetramorium nr grassii	1		1		8		1	1	1	1	1	2	8	1
Tetramorium thoth	2	3	1	1	1	4	5	2		6	8	3	1	1
Tetramorium UKZN_42		1				1					1	1		
Total no. records	252	193	221	239	257	277	217	227	264	529	410	448	257	503
Total no. species	35	32	36	35	38	43	38	36	42	49	46	47	38	46
Mean no. species	14,3	12,0	12,3	13,6	13,5	13,0	11,6	12,6	12,7	21,4	17,8	19,0	13,6	21,5

Appendix D: List of species and their corresponding abbreviations used in Figure 2.8

Abbreviations	Species
Bothro1	*Bothroponera* UKZN_01
Camp2	*Camponotus* UKZN_02 (*cinctellus* gp.)
Camp6	*Camponotus* UKZN_06 (*niveosetosus* gp.)
Careb2	*Carebara* UKZN_02
CremRuf	*Crematogaster rufigena*
DiscoOcu	*Discothyrea oculate*
LepiCr	*Lepisiota crinite*
Lepi8	*Lepisiota* UKZN_08 (*spinosio*r gp.)
Lepi11	*Lepisiota* UKZN_11
Lept3	*Leptogenys* UKZN_03
MesCaf	*Mesoponera caffraria*
Mono2	*Monomorium* UKZN_02 (*salamonis* gp.)
Mono3	*Monomorium* UKZN_03
Mono8	*Monomorium* UKZN_08
Phei2	*Pheidole* UKZN_02 (*megacephala* gp.)
Phei3	*Pheidole* UKZN_03
Plagi1	*Plagiolepis* UKZN_01
Sole1	*Solenopsis* UKZN_01
Sole2	*Solenopsis* UKZN_02
Sole6	*Solenopsis* UKZN_06
Tapino5	*Tapinolepis* UKZN_05
Tetra3	*Tetramorium* UKZN_03 (*setigerum* gp.)
Tetra7	*Tetramorium* UKZN_07 (*setigerum* gp.)
Tetra11	*Tetramorium* UKZN_11 (*simillimum* gp.)
Tetra19	*Tetramorium* UKZN_19 (*squaminode* gp.)
TetraEre	*Tetramorium nr erectum*

Appendix E: Vegetation data collected in each of the three replicates of limestine ammonium nitrate (LAN) and ammonium sulphate (AS) fertilizer treatments at Ukulinga research farm

Treatment	%Bare ground	% Biomass
LAN_low1	17,5	8,499895
LAN_low2	23,75	7,321692
LAN_low3	0	4,860088
AS_low1	35	8,436777
AS_low2	35,75	6,690511
AS_low3	0	6,501157
Control1	10	6,501157
Control2	45	5,533347
Control3	60	6,669472
LAN_high1	28,75	7,7004
LAN_high2	3,25	7,216495
LAN_high3	35	3,51357
AS_high1	61,25	10,14096
AS_high2	22,5	5,1336
AS_high3	58,75	5,280875
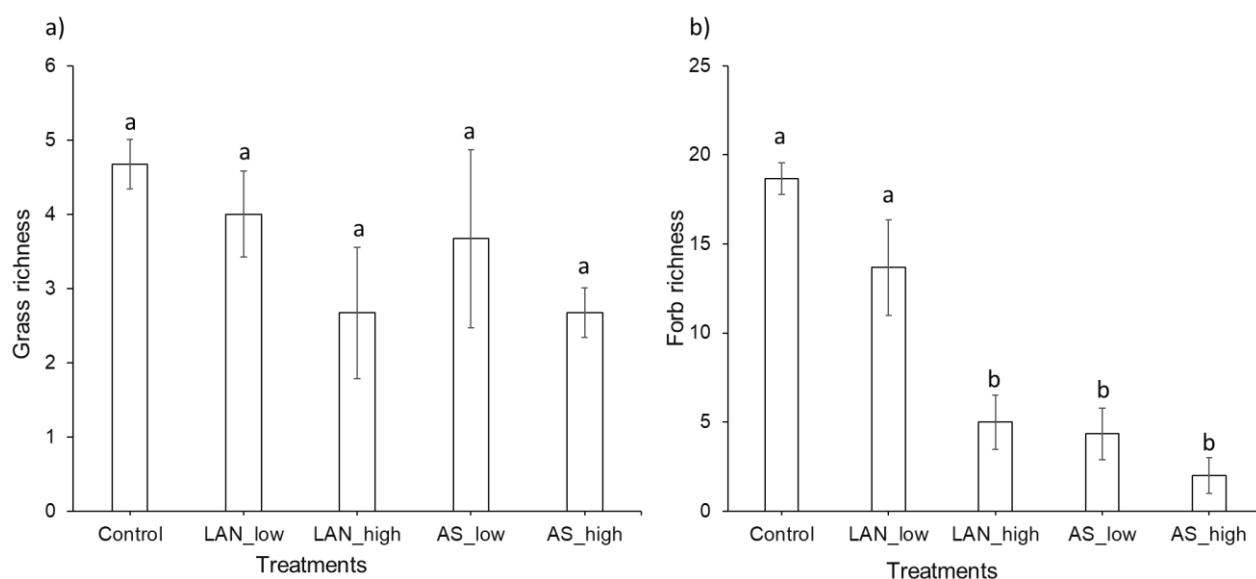
Appendix F: Mean (±SE) total grass species richness (a) and forb species richness (b) in relation to fertilizer treatments in the Ukulinga research farm, Pietermaritzburg, South Africa.
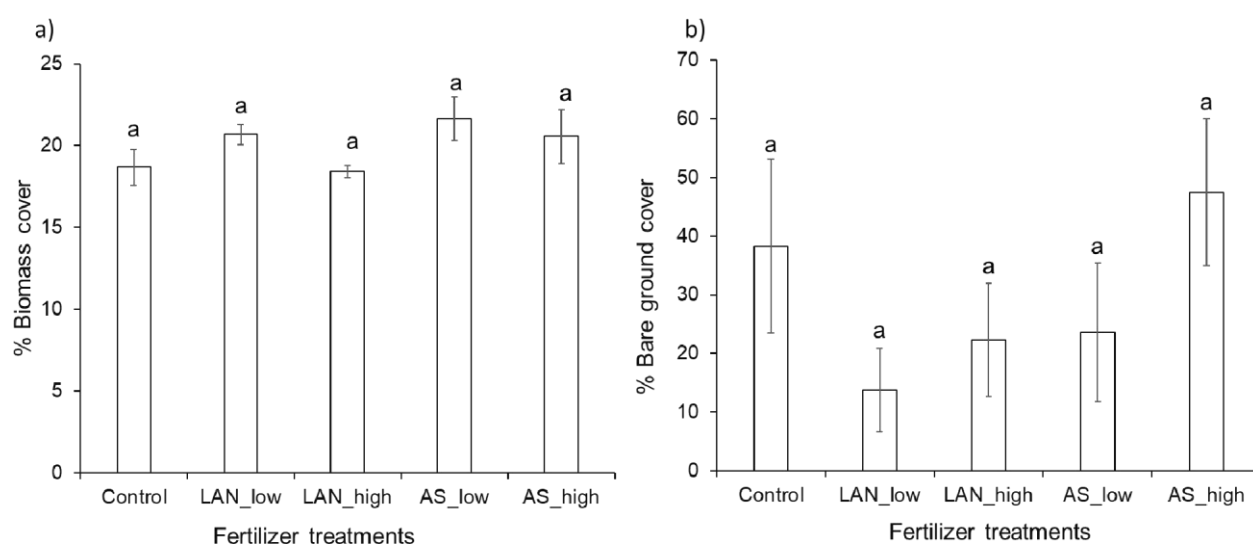
Appendix G: Mean (±SE) percentage plant biomass (a) and bare ground cover (b) in relation to fertilizer treatments in the Ukulinga research farm, Pietermaritzburg, South Africa.

Appendix H: Analysis of similarity for ant assemblages between the control and the fertilizer treatments. Significant treatments are highlighted in bold

Treatments	*R* statistics	Significant level (*P*)
Control vs LAN_high	0.091	0.232
Control vs AS_high	0.219	0.067

Control vs AS_low	0.006	0.418
Control vs LAN_low	-0.056	0.71
LAN_high vs AS_high	-0.071	0.66
LAN_high vs AS_low	-0.048	0.593
LAN_high vs LAN_low	0.245	**0.024**
AS_high vs AS_low	0.168	0.113
AS_high vs LAN_low	0.256	**0.041**
AS_low vs LAN_low	0.215	0.061

Appendix I: List of species and their corresponding abbreviations used in Figure 3.6

Abbreviations	Species
AnoCusto	*Anoplolepis custodiens*
CardicE	*Cardiocondyla emeryi*
LepiCape	*Lepisiota* UKZN_03(*capensis* gp.)
Leptog03	*Leptogenys* UKZN_03
MesCaf	*Mesoponera caffraria*
Mono02	*Monomorium* UKZN_02 (*salamonis* gp.)
Mono03	*Monomorium* UKZN_03
Mono08	*Monomorium* UKZN_06
MeranMag	*Meranoplus magrettii*
NylaNata	*Nylanderia natalensis*
Phei02	*Pheidole* UKZN_02 (*megacephala* gp.)

Pheid03	*Pheidole* UKZN_03
Plagi1	*Plagiolepis* UKZN_01
Solen01	*Solenopsis* UKZN_01
Solen03	*Solenopsis* UKZN_03
Solen05	*Solenopsis* UKZN_05
Technop	*Technomyrmex cf. pallipes*
Tetra01	*Tetramorium* UKZN_01 (*notiale* gp.)
Tetra03	*Tetramorium* UKZN_03 (*setigerum* gp.)
Tetra11	*Tetramorium* UKZN_11 (*simillimum* gp.)
Tetra15	*Tetramorium* UKZN_19 (*sericeiventre* gp.)
Tetranr	*Tetramorium nr erectum*