Effects of biotic resistance and resource availability on the invasion success of the Argentine ant, *Linepithema humile* (Mayr), in the Cape Floristic Region, South Africa.

by

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ABSTRACT

The invasive Argentine ant, *Linepithema humile*, is widespread and has been introduced into the Cape Floristic Region (CFR) of South Africa. It has successfully established and spread into both urban and natural environments. Even with its potential negative effects on the CFR, a biodiversity hotspot, very few studies have focused on this ant in South Africa. Even less is known about the indigenous ants to the CFR highlighting the paucity in our knowledge of resident ant community structure and the threat of *L. humile* on our native ants and ultimately the CFR. In the Fynbos biome, *L. humile* occupies distributions mutually exclusive to those of many of the dominant native ants, as well as to *Pheidole megacephala* which occupies the eastern escarpment of the country. We investigated resource exploitation: i) under controlled laboratory conditions, ii) floral nectar utilisation in the field and iii) diet switching in response to levels of *L. humile* invasion, as well as interspecific interactions between resident ants and *L. humile*. We used laboratory bioassays to ascertain whether resident ants posed any biotic resistance to the spread of *L. humile*. Fynbos ants were not competitive towards *L. humile* despite equalised colony sizes, suggesting no biotic resistance from this community. *Linepithema humile* was able to recruit far more workers than three of the resident Fynbos native ants studied and interfered with their recruitment through aggressive behaviour. If this ineffectual competition from native Fynbos ants under these laboratory conditions is extrapolated to field conditions, it may be one factor currently contributing to the successful invasion of the Fynbos by *L. humile*. On a more positive note, *P. megacephala* showed competitive superiority and *L. humile* suffered huge mortality rates, implying that this resident ant species may actually be offering biotic resistance to *L. humile*. The abundance of floral nectar in the Fynbos increases during winter and so we measured the foraging activity of the native dominant ant *Anoplolepis custodiens* and *L. humile* on nectar producing proteacea species as well as nest density around the flowering plants. In addition, the ground foraging activity of ants in the study plots and floral composition of these protea plants were assessed. Elemental stable-isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ and C:N ratios, which are the contribution of carbohydrates and protein to the diet, was used to study the foraging ecology of *L. humile* and some of Fynbos native ant species along an invasion continuum. *Linepithema humile* effectively exploited Fynbos floral resources, showed diet flexibility by feeding on carbohydrate resources in winter but also supplemented their diet with protein, likely from predation or scavenging on native arthropods. *Linepithema humile* altered the diets of some native ant species and also changed species assemblages both on the ground and in the Proteacea inflorescences. *Linepithema humile* responded more efficiently to fluctuating resources provided by floral nectar than native Fynbos ants and outcompeted resident ants through aggression when competing for a shared resource. This aggression of *L. humile*, together with their ability to monopolise fluctuating carbohydrate resources promotes ecological dominance and invasion success of this ant species, especially in areas with nectar producing Proteacea species.

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ABSTRAK

Die Argentynse indringermier, *Linepithema humile*, is wydverspreid en is onder andere na die Kaapse Blommeryk (KBR) van Suid-Afrika gebring. Die miere het hulself suksesvol gevestig en het versprei na beide stads- en natuurlike omgewings. Selfs met die potensiële negatiewe effekte op die KBR, 'n biodiversiteitskern, het baie min studies sovêr op hierdie mier in Suid-Afrika gefokus. Selfs minder is bekend oor die inheemse miere in die KBR, wat dui op die gebrek aan kennis van die oorspronklike miergemeenskapstuktuur en die bedreiging wat *L. humile* inhou vir ons inheemse miere en uiteindelik ook vir die KBR. In die Fynbosbioom, beset *L. humile* verspreidings wat wedersyds uitsluitlik teenoor die verspreiding is van baie van die dominante inheemse mierspesies, asook *Pheidole megacephala* wat die oostelike eskarp van die land beset. Ons het hulpbronontginning ondersoek: i) onder beheerde laboraturimtoetstande, ii) blomnektargebruik in die veld en iii) dieetveranderinge as 'n reaksie op die vlakke van *L. humile* inval, en ook die interspesie wisselwerking tussen die inheemse miere en *L. humile*. Ons het laboraturiumbiotoetse gebruik om vas te stel of inheemse miere enige biotiese teenstand bied teen die verspreiding van *L. humile*. Fynbosmiere het nie met *L. humile* gekompeteer nie ten spyte van gelykgemaakte koloniegroottes, wat geen biotiese weerstand deur die gemeenskap aandui nie. *Linepithema humile* was in staat om veel meer werkers te werf as drie van die inheemse Fynbosmierspesies wat bestudeer is, en het ingemeng met hulle werwing deur aggresiewe gedrag. As hierdie oneffektiewe kompetisie van die inheemse Fynbosmiere onder laboratoriumtoestande ge-ektrapoleer word na veldtoestande, sal dit moonlik 'n faktor wees wat bydra tot die suksesvolle inval van die Fynbos deur *L. humile*. Op 'n meer positiewe noot, *P. megacephala* het superioriteit teenoor *L. humile* getoon in kompetisie, en die mortaliteitssyfers *van L. humile* was enorm , wat impliseer dat hierdie inheemse mierspesie tog teen *L. humile* biotiese weerstand bied. Die Fynbosblomnektar vermeerder in die winter, daarom het ons die kos-soek aktiwiteit van die inheemse dominante mier *Anoplolepis custodiens* en *L. humile* op nektarprodiserende Proteaceae spesies, asook nesdigtheid om die blomplante ondersoek. Ons het ook die grond kos-soek aktiwiteit van miere in die studieplotte en blomsamestelling van proteaplante geassesseer. Element stabiele-isotoopanaliese van $\delta^{13}C$ en $\delta^{15}N$ en C:N verhoudings, wat die bydrae van koolhidrate en protein tot die dieet is, is gebruik om die kos-soek ekologie van *L. humile* en inheemse Fynbosmierspesies te ondersoek asook die invalskontinuum. *Linepithema humile* het Fynbosblomhulpbronne effektief ontgin, het aanpasbaarheid in hul dieet getoon deur te voed op koolhidraadbronne in die winter maar ook deur hul dieët aan te vul met proteïne, bes moontlik deur predasie of aas op inheemse geleedpotiges. *Linepithema humile* het die dieet van sommige inheemse mierspesies verander en ook spesiesamestellings op die grond sowel as in die Proteaceae-blomwyses. *Linepithema humile* het meer effektief reageer op wisselende hulpbronne wat beskikbaar gestel word deur blommenektar as die inheemse Fynbosmiere en het die inwonermiere uitgekompeteer deur aggressie wanner kompetisie vir 'n gedeelde hulpbron voorgekom het. Hierdie agressie van *L. humile*, saam met hulle vermoë om wissellende koolhidraadhulpbronne te monopoliser, bevorder die ekologiese dominansie en invalssukses van hierdie mierspesie, veral in gebiede met nektarproduserende Proteaceae spesies.

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"Success is the preserve of those who are steadfast in their desire, for they become great because when you follow your passion, success follows you."!

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DEDICATION

To inspire **my daughter**, Mandlakazi Oratile Mothapo, to believe in the power of dreams and the knowledge that nothing is impossible

To honor **my father**, David Lesetša Mothapo, for the immeasurable love that gave me strength and determination

To **God** for the end of an incredible chapter and the beginning of a new one

In the gift of life and love that He has given me

through

my newborn **son**, Simphiweyinkosi Katlego Mothapo, born 15 November 2013

Invictus

Out of the night that covers me,
Black as the Pit from pole to pole,
I thank whatever gods may be
For my unconquerable soul.

In the fell clutch of circumstance
I have not winced nor cried aloud.
Under the bludgeoning of chance
My head is bloody, but unbowed.

Beyond the place of wrath and tears
Looms but the Horror of the shade,
And yet the menace of the years
Finds, and shall find me, unafraid.

It matters not how strait the gate,
How charged with punishment the scroll,
I am the master of my fate;
I am the captain of my soul.

-William Ernest Henley

"I'm blessed and highly favoured"

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CHAPTER 1: GENERAL INTRODUCTION

INTRODUCTION

Community assembly

Competition between species is one of the most important factors affecting the structure of ecological communities (Elton 1958; Brown & Davidson 1977; Gurnell *et al.* 2004). Typically, co-occurring species within a community utilise available resources and space differentially to promote co-existence, and species diversity (Tillman 1994; Chase *et al.* 2002; Palmer *et al.* 2003.). However, species within ecological communities compete with each other for resources, and these interactions can lead to limitations placed on interacting species, determining the extent to which species can co-exist, which Elton (1958) termed assembly rules. The intensity of competition between two co-occurring species within a community is mainly dependent on the degree to which both species share niche requirements in terms of shared resources (Schmitt & Holbrook 2003). Thus, if species co-occur within the same environment in the ecological community and have similar resource requirements, interspecific competition will result in the detriment of one of the species in terms of overall fitness unless they develop strategies that allow them to co-exist, known as competitive exclusion and interspecific trade-offs (Mooney & Cleland 2001; Palmer *et al.* 2003; Tilman 2004).

Species richness and community diversity of ecological communities are influenced by the ability of the species within the environment to partition resources, which in turn promote co-existence patterns (Tilman 1994). These resources may vary spatially through environmental heterogeneity, which allows species with niche overlap to co-occur through mutually exclusive distributions within the same community by partitioning resources both spatially and temporaly (Abrams & Wilson 2004). Resources may also be temporally variable, or occur in sufficient abundance to allow competing species to utilise them concurrently (Tilman 1994; Kneitel & Chase 2004). In this way, species can avoid conflict and maintain community diversity and species richness (Bonesi & Macdonald 2004). Ultimately, over time, species within a community become specialised in utilising particular resources and niche space (Schmitt & Holbrook 2003)

Community invasibility

Invasive species introduced into a recipient environment, may have an impact on the recipient environment or they may persist within the new environment and have no notable impact on the recipient environment (Holway et al. 2002). Invasion success, the ability of a species to successfully

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reproduce after colonisation to the point where it can spread and naturalise, is predicted by the characteristics of the recipient environment and those of the colonising species such as propagule size and life history traits (Lonsdale 1999; Sakai *et al.* 2001; Shea & Chesson 2002; Statchowitz & Tilman 2005). The recipient environment's susceptibility to invasion is predicted by the niche opportunities it can provide e.g. resource opportunities and favourable environmental conditions and/or the combination of both these factors (Mack *et al.* 2000; Richardson *et al.* 2000a). An introduced species must first overcome a range of abiotic and biotic barriers in the recipient environment to become a successful invader with noticeable impacts (Richardson *et al.* 2000b). These barriers affect its ability to successfully establish after arrival, persist (survive and reproduce), spread and naturalise from the site of introduction (Richardson *et al.* 2000b).

Invasive species are often introduced as small propagules that require physiological tolerances to the abiotic environment which will affect survival, withstand biotic interactions with native species in the recipient environment and successfully acquire available resources present within the recipient environment (Moller 1996; Davis *et al.* 2000; Mack *et al.* 2000; Chapman & Bourke 2001; Shea & Chesson 2002; Lee 2002; Prenter *et al.* 2004). These species typically have fast growth rates, high tolerances for environmental variation as well as strong competition for resources (Davis *et al.* 2000; Sakai *et al.* 2001; Pyšek & Richardson 2007), which likely gives them an advantage during the colonising stages (Sakai *et al.* 2001). Moreover, the lack of natural enemies on arrival also has a positive influence on the ability of small propagules to increase their densities from small incipient propagules to such high densities that they begin to have an impact on the recipient environment (Shea & Chesson 2002; Lockwood *et al.* 2005). Therefore, the interplay between abiotic and biotic factors may affect the ability of introduced species to persist within the recipient environments and impose distributional limits which may affect their ability to spread and become invasive (Hölldobler & Wilson 1990; Moller 1996; Mack *et al.* 2000; Shea & Chesson 2002). However, many invasive species survive most of the critical stages of the invasion process, establishment and persistence (McGlynn 1999; Richardson *et al.* 2000b), due to their association with areas of high anthropogenic influence which often have high resource availability and limited biotic resistance (Elton 1958; Holway *et al.* 1998). Consequently, they are called disturbance specialists since they thrive in these areas whereas native species are unable to tolerate or survive such environments (Elton 1958; Colautti *et al.* 2006; King & Tschinkel 2008). Thus, human modified habitats present a niche opportunity which is used by invasive species (Hölldobler & Wilson 1990; Torchin *et al.* 2002), and alternatively provide source pools for invasive species to spread into natural environments (McNeely *et al.* 2000; Statchowitz & Tilman 2005).

Several hypotheses have been proposed to explain the ability of introduced species to successfully establish and spread into natural environments and the factors that facilitate this spread (reviewed in Catford *et al.* 2009). These hypotheses explain interdependent factors that promote the success of

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these introduced species. The *Enemy Release Hypothesis* posits that introduced species are able to survive new environments due to the release from their natural enemies (Elton 1958; Giraud *et al*. 2002; Lockwood *et al.* 2005); the *Ecological Niche Hypothesis* posits that abiotic and biotic conditions define suitable niche space for an organism and introduced species should readily establish in ecologically suitable environments (see Le Breton *et al.* 2007); the *Increased Resource Availability Hypothesis* and the *Empty Niche Hypothesis* are similar in that they both suggest that introduced species are successful because they exploit resources which native species do not use or are inefficient at using (Davis *et al.* 1998; Hierro *et al.* 2005; Blumenthal 2006), while the Increased Resource Availability Hypothesis also suggests that competition might be more relaxed due to resource abundance (Davis *et al.* 1998); the *Biotic Resistance Hypothesis* posits that species rich communities are more resistant to invasion due to high competition from resident species (Elton, 1958; Levine & D'Antonio 1999; Statchowitz & Tilman, 2005); and lastly, the *Limiting Similarity Hypothesis* which states that invasive species are successful because they are functionally different to native species, especially those ecologically dominant invasive species (Davidson 1998; Emery 2007). Thus, based on the abovementioned hypotheses, colonising species which are different to resident species in one or more traits have a higher likelihood of establishment (Davis *et al.* 2000).

Resource availability

The ability of introduced species to capitalise on resources available within the recipient environment, and to utilise unused resources can increase their survival and consequently establishment success (Davis *et al.* 2000; Tilman 2004; Mata *et al.* 2013). However, since resources are spatially variable in an environment and may fluctuate over time (Han *et al.* 2012; Mata *et al.* 2013), the partitioning of resources among resident species therefore affects resource availability which in turn influences the invasibility of the resident community (Elton 1958). Consequently, resource availability is one of the most underappreciated factors contributing to the invasion success of introduced species in natural environments (Davis *et al.* 2000; Richardson *et al.* 2000b).

Competition for limited and/or shared resources, as well as the availability of resources within an environment contributes to the invasibility of a community and the establishment success of introduced species (Elton 1958; Davis *et al.* 2000). The ability of introduced species to respond more efficiently to changes in resource availability can further enhance propagule survival and establishment (Davis *et al.* 2000; Han *et al.* 2012; Mata *et al.* 2013). Resource supply may fluctuate with the flowering and fruiting periods, offering a temporarily abundant resource, in addition to already available stable resources such as honeydew from exudate producing insects within that environment (Davis *et al.* 2000; Lach 2013). The efficient uptake of these periodically available resources can increase invasibility (Davis *et al.* 2000). Resident species may completely exploit all available resources within an area reducing invasibilty of that environment, however introduced

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species may be better at exploiting those available resources or resident species may not be able to exploit all the resources leaving room for newly arriving species to occupy these empty niches (Davis *et al.* 2000). Moreover, if the uptake of this resource by resident species is slower than the supply then colonising species that respond quickly to this may improve their establishment (Dukes & Mooney 1999; Davis *et al.* 2000). Thus, invasive species generally may increase their chances of establishment by responding effectively to fluctuating resources, and utilising those resources not fully exploited by resident species (Tilman 2004).

Competition for limiting resources is usually high among species within a community, however, increased resource availability within a recipient community reduces competition between species and increases invasibility (Han *et al.* 2012), while increased species diversity and abundance reduces resource availability and results in high competition with a concomitant decrease in invasibility (Elton 1958; Mata *et al.* 2013). The intensity of biotic interactions between resident species and colonising species is likely to be related to the amount of available resources (Davis *et al.* 2000). Communities with high resource abundance will have less intense competition and possibly higher likelihood of invasion (Jiang & Morrison 2004; Blüthgen *et al.* 2009). This is in support of the *Empty Niche Hypothesis* and the *Increased Resource Availability Hypothesis*, largely because invasive species often have higher reproductive rates and are more likely to have higher reproductive output if they establish in an environment with high resource availability (Shea & Chesson 2002; Blüthgen *et al.* 2004). Ultimately, community invasibility is directly influenced by species diversity, the competitive ability of these resident species and resource availability.

Resident species characteristics

Elton's 1958 *Biotic Resistance* hypothesis posits that susceptibility of a recipient community to invasion is influenced by species diversity within that particular environment (Elton 1958; Levine & D'Antonio 1999). The view of this hypothesis is that due to the saturation of the community by the presence of many different species, newly introduced species are unlikely to establish due to potential competition as well as the lowered availability of resources within the environment (Davis *et al.* 2000; Levine *et al.* 2004). Colonising species have to compete for niche spaces that are already occupied and for resources that are already being utilised by resident species (Elton 1958; Stachowicz *et al.* 1999; Davis *et al.* 2000; Shea & Chesson 2002; Tilman 2004). Biotic resistance may act as a barrier to invasion particularly at the establishment phase when introduced species' population sizes are at their lowest (Kennedy *et al.* 2002), although mixed results have been found in ant studies of biotic resistance to invasive ant species in Australian ant communities (Walters & Mackay 2005; Hoffmann et al. 2009). In this way, the establishment of the invader can be slowed down or completely prevented (Elton 1958; Levine *et al.* 2004). Therefore, in order for an introduced species to successfully establish and become invasive it must outcompete those species that share similar niche

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requirements and life history traits and (Davis *et al.* 2000; Shea & Chesson 2002). Therefore, interspecific exclude them from the shared resources competition is the main predictor of biotic resistance, even though environmental factors may also be at play (Mitchel *et al.* 2006).

Ant community structure

Interspecific competition is considered an important factor in shaping ant community structure (Hölldobler & Wilson 1990). Ecologically dominant species shape the ecosystem function through their interactions with other ant species over resources and space, which consequently affects the distribution and the activity patterns of other ant species within the community (Hoffmann & Andersen 2003; Sanders *et al.* 2003). These competitive interactions structure the ant community with few ecologically dominant species which are very abundant within the habitat, a few subdominant species as well as several species that are inferior, cryptic or specialised (Andersen 1992; Davidson 1998). *Ecological Dominance* by a species occurs when an ant species makes up a large proportion of the ant community biomass and can be found in very high abundances at resources (Davidson *et al.* 2003), while *Behavioural Dominance* occurs when an ant species demonstrates extreme aggressive behaviour towards other ant species within the habitat, often deterring them from resources (Davidson 1998). The combination of these two aspects of dominance may allow a species to structure an entire community, with detrimental impacts if the species is invasive or non-native (O'Dowd *et al.* 2003).

Invasive ants

Social hymenoptera are among the most successful of animal invaders with regards to geographic distribution, ecological and economic damage, as well as proportion that become invasive (Moller 1996; Payne *et al*. 2004; Heinze *et al*. 2006). Their success can be attributed to their reproductive potential and their ability to easily spread over large distances (Moller 1996). Indeed at least five ant species, yellow crazy ant (*Anoplolepis gracilipes*), red imported fire ant (*Solenopsis Invicta*), Argentine ant (*Linepithema humile*), African big headed ant (*Pheidole megacephala*), and the little fire ant (*Wasmannia auropunctata*), are listed amongst the top 100 worst invaders in the world (Global Invasive Species Database, 2013). Although many of these ants show strong affinity for human modified habitats where there is limited biotic resistance and high resource availability (Elton 1958; Hölldobler & Wilson 1990; Passera 1994; Suarez *et al.* 2005), they have also penetrated natural ecosystems (Human & Gordon 1999; Hoffmann *et al.* 1999; Holway *et al.* 2002a). They also have had negative impacts on natural ecosystems through direct competition, predation and eventual displacement of native ant species (Hoffmann *et al.* 1999; Holway *et al*. 2002a; Sanders *et al.* 2003); as well as indirectly through the disruption of plant-insect interactions (Bond & Slingby 1984; Carpintero *et al.* 1998; Christian 2001).

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The successful invasion by introduced ant species is determined by the interactions with native ants within the recipient environment as well as abiotic conditions (Walters 2006; Menke *et al.* 2007). Recipient environments that are species rich and have ant species that are competitively similar to the introduced species have low susceptibility to invasion (Elton 1958; Hoffman *et al*. 1999: Walters & Mackay 2005; Wetterer *et al*. 2006). Ant species with similar niche requirements do not often co-exist due to high conflict, particularly over resources (Andersen 1995; Hölldobler & Wilson 1990). For example, in Australian ant communities, native dominant ant species of the genus *Irydomyrmex* have been shown to limit the ability of the invasive Argentine ant, *L. humile*, to successfully spread into some areas where the native ant species dominates (Walters & Mackay 2005). *Iridomyrmex* shares the same niche requirements as *L. humile* in terms of resource preferences, nesting and behavioural characteristics and has been shown to outcompete *L. humile* (Thomas & Holway 2005; Walters & Mackay 2005) and other invasive ants (Hoffmann *et al*. 1999; Hoffmann & Andersen 2003). However, this resistance to *L. humile* invasion by *Iridoyrmex* is further facilitated by environmental conditions because the areas in which it dominates are usually much drier and largely intolerable to Argentine ants (Thomas & Holway 2005; Walters 2006). Therefore, native and invasive species with comparable biologies will probably not co-exist with native species, potentially limiting the spread of the invader (Hölldobler & Wilson 1990; Hoffmann & Andersen, 2003).

The Invasive Argentine ant, *Linepithema humile*: background and impacts in the Fynbos

The Argentine ant, *Linepithema humile*, is one of the most well studied invasive ant species and considered one of the most harmful (Skaife 1955; Vega & Rust 2001; Pyšek *et al*. 2008; Wetterer *et al.* 2009). Throughout its introduced range, *L. humile* is associated with the displacement of native fauna and negative impacts on ecosystem function (Bond & Slingby 1984; Human & Gordon 1996; Holway 1998; Human & Gordon 1999; Blancaford & Gomez 2005). Through active predation, resource exploitation and interference competition, *L. humile* displaces most ground-dwelling native ant species and other slow moving arthropods (de Kock & Giliomee 1989; Cole *et al*. 1992; Human & Gordon 1999; Zee & Holway 2006; Tillberg *et al*. 2007). In addition, *L. humile* shares a suite of characteristics with other invasive ant species that are thought to facilitate their ecological success, such as strong competitive ability, omnivory and polygyny, as well as the ability to form supercolonies (Porter & Savignano 1990; Suarez *et al*. 1998; Human & Gordon 1999; Chapman & Bourke 2001; Giraud *et al*. 2002: Holway *et al*. 2002; Abbott *et al*. 2007; Rowles & O'Dowd 2007). Extreme polydomy and polygyny are associated with unicolonial populations; consequently unicolonial ant species are able to attain extremely high worker abundances (Suarez *et al*. 1999; Holway *et al*. 2002; O'Dowd *et al*. 2003; Le Breton *et al*. 2007; Sarty *et al*. 2007), which contributes to their interspecific dominance (Holway *et al*. 2002). Thus, *L. humile* is able to exert pressure on native ants through numerical dominance (Morrison 1996, 2000; Holway & Case 2001). For example,

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large numbers of workers can be mobilised and are able to discover, recruit to and recover food resources faster and more efficiently than most resident ant species (Human & Gordon 1996; Macom & Porter 1996; Chapman & Bourke 2001; Holway & Case 2001).

Linepithema humile maintains their territories through high worker numbers, as well as highly aggressive behaviour (Human & Gordon 1999; Holway *et al.* 1998), and demonstrates behavioural plasticity in competitive situations where the species is able to assess the risk of competition by either fleeing or fighting (Le Brun *et al.* 2007). Blight and colleagues (2010) recently showed that *L. humile* used death-feigning behaviour, or thanatosis, when the risk of competition with the native ecological dominant ant *Tapinoma nigerrimum* was high when an individual is outnumbered. Death feigning is a self-defence method often used by prey species when there is a threat of a predator or dangerous competitor. Thanatosis is little understood in ants and has only been observed in *Solenopsis invicta* (Casill *et al.* 2008). In addition, *L. humile* has been shown to switch trophic positions once they have successfully established in a new environment (Tillberg *et al*. 2007). At the onset of the invasion, they are highly carnivorous, actively predating on ground-dwelling ants and arthropods, and once established, they switch to a diet that predominantly includes a wide range of plant and animal exudates (Tillberg *et al.* 2007). The protein is important for queen production and larval growth (Aron 2001), while the carbohydrate is thought to sustain these extremely large colonies (Bristow 1991; DiGirolamo & Fox 2006; Addison & Samways 2007). However, very little is known about the role of resource limitation on the physiology and colony function of ants, the relative importance of these protein and carbohydrate resources to colony function (Lach *et al*. 2009).

Since Tillberg *et al* (2007), it has been shown that incipient colonies of *L. humile* require a steady supply of carbohydrate and proteins to maintain colony growth, with carbohydrates being more important for incipient colony survival (Shik & Silverman 2012). Unlike other ants, *L. humile* activily forage throughout the day and night, potentially preventing native ants access to resources (Human & Gordon 1996; Roura-Pascual *et al.* 2011). They have a foraging strategy called *Dispersed Central-Place foraging* (DCF) where nests are distributed within an area according to the spatial heterogeneity of food resources (Holway & Case 2000), and this has recently been shown for other polydomous species (Buczkowski & Bennett 2006). By moving nests closer to food sources, dispersed cetral-place foragers are able to reduce travel costs and to efficiently exploit food resources, being able to monopolise stable and clumped food sources (Buczkowski & Bennett 2007). This foraging strategy is thought to facilitate competitive dominance of communities by *L. humile*, yet, this aspect of *L. humile's* biology and of other invasive ants is poorly studied (Buczkowski & Bennett 2007). The contribution of this foraging strategy and the role of carbohydrate resources in shaping ant community structure, facilitating invasion of natural communities and the effects on colony performance remain unquantified .

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Linepithema humile is thought to have been introduced into South Africa in the late 1800s with horse fodder during the Anglo-Boer war. The first record of the Argentine ant dates to 1901 in Stellenbosch, Western Cape (Skaife 1955; Prins *et al*. 1990). The ant has since spread into both urban and natural areas throughout the country (de Kock & Giliomee 1989; Luruli 2007). However, it is largely associated with human–influenced areas (Luruli 2007). Recent studies using behavioural, chemical and genetic analyses reveal that the ant is unicolonial and forms two behaviourally distinct supercolonies in South Africa (Lado 2007; Mothapo & Wossler 2011). The ant is distributed throughout most of South Africa; however, it is not present in the sub-tropical regions in the Eastern parts of South Africa (Luruli 2007). Climate data shows that *L. humile* has not established in all areas that are climatically suitable for them in South Africa (Roura-Pascual *et al*. 2004), suggesting that there are other factors that may be limiting the spread of this ant within the country. One explanation for the current *L. humile* distribution in South Africa may be biotic resistance from resident species. Luruli (2007) showed that the geographical distributions of *L. humile* and the African big-headed ant, *Pheidole megacephala* are mutually exclusive throughout South Africa. *Pheidole megacephala* is present in those regions where the Argentine ant is not found (Luruli 2007, see Chapter 3), although these regions have been predicted to be suitable for this ant species (Roura-Pascual *et al*. 2004), suggesting that *P. megacephala* may be excluding *L. humile* from these areas. *Pheidole megacephala* and *L. humile* share a suite of characteristics as successful invasive ant species, however, little is known about *P. megacephala* as compared to *L. humile* (Wetterer 2007; Fournier *et al.* 2009). Both ant species are behaviourally and numerically dominant where they have invaded elsewhere in the world and are highly aggressive to other ant species (Hoffmann *et al.* 1999; Kirschenbaum & Grace 2008). Moreover, where these two species co-occur on tropical islands where they have been introduced and occupy mutually exclusive distributions (Lach 2008, Lach *et al.* 2009), they are both highly aggressive and are able to displace each other depending on the suitability of abiotic conditions (Haskins & Haskins 1965; Crowell 1968; Krushelnycky *et al*. 2005).

Within the Cape Floristic Region (CFR), *L. humile* is found in urban, agro-ecosystems and natural environments (Luruli 2007). Previous studies have looked at the impact of *L. humile* on seed dispersal where it was demosntsrated that *L. humile* did not play a role in seed dispersal of important proteaceae species with potential detrimental effects on the CFR (Bond & Slingby 1984; Witt & Giliomee 1999; Christian 2001). It was found that *L. humile* displaces several native ant species that fulfil important ecological roles in the ecosystem function of Fynbos plants such as *Pheidole capensis* (Mayr), *Anoplolepis custodiens* (Smith), *Anoplolepis steingroeveri* (Forel) (Bond & Slingby1984; de Kock & Giliomee 1989; Christian 2001; Witt & Gilliomee 2004; Luruli 2007). These ants are involved in seed dispersal of many Fynbos plants. The distributions of *L. humile* and these three native ant species are mutually exclusive (Luruli 2007). These species share similar biological characteristics with *L. humile* such as foraging ability, nesting preferences, omnivory and high affinity for trophobiont exudates

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(Addison & Samways 2000). Although many Fynbos ant species are eliminated from invaded areas, a few ant species such as *Ocymyrmex barbiger* and *Tetramorium quadrispinosum* are able to co-exist with *L. humile* likely because they have high thermal tolerances which differ from *L. humile* and allow them to be active at times when *L. humile* is unable to forage (Witt & Giliomee 1999; Christian 2001). *Monomorium Sp. 8* and *Meranoplus peringueyi* are found in high abundances in sites invaded by *L. humile* and may be using other behavioural strategies that allow them to co-exist with *L. humile* (Skaife 1955; Witt & Giliomee 1999; Luruli 2007). *Linepithema humile* also interferes with the floral visitation by floral arthropods that play important pollination roles in the Fynbos, and this may have significant future consequences for this biodiversity hotspot (Visser *et al*. 1996; Lach 2007, 2008).

MOTIVATION

Understanding the factors that influence the establishment and spread of invasive species in natural communities is one of the major challenges in invasion biology. Much of our knowledge around the impacts of *L. humile* on native Fynbos ant species is largely inferred from distribution data using bait and pitfall traps. Empirical studies on the actual interactions between native ants and *L. humile* in terms of competition for resources and nesting space are lacking. By investigating the interactions between *L. humile* and Fynbos native ant species we can potentially explain ant distribution patterns and the potential for native ant species to limit invasion of *L. humile* through biotic resistance. The Fynbos is rich and abundant in carbohydrate resources from floral nectar (Cowling *et al.* 1996); however, only two studies have quantified the ability of native ants versus *L. humile* in utilising these available resources (Lach 2007, 2013). By studying the ability and efficiency of native and invasive ant species to utilise available resources we may gain insight to the mechanisms and processes underlying the invasion success of *L. humile* in natural communities. The indirect threat of extinction of many Fynbos plants as a consequence of the displacement of native ant species that play important roles in mymercochory can further be exacerbated by resource availability in the Fynbos in terms of floral nectar resources, which may facilitate the further spread of *L. humile* into pristine environments with a concomitant increase in these negative impacts.

It is imperative to understand competition between resident ant species and an introduced species in terms of foraging success which can in turn affect colony survival. Competitive interactions between resident species and introduced species are important aspects to investigate in order to determine the invasibility of a recipient community. Quantification of resource availability and partitioning of these resources between resident species and introduced species can assist in understanding the efficiency of resource use by introduced species compared to those resident species in the community. Firstly, this study ascertained whether competitive pressure from *L. humile* alters the foraging behaviour of native species so as to minimise competitive pressure from this invader. Secondly, this study also investigated several important mechanisms behind *L. humile's* successful

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spread into natural environments, and provides important observations of the ecology and behaviour of four native ant species of the Fynbos about which very little is known.

The first chapter introduced invasive ants in general, aspects of the recipient environment that promote or limit invasion success, as well as the study organism. The chapters that follow cover more relevant topics in further depth. The thesis is structured as standalone manuscripts and subsequently there may be occasional overlap across chapters and references. In addition, the term ”we” is used frequently throughout the chapters, which is my acknowledgement of my supervisor’s contribution in the entire research process, though the results reported in this thesis are my original work.

The foraging efficiency, measured as the ability to discover a resource, recruit nestmates to a resource as well as the ability to defend a resource, was compared between *L. humile* and native Fynbos ant species using aggression and resource competition (interference and exploitation) bioassays, and is presented in chapter 2. The current distribution of *L. humile* in South Africa, with the African big headed ant, *Pheidole megacephala*, dominating the eastern escarpment and mutually exclusive of *L. humile*, was investigated in chapter 3 also using aggression and resource competition bioassays. The availability of resources is thought to be a factor that may limit the spread or invasion success of invasive ants in natural environments. We predict that the presence of an abundant and temporarily available carbohydrate resource is important in maintaining high local densities of *L. humile* and may facilitate their spread into natural communities (see Rowles & Silverman 2009). In order to understand the foraging biology of *L. humile* in the Fynbos, we compared their patterns of floral resource use with that of the native ant *Anoplolepis custodiens* using stable isotope analysis in order to ascertain whether the availability of floral carbohydrate resources can potentially facilitate the invasion of *L. humile* in the Fynbos (chapter 4). We also use stable isotope analysis to ascertain the level of trophic niche separation and/or diet shifts between Fynbos native ant species and *L. humile* in three invasion categories: (i) uninvaded Fynbos, (ii) invaded Fynbos and (iii) pine forests (Chapter 5). The overall implications of the study findings are discussed in Chapter 6.

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CHAPTER 2: The Argentine ant, *Linepithema humile*, interferes with the foraging success of native ant species in a biodiversity hotspot.

ABSTRACT

The Cape Floristic Region (Fynbos) is a biodiversity hotspot characterised by high levels of endemism, with nearly 53% of Proteaceae species dependent on native ant species for seed dispersal. The potential impact of the invasive Argentine ant, *Linepithema humile*, on the native ant community was inferred using an empirical laboratory based study which evaluated the Fynbos native ant species and the Argentine ant vis-à-vis resource exploitation and interference competition, a function of their competitive ability and potential foraging success. We tested the ability of each species to find, recruit nestmates to and assimilate a resource, as well as compete for the same resource by pairing a native ant nest with that of *L. humile*. Levels of interspecific aggression were recorded, a proxy for competition between the two species. *Linepithema humile* recruited the greatest number of workers but they did not discover the bait more quickly nor did they consume more of the bait than native ant species when foraging in the absence of a potential competitor. During resource competition, the native ant *Anoplolepis custodiens* was better at discovering the bait compared to *L. humile*. However, once *L. humile* located the bait, they rapidly displaced native ants and monopolised the bait within the first 10 minutes of the trial, dominating the baits through high aggression. *Pheidole capensis* and *A. custodiens* are the dominant ant species in the Fynbos and occupy mutually exclusive distributions to that of *L. humile* and therefore supposedly limit Argentine ant invasion. Both native species, however, suffered significant mortality through Argentine ant aggression and subsequently their inability to compete with *L. humile* paints a bleak picture for the future of this biodiversity hotspot. This study showed no support for biotic resistance to Argentine ant invasion in the Fynbos.

Keywords: Resource competition, foraging efficiency, biotic resistance, *L. humile*, Fynbos.

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INTRODUCTION

In ant communities, interspecific competition is considered an important factor shaping community structure (Hölldobler & Wilson 1990; Parr *et al.* 2005). Interspecific competition results in a hierarchy where behaviourally and ecologically dominant ant species define ecosystem function through their competitive interactions with other ant species over resources and space (Andersen 1992, Andersen & Patel 1994, Andersen 1995). Interspecific competition thus affects the distribution and activity patterns of other ant species within the community (Andersen & Patel 1994; Sanders *et al.* 2001; Hoffman & Andersen 2003; Sanders *et al.* 2003). Ecological dominance of a species is when an ant species makes up a large proportion of the ant community biomass and can be found in very high abundances at resources (Davidson *et al.* 2003), while behavioural dominance is when an ant species demonstrates extreme aggressive behaviour towards other ant species within the habitat, often deterring them from resources (Davidson 1998; Blüthgen *et al.* 2004). The combination of behavioural and ecological dominance may allow an ant species to structure the ant community (Morrison 1996; Cerda *et al.* 2012; Parr & Gibb 2012). This may have detrimental impacts with the complete displacement of some ant species, especially if the dominant species is an invader (Sanders *et al.* 2001; O'Dowd *et al.* 2003; Walters & Mackay 2005). Globally, invasive ant species commonly reduce the abundance and species richness of native ants in communities where they have invaded (Holway 1999; Holway *et al.* 2002; Carpintero *et al.* 2005; Carpintero & Reyes-Lopez 2008; Abril & Gómez 2009). However, some ant communities with highly competitive native ant species have low invasibility as a result of biotic resistance (Elton 1958; Hoffman *et al.* 1999; Thomas & Holway 2005; Walters & Mackay 2005; Wetterer *et al.* 2006; Roura-Pascual *et al.* 2011).

Invasive Argentine ants, *Linepithema humile*, are successful in their introduced ranges largely due to the numeric dominance they attain and the high aggression they display towards native ant species (Human & Gordon 1996, 1997; Holway 1999; Holway & Case 2001). They outcompete the majority of native ants at resource exploitation, being able to discover, monopolise and retrieve resources faster than most native ant species (Human & Gordon 1996, 1997; Buczkowski & Bennett 2008). Moreover, they defend resources through the use of chemical defence and physical aggression thereby displacing native ants from resources (De Kock 1990; Holway 1999; Zee & Holway 2006; Buczkowski & Bennett 2008). *Linepithema humile* however do not always outcompete native species when introduced into communities with ecologically similar species, since the native ant species which are comparably competitive and aggressive to *L. humile* are capable of resisting invasion (Walters & Mackay 2005; Thomas & Holway 2007; Blight *et al.* 2010). Environmental conditions have been shown to significantly affect the likelihood of *L. humile* displacing *Iridomyrmex* species in Australia, over and above the competitive ability of these ants over *L. humile* (Thomas & Holway 1997). Thus, there is a combination of both environmental and behavioural factors that influence successful invasion by introduced species, for example *L. humile*. *Linepithema humile*, like other invasive species, arrive as small propagules which must overcome a range of ecological conditions in the

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recipient environment such as physiological tolerance to the abiotic environment and the ability to withstand biotic interactions with native species (Moller 1996, Way *et al.* 1997; Mack *et al.* 2000; Richardson *et al.* 2000; Chapman & Bourke 2001; Shea & Chesson, 2002; Walters & Mackay 2005). What gives them the competitive edge to outcompete native ant species, successfully establish, spread and naturalise to become invasive? These aspects are central in understanding why some communities are better able to resist invasion than others (Walters & Mackay 2006; Rowles & O'Dowd 2007).

Linepithema humile has successfully established in many parts of the world with a Mediterranean and sub-tropical type climate (Passera 1994; Suarez *et al.* 1998; Vega & Rust 2001; Holway *et al.* 2002), as a direct consequence of human activities (Suarez *et al.* 2001). Its distribution is typically associated with human-influenced environments, however, it has also successfully spread into pristine natural environments in some regions (Suarez *et al.* 1998, Holway 1998; Suarez *et al.* 2001; Luruli 2007). Abiotic factors such as climatic suitability, water availability and altitude are determinants of *L. humile* establishment, survival and distribution (Holway *et al.* 2002; Roura-Pascual *et al.* 2004; Thomas & Holway 2005; Menke & Holway 2006; Brightwell *et al*. 2010; Roura-Pascual *et al.* 2011). Conversely, the influence of biotic factors such as interactions with native species cannot be ignored (Walters & Mackay 2005; Rowles & O'Dowd 2007), since they will have to compete with native ants for resources (food, nesting space) (Walters & Mackay 2005; Blight *et al.* 2010).

Resource Exploitation is defined as the ability of an ant species to discover a resource and utilise it before other ant species arrive (Hölldobler & Wilson 1990; Davidson 1998; Human & Gordon 1999; Holway & Case 2001), while *Interference Competition* describes the ability of a species to utilise a range of behavioural mechanisms which enables it to interfere with the foraging ability of other ants by actively removing or excluding them from a resource (Hölldobler & Wilson 1990; Davidson 1998; Le Brun *et al.* 2007). Thus, species unable to compete with *L. humile* are either excluded from the community or are forced to utilise other resources (Andersen 1995, Holway 1999).

Linepithema humile was first introduced into Stellenbosch, South Africa in the late 1800s (Skaife 1979; Prins *et al.* 1990) and has since spread over large urban parts of the country (De Kock & Giliomee 1989; Luruli 2007). Although it is largely associated with human–influenced areas (Luruli 2007), it has spread into pristine environments (Buys 1987; De Kock 1990; Visser *et al.* 1996; Lach 2007, 2008). The Cape Floristic Region (CFR) is one of the six floral kingdoms of the world and is considered a unique biodiversity hotspot (Cowling *et al.* 2006). The Fynbos biome is the largest of the biomes of the CFR and the presence of the Argentine ant poses a serious threat to the future of Fynbos plant communities (Bond & Slingsby 1984), and the biome as a whole (De Kock *et al.* 1992). *Linepithema humile* do not co-occur with native Fynbos ant species that play a key role in the seed dispersal of many Fynbos plants (Bond & Slingby 1984; Donnelly & Gilliomee 1985; De Kock 1990; Christian 2001), such as *Pheidole capensis* (Mayr), *Anoplolepis custodiens* (Smith) and *Anoplolepis steingroeveri* (Forel) (Slingby & Bond 1984; De Kock & Giliomee 1989; Christian 2001; Witt &

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Gilliomee 2004; Luruli 2007). Moreover, they also interfere with the floral visitation by arthropods (bees, butterflies and beetles) that play important pollination roles in the Fynbos, and this may have significant future consequences for the germination of plants in this biodiversity hotspot (De Kock & Giliomee 1989; Lach 2007, 2008).

Globally, few studies have explicitly compared the direct competitive interactions of native ants against *L. humile* (Holway 1999; Human & Gordon 1996, 1997), or measured the direct impact this invader has on the foraging strategies of native ants or the mechanisms by which this invader excludes native ant species from an area (Morrison 1999; Buczkowski & Bennett 2008; Abril & Gómez 2009; Blight *et al.* 2010). Furthermore, we know very little of the foraging ecology of the native ant species in the Fynbos, and even less on the direct impact *L. humile* has on the foraging behaviour of these native ant species. This study investigated the response of Fynbos native ant species to *L. humile* by measuring their foraging success and ability to compete with *L. humile* for shared resources under laboratory conditions. We used equal sized colonies of both *L. humile* and native ants to mimic the small propagule size of *L. humile* that would interact with native ant species at the introduction phase so as to understand the factors that promote successful establishment, spread, naturalisation and invasion. By controlling for abiotic conditions in the laboratory, we evaluate the importance of the biotic interactions between *L. humile* and the native ant species. Biotic resistance from native ant species has been shown in Australian ant communities where native ant species limit the ability of *L. humile* to successfully invade some areas (Walters & Mackay 2005). Biotic resistance to *L. humile* invasion has not been previously tested in South African ant communities (see chapter 3 for additional studies on biotic resistance in South Africa). Therefore, investigating the interactions between *L. humile* and Fynbos native ant species within natural environments (where indigenous ants are abundant) we can make inferences on the current *L. humile* distribution patterns in the Fynbos and hypothesise on the potential of native ant species to limit invasion of *L. humile* into undisturbed Fynbos through biotic resistance.

METHODS

Ant collection and laboratory maintenance.

This laboratory based study compared the foraging efficiency of native ants to that of *L. humile*, controlling for colony sizes to mimic interactions with potential incipient colonies of *L. humile*. We standardised colony sizes so as not to confound these data with numeric dominance of one species over another (Rowles & O'Dowd 2007). Fynbos native ant colonies are generally small, no more than 50-600 individuals with the exception of *P. capensis* and *A. custodiens* which can be populous (Skaife 1962, Addison & Samways 2006 for *A. custodiens*, N.Mothapo pers. Obs.). *Pheidole capensis* and *A. custodiens* are polymorphic (Skaife 1952), with *A. custodiens* having continuous polymorphism with minors, medias and majors occurring together (Addison & Samways 2006). In general, very little is known of the social structure, ecology and morphology of most native Fynbos ant species. Nests of *L.*

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humile (Mayr) and four common Fynbos native ant species (*Tetramorium quadrispinosum* (Emery), *Pheidole capensis* (Mayr), *Anoplolepsis custodiens* (Smith) and *Lepisiota capensis* (Mayr) were collected in the Jan Marais Park in Stellenbosch (33°93' S, 18°87' E) and the Jonkershoek Nature Reserve in the Jonkershoek valley (34°58' S, 18°56'E), 15 km south-east of Stellenbosch, South Africa. Nests of the focal ant species were located by overturning small rocks and locating small mounds of soft soil when ants were observed in the vicinity. The nest was dug out using gardening trowels and transferred to 4.5l plastic containers and transported to the laboratory. Nests of *P. capensis* were collected as far apart as possible so as to avoid collecting from the same colony as these ants are also polydomous (Donnelly & Giliomee 1985, Addison and Samways 2006). *L. humile* in South Africa form two supercolonies, the nests collected originate from one supercolony as the ants belong to the large supercolony occurring within the Western Cape (see Mothapo & Wossler 2011).

Experimental colonies consisting of 500 workers, a single queen and no brood, were established from the original colony by extracting them directly from the soil using an aspirator; and kept in 4.5l plastic containers. A small layer of soil from the original nest was added to provide ants with nesting material. The colonies were maintained in the laboratory under the following conditions: 24°C±2°C, 40% RH and a 12L: 12D light-dark cycle; they were provided with 0.25M sugar water on the first day of the set-up and at least twice a week until experiments ended. Water was provided *ad libitum* while solid food was only provided during experimental trials. All experiments were conducted in plastic arenas (60 x 120 x 15 cm) layered with Plaster of Paris on the floor and lined with Fluon™ (Fluoropolymer Dispersion, Whitford Plastics LTD, England) on the sides to prevent ant escape. The Plaster of Paris was regularly cleaned to remove any residue odors and dead ants. It was also moistened to prevent dust and maintain a moist surface before each experiment to improve traction during walking. .

Experimental procedure

The foraging efficiency and competition for resources of the focal native species and *L. humile* were evaluated in two experiments. The first was to establish baseline data on the foraging efficiency for each species on a resource. The second was to investigate the foraging efficiency of each native species when competing with *L. humile* for the same resource, with all interspecific interactions recorded. Three variables associated with foraging success in ants were measured (Davidson 1998): (1) Discovery time: time taken to discover the bait, (2) Recruitment intensity: recruitment of nestmates to the bait and (3) Resource consumption: the amount of bait taken by the ants within a given time.

Foraging efficiency of each species

The foraging efficiency of each focal species was tested independently. We collected nests of *L. humile* (n = 5), *T. quadrispinosum* (n = 8), *P. capensis* (n = 6), *A. custodiens* (n = 6) and *L. capensis* (n = 6) in the field and transported them to the laboratory for sorting and acclimation for the baseline

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study. The experimental colonies were established from these nests as described above. All workers were starved for 72 to 96 hours prior to experimentation to ensure that the ants were hungry enough to start foraging during the experiment (Buczkowski & Bennett 2008; Blight *et al.* 2010; Mathieson *et al.* 2012). The ants were allowed to roam the arena for 24 hours before the experiments were initiated. This was done by placing each nest box in the arena and partially opening the container lid and connecting an artificial bridge made of cardbox from the top of the container to the arena floor. The ants used this to exit and re-enter the nesting box. We mixed bait consisting of tuna, honey and water in the ratio of 3:1:2. This bait was found to be attractive to the abovementioned experimental ant species in field based baiting experiments (Luruli 2007; Vorster 2011). The bait represents a clumped resource (introduced as a single clump) which is too large for an individual ant to move and requires the recruitment of a large proportion of the colony members for efficient retrieval (Holway 1999).

The single bait was placed 60cm from the nestbox, a 5cm radius was also delineated around the bait location, and only the ants present within this circumference around the bait were recorded during the experiments. We then weighed 2g of the bait using a microbalance (Explorer-OHAUS with a weighing range of 0.001-410 g) and placed it on weighing paper (2x2cm). For each trial, we weighed (before and after each trial) the experimental bait and a second bait as a control to measure loss by desiccation. The control bait was placed outside of the arenas during the experimental trial period. We used three measures of foraging success as mentioned above Discovery time, Recruitment intensity over 90 minutes and Resource consumption to determine the foraging efficiency of each ant species in the absence of a competitor. Timing was initiated immediately after placing the bait in the demarcated area within the arena, ensuring no ants were within a 30 cm radius of the demarcated area. Discovery time was recorded immediately when the first ant arrived at the bait. The numbers of ants present within the circumference around the bait were recorded by counting every 10 minutes for one minute at 20 second intervals over a period of 90 minutes. Each count interval, the one minute reading, was taken as an independent reading.

Interspecific resource competition

The ability of the focal native ant species to compete with *L. humile* for resources was evaluated. Another set of experimental colonies were collected and paired as follows: *L. humile* X *T. quadrispinosum* (n = 5), *L. humile* X *P. capensis* (n = 7), *L. humile* X *A. custodiens* (n = 7) and *L. humile* X *L. capensis* (n = 6); sample sizes differed due to the nest availability of native ant species. Each nest was placed on either end of the arena and opened simultaneously with the bridge attached to the top of the container and the arena floor to allow ants freedom of movement. Discovery time and recruitment intensity were measured as before, but resource consumption was not measured for these trials as we could not differentiate the amount of resource used by each species.

During the 90 minute trial, we recorded all interactions between the two species by scanning for 10 seconds every two minutes for ten minutes at 10, 40 and 70 minute intervals of the trial. Interactions

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between the ants were scored according to the categories defined by Suarez *et al.* (1999) where 1= antennation, 2= avoidance, 3= aggression (pulling, biting, abdominal curling to deposit or spray chemical compounds) and 4= fighting (progressive aggression, fighting until one or both ants are dead). Categories 1 and 2 were regarded as non-aggressive interactions, while 3 and 4 were aggressive interactions. The number of dead ants within the 5cm circumference was recorded for each species at the end of each trial.

Statistical analysis

Foraging efficiency of each species

The discovery time to a resource of each species in the absence of a competitor was compared using a One Way ANOVA (with posthoc analyses using Tukeys HSD test when statistical significance was reached). Recruitment curves, showing the pattern of recruitment to a resource over the 90 minute trial period, are presented as Mean±SE. We corrected the resource assimilation data by subtracting the weight loss by desiccation from the experimental bait weight to determine the actual amount of food available and utilised by each ant species. We then used a Kruskal-Wallis test to compare the amount of bait retrieved by each species in the absence of a competitor. The discovery times of each species during paired interactions with *L. humile* were compared using an Independent Samples T-test. A two-way repeated measures ANOVA with a Greenhouse-Geisser correction was used to ascertain the effect of competitor presence on recruitment patterns (recruitment effort over time) and recruitment intensity by comparing a species' baseline recruitment to that when it competed with *L. humile* for a resource over the 90 minute trial. The Greenhouse-Geisser correction is used when the assumption of sphericity (equal variances) is violated and calculates new degrees of freedom and significance value (p) so that a valid F-ratio can be obtained. Posthoc tests were done using Least Square Difference (LSD). The four aggression categories were converted to binary data of aggression and non-aggression. The number of aggressive interactions was summed across the 90 minute trial period and presented as bar graphs. The proportion of dead ants during resource competition was calculated for each species; arcsine transformed and compared using a Mann-Whitney U test. All analyses were conducted in SPSS 20.0 statistical software and statistical significance was accepted at $p < 0.05$.

RESULTS

Foraging efficiency of each species

All of the ants species tested were equally fast in discovering a resource in the absence of a competitor ($F_{(4, 30)} = 0.82$, $p > 0.05$, Figure 1). *Linepithema humile*, however, was very successful at recruiting colony members to the resource within the first 50 minutes of the trial (Figure 2). *P. capensis* also recruited large numbers of workers throughout the trial, but took longer to do so (Figure 2). The observed behaviour is typical of both *L. humile* and *P. capensis* as they are known for their mass recruitment strategy when foraging (Christian 2001). Neither *T. quadrispinosum, A. custodiens* nor *L. capensis* showed high levels of recruitment despite the lack of a competitor. Even though the

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recruitment of some of the native ants was not very high relative to *L. humile* , the few ant workers that were present at the bait were able to consume equal amounts of bait compared to the mass recruiters *L. humile* and *P. capensis* over the 90 minute trial (Kruskal-Wallis $\chi^2_{(4)} = 6.10$, $p > 0.05$). Strangely, the number of *L. humile* at the bait drops off after 50 minutes; this could potentially be due to these ants reaching satiation levels.

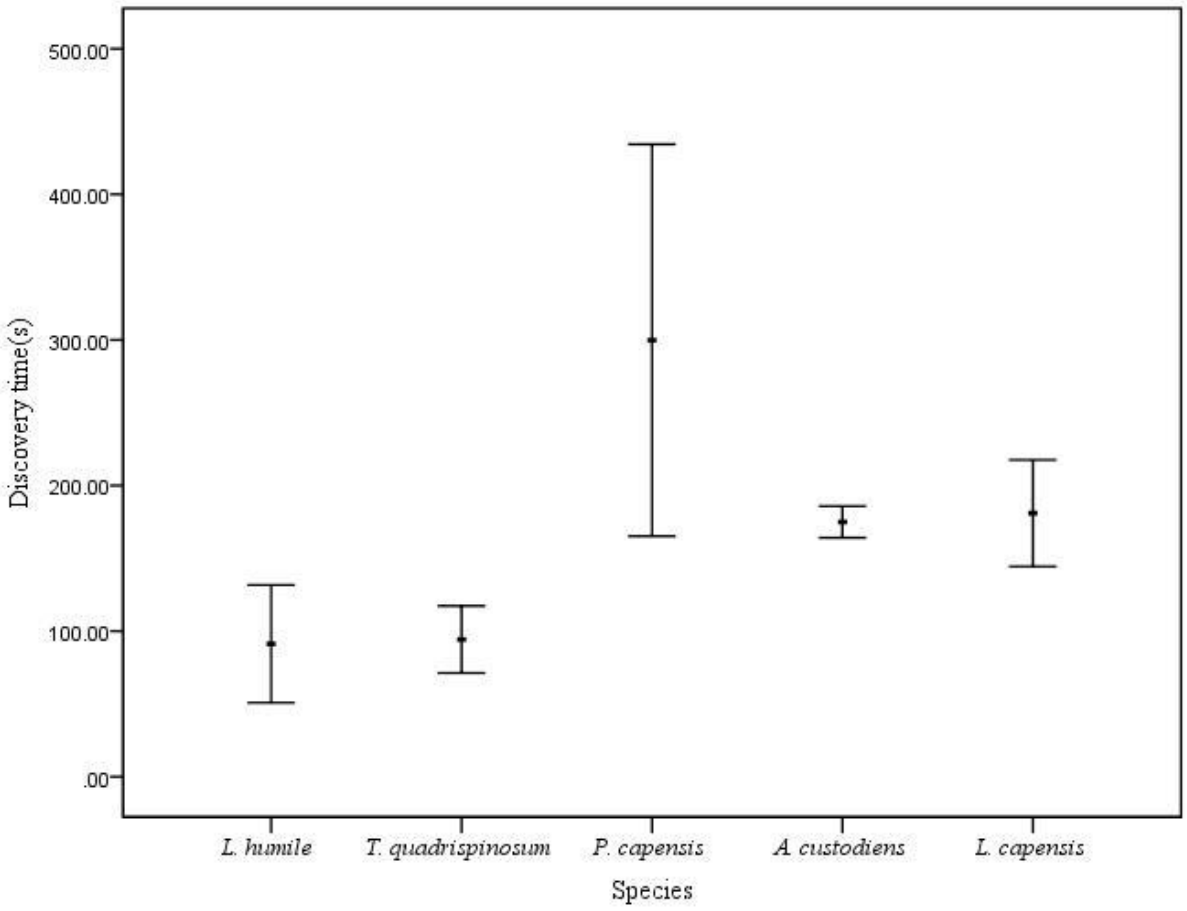

Figure 1. Discovery time (Mean±SE) of each species to a clumped resource in the absence of a competitor. There was no significant difference in the discovery time for each species in the absence of a competitor (One-Way ANOVA).

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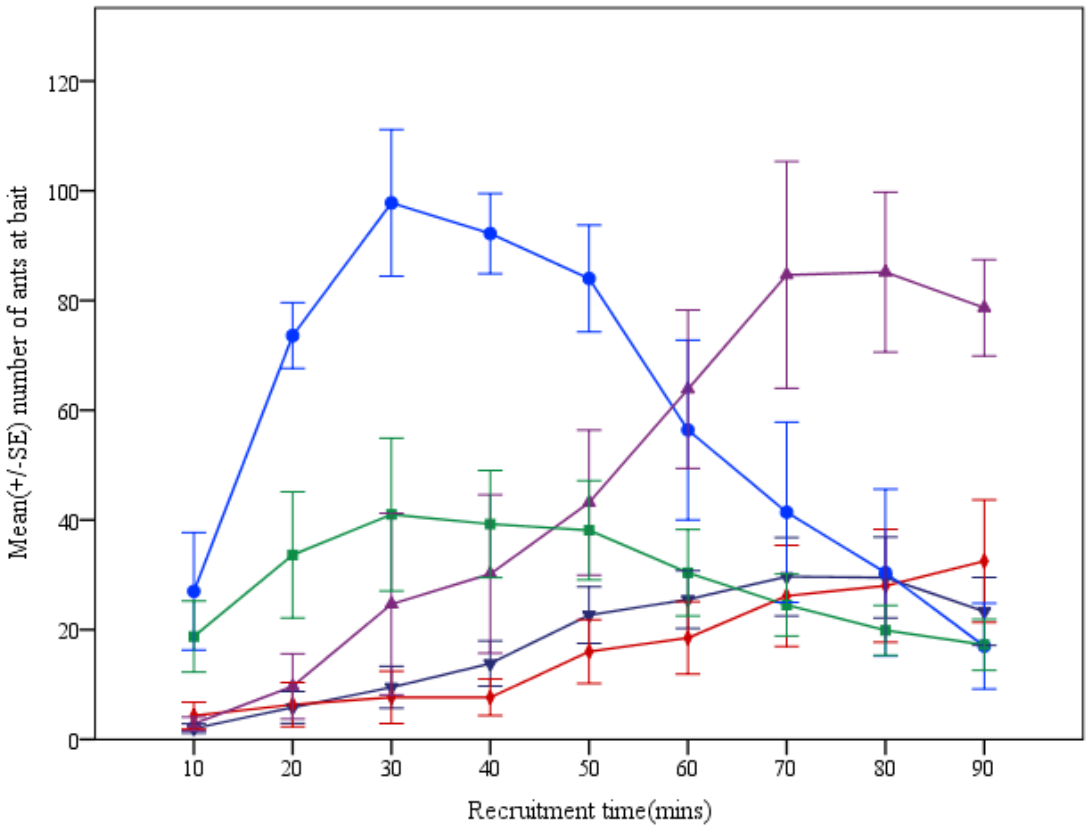

Figure 2. Recruitment curves for the five ant species representing the average pattern of recruitment to a fixed resource (bait) in the absence of a competitor. Means and SE bars for each time interval over 90 minutes. *Linepithema humile* (●), *Pheidole capensis* (▲), *Tetramorium quadrispinosum* (■), *Anoplolepis custodiens* (▼), *Lepisiota capensis* (♦).

Interspecific resource competition

Anoplolepis custodiens (331.9±240.8s) was fastest at discovering the bait during its interactions with *L. humile* (818.3±293.5s; $t_{(12)} = 3.4$, $p < 0.01$) compared to the other native ant species when competing with *L. humile* (Figure 3a-d).

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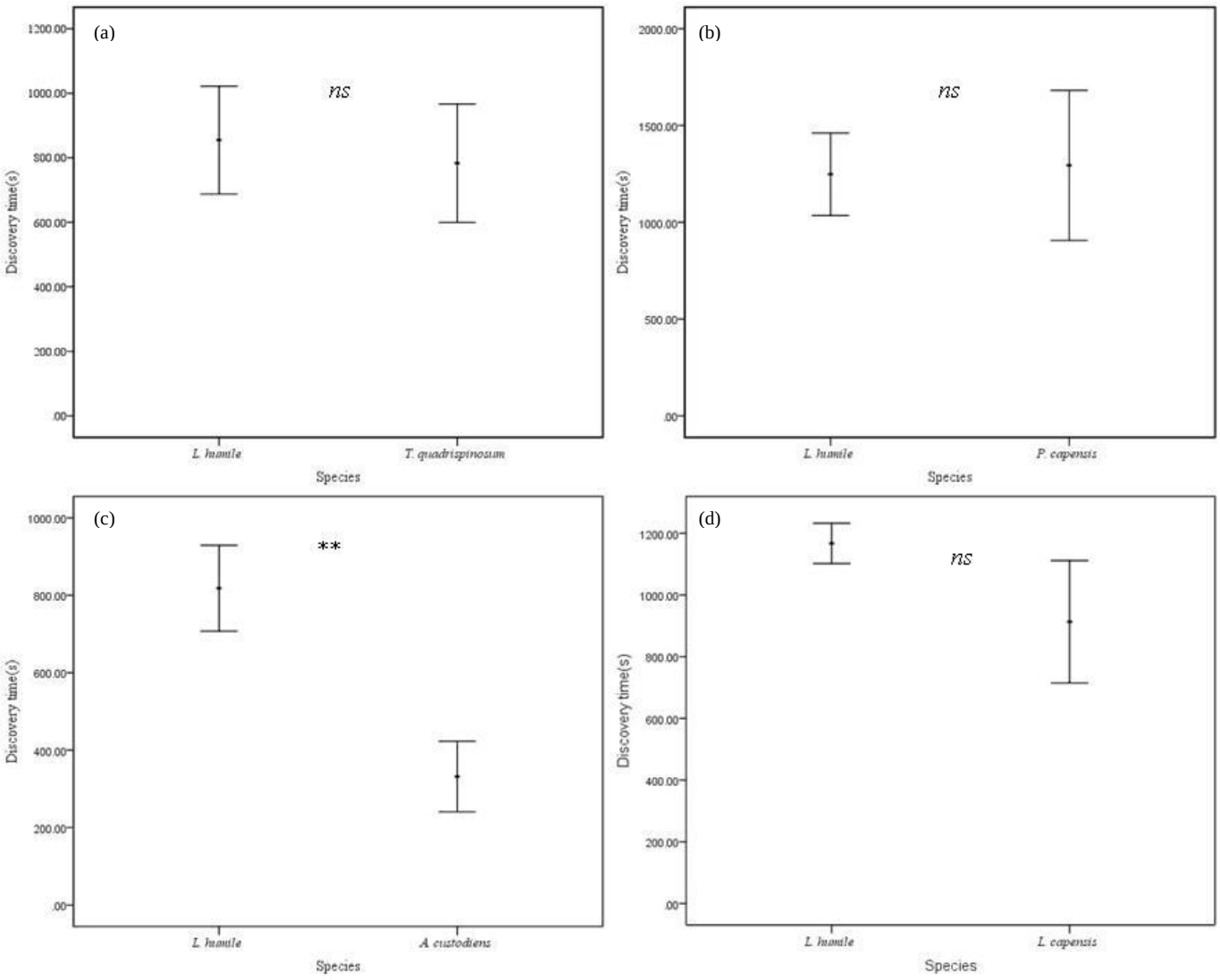

Figure 3a-d. Discovery time (Mean±SE) of each ant species to a clumped resource during interactions with *L. humile*. The native ant *A. custodiens* discovered the resource significantly faster than the *L. humile* during the interaction experiment. Independent Samples T-Test (**p < 0.01). Note the discovery times are considerably lower during this assay compared to the baseline since the ants did not have access to the arena prior to experimentation.

The recruitment effort of three native ant species tested changed significantly over time: *P. capensis* ($F_{(1.78, 19.54)}$ = 12.54, ε = 0.22, p = 0.001), *A. custodiens* ($F_{(1.94, 21.37)}$ = 4.44, ε = 0.24, p = 0.03) and *L. capensis* ($F_{(1.30, 12.98)}$ = 7.86, ε = 0.16, p = 0.01), but not for *T. quadrispinosum* ($F_{(1.99, 21.99)}$ = 3.06, ε = 0.25, p = 0.07). *Linepithema humile* showed a change in foraging effort with time, when foraging with the native ants *T. quadrispinosum* ($F_{(1.73,13.86)}$ = 9.70, ε = 0.22, p = 0.003), *P. capensis* ($F_{(1.26, 12.62)}$ = 8.41, ε = 0.16, p = 0.009), *A. custodiens* ($F_{(1.69, 16.86)}$ = 10.48, ε = 0.21, p = 0.002) and *L. capensis* ($F_{(1.82, 16.33)}$ = 21.55, ε = 0.23, p ≤ 0.001). Even though *L. humile* did not locate the bait quickest, once *L. humile* discovered it, it had a severe negative effect on the recruitment intensity of native species (Figure 4a-d). The native ant species *T. quadrispinosum* ($F_{(1)}$ = 5.59, p <0.05), *P. capensis* ($F_{(1)}$ = 23.20, p < 0.001), and *L. capensis* ($F_{(1)}$ = 6.72, p < 0.05) recruited significantly fewer workers in the

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presence of *L. humile* (Figure 4a-d). However, the recruitment intensity of *A. custodiens* ($F_{(1)} = 4.37$, $p > 0.05$) was not significantly affected by *L. humile* presence, likely due to this ant not being a mass recruiter like *L. humile* and *P. capensis* and thus using a different foraging strategy. Conversely, the recruitment intensity of *L. humile* was not adversely affected when foraging with any of the native ant species. Of all the native ant species, *T. quadrispinosum* was the only species able to recruit more than 11 workers during the entire trial period when in the presence of *L. humile* (Figure 4a), while *L. capensis* only recruited a maximum of 2 workers (Figure 4d).

Linepithema humile were highly aggressive towards all of the native ants, with the lowest proportion of aggression towards *T. quadrispinosum* (Figure 5). This aggression resulted in high mortality of two of the native ant species, *A. custodiens* ($U = 0.00$, $z = -3.13$, $p < 0.01$) and *L. capensis* ($U = 0.00$, $z = -3.08$, $p < 0.01$). In contrast, the mortality rates of *P. capensis* was relatively low (8%) yet significant ($U = 10$, $Z = -1.93$, $p \leq 0.05$), despite the high levels of aggression between *L. humile* and *P. capensis* (Figure 5). Only *T. quadrispinosum* was not lethally affected when interacting with *L. humile* ($U = 9$, $Z = -0.90$, $p > 0.05$; Figure 5).

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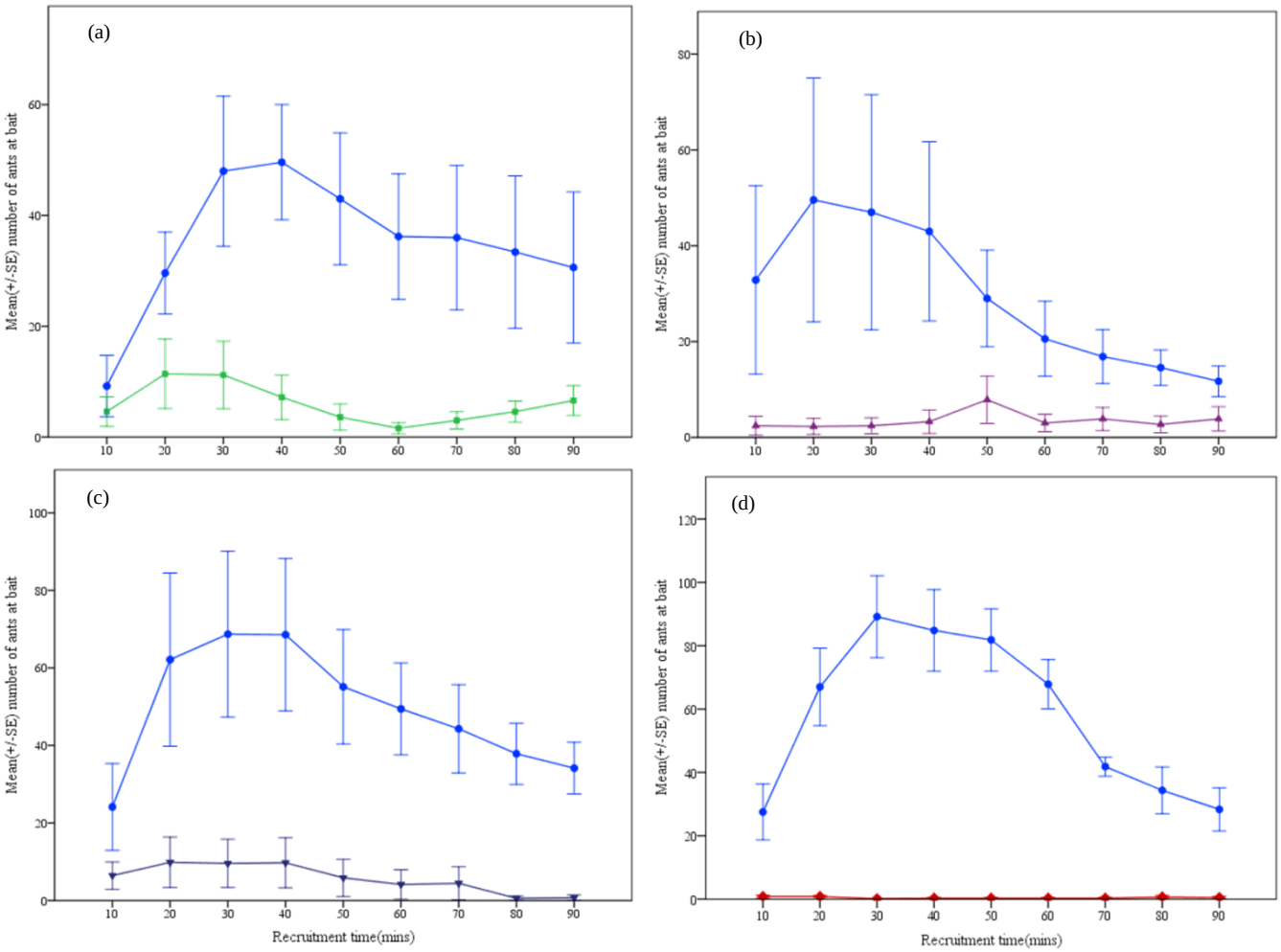

Figure 4a-d. Recruitment curves (Mean±SE) showing the recruitment patterns of each native species during interactions with *L. humile*. *Linepithema humile* significantly affected the recruitment effort of three native species (b, c and d) based on Two way repeated measures ANOVA (a) *Linepithema humile* (●) and *Tetramorium quadrispinosum* (■), (b) *Linepithema humile* (●) and *Pheidole capensis* (▲), (c) *Linepithema humile* (●) and, *Anoplolepis custodiens* (▼), (d) *Linepithema humile* (●) and *Lepisiota capensis* (♦).

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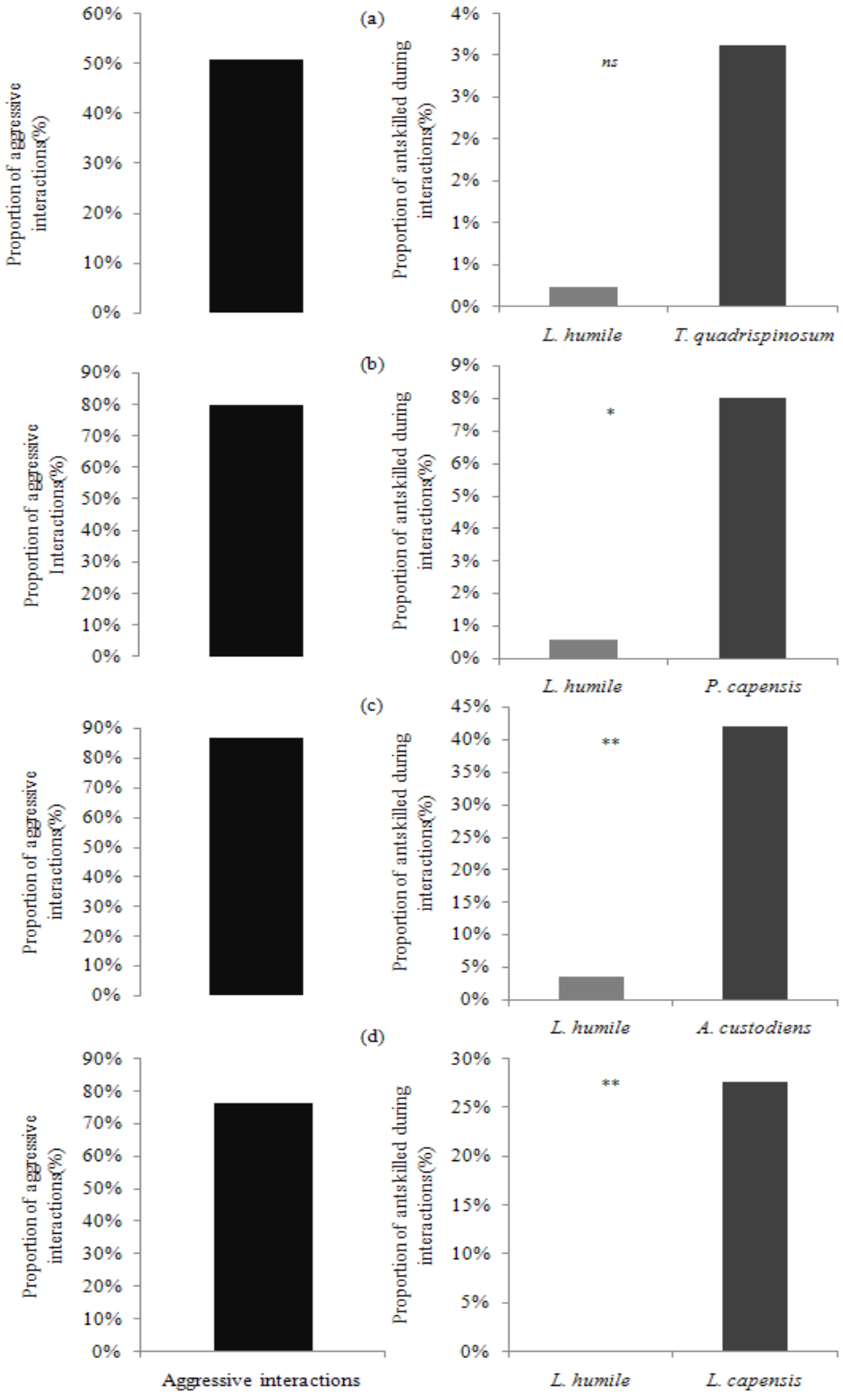

Figure 5a-d. Proportion of aggressive interactions and ants killed during interactions between *Linepithema humile* and the four native ant species. *Linepithema humile* was very aggressive towards all the native ants, however, more tolerable towards *T. quadrispinosum*. Statistical significance based on Mann-Whitney U Test, *p<=0.05, **p<0.01.

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DISCUSSION

Linepithema humile outcompeted the Fynbos native ant species through interference competition by maintaining high recruitment intensity and via aggressive interactions. The indigenous Fynbos ant species are able to forage competently through adequate recruitment and resource consumption when not faced with *L. humile* as a competitor. However, the number of workers recruited to the resource was negatively affected by the presence of *L. humile* for three of the native ant species *A. custodiens*, *P. capensis* and *L. capensis*. *Linepithema humile* outcompeted the ecologically dominant native ant species through competition for shared resources. Previous studies done in the Fynbos have focused on how *L. humile* has affected the native ant species diversity and abundance (De Kock & Giliomee 1989; Luruli 2007; Schoeman & Samways 2011), however, there have been no empirical studies on the impacts this invader has on the foraging success of Fynbos native ants. In other parts of the world, both field an laboratory studies have indicated the ability of L. *humile* to affect foraging success of native ant species, excluding them from resoures using both exploitative and interference competition (Holway 1999; Human & Gordon 1999). There is a paucity in studies investigating the mechanisms that promote displacement of native ant species and therefore this study is one of a few considering the mechanisms behind *L. humile* success (Blight *et al.* 2010, Vonshak *et al.* 2012). The ability of incipient *L. humile* colonies to potentially compete for a resource with native ant species was investigated to understand the factors that may favour their establishment, survival and spread in the Fynbos. This is the first study investigating resource exploitation and interference competition between *L. humile* and native ant species of the Cape Floristic Region (Fynbos) and clearly shows *L. humile* to be the superior competitor, and may potentially explain the differences observed in ant species composition between invaded and uninvaded areas in the Fynbos (Luruli 2007; Vorster 2011).

Despite controlling for colony size, *L. humile* still recruited in very high numbers compared to the native ant species used in this study, which ensured its monopolisation and domination of the bait. *L. humile* negatively influenced the foraging behaviour of native ants by completely preventing one of the native species, *L. capensis*, from having access to the bait. Moreover, they also raided both the nests of *L. capensis* and *A. custodiens* during the experiments, a behaviour not uncommon to *L. humile* (de Kock 1990; Zee & Holway 2006). Nest raiding suggests a high intolerance for epigaiec species by *L. humile* (Human & Gordon 1996; De Kock 1990; Zee & Holway 2006). *Tetramorium quadrispinosum* co-exists with *L. humile* (Witt 1993; Luruli 2007), while *P. capensis* and *A. custodiens* (de Kock 1990; Witt & Giliomee 1999; Luruli 2007) never co-exist with *L. humile*. This study shows no support for biotic resistance to *L. humile* invasion in the Fynbos, but supports previous findings that *T. quadrispinosum* can coexist with *L. humile* (Witt & Giliomee 1999; Christian 2001; Luruli 2007).

Competition between ant species with comparatively similar competitive ability and ecological niche requirements influences ant community structure, with the ant distribution patterns and

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assemblage of the ant community being determined by those ant species that are behaviourally and ecologically dominant (Cerda *et al.* 2012; Parr & Gibb 2012). This in turn affects the partitioning of resources and space through asymmetric competition, thus, co-existence between sympatric ant species within an ant community can be determined by resources, temperature, physiological tolerances, nesting requirements and body size (Hölldobler & Wilson 1990). Species must differ in resource and niche requirements to coexist peacefully (Abril & Gómez 2009). The native ants used in this study do not differ in terms of physiological tolerances, particularly *L. humile*, *A. custodiens* and *P. capensis*, being generally active at the same times of the day and having similar thermal tolerances (Witt *et al.* 2004), which is likely to bring them into conflict. *Tetramorium quadrispinosum* has higher thermal tolerances and can be active during the hottest parts of theday when *L. humile* is not active (Witt 1999; Witt *et al.* 2004). Strong competition is likely to arise between species that are similar in their ecological niche requirements or are both ecologically dominant within the ant community (Andersen 1995), when resources are limited, or when species share a resource or activity patterns. *Linepithema humile* is a highly competitive ant species which forms populous colonies and is highly aggressive (Holway *et al.* 2002). Moreover, *L. humile* are highly intolerant of other ant species, particularly those that have similar ecological niche requirements (Human & Gordon 1996, 1999). The native ants *A. custodiens* and *P. capensis* have been shown to share similar ecological niche requirements as do *L. humile* and thus are unable to co-exist with them (Witt & Giliomee 1999; Majer *et al.* 2004; Schoeman & Samways 2011); while both *L. capensis* and *T. quadrispinosum* are both Opportunists (see Andersen 1995 for functional group classifications, Majer *et al.* 2004), and are able to co-exist with Dominant Dolichoderinae, *L. humile*, having developed physiological, behavioural and ecological mechanisms that allow them to survive alongside *L. humile* (Andersen 1995; Hoffman & Andersen 2003; Majer *et al.* 2004). *Tetramorium quadrispinosum* was not as severely affected as the other native ant species in this study (see Figures 2, 3 and 4), being able to recruit to a shared resource in the presence of *L. humile* and suffering relatively low mortality rates (see Donelly 1983; Bond & Slingsby 1983, 1984; De Kock 1989; Luruli 2007; Vorster 2011). *Lepisiota capensis* on the other hand was not able to recruit and suffered high mortality in the presence of *L. humile. Lepisiota capensis* workers are similar in size to *L. humile* and also have similar foraging activity patterns and this may bring them into conflict over resources where they co-occur (Donelly 1983; Witt & Giliomee 1999; Witt & Giliomee 2004). This may further explain why they were not able to successfully compete with *L. humile* for a shared resource. Strategies adopted by *T. quadrispinosum* are to close their nest entrances in the field as a mechanism to prevent predation (Witt 1993) and in this study, they were observed on numerous occasions feigning death in the presence of *L. humile*. Thanatosis, death feigning, has been observed with *Solenopsis invicta* (Casill *et al.* 2008) and recently with *L. humile* (Blight *et al.* 2010), and is thought to be a submissive behaviour which highlights the dominance or superiority of the competitor (Blight *et al.* 2010).

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The foraging behaviour of the native ants within the Fynbos is poorly understood (Witt & Giliomee 1999) but it is clear that some of the native species are able to recruit large numbers of workers to an available resource, for example, *P. capensis* when foraging without competition (Figure 2). Irrespective of whether initial recruitment rates of native ants were low or high; their number of workers recruited was negatively influenced, even lethal confrontations ensued, by the presence of *L. humile* (Figure 4). The presence of dominant and functionally similar ant species within a recipient environment may limit the establishment and spread of *L. humile* (Walters & Mackay 2005; Blight *et al.* 2010; Rowles & O'Dowd 2007). *Pheidole capensis* and *A. custodiens* are functionally similar to *L. humile*, and have been suggestd to potentially limit the spread of *L. humile* in the Fynbos (Luruli 2007). However, the Fynbos native ants tested in this study are unable to compete with *L. humile*. *Pheidole capensis* and *A. custodiens* are the most abundant and behaviourally dominant native ant species in the Fynbos (De Kock 1990; Witt & Giliomee 1999; Majer *et al.* 2004) and from the most current distribution data (Vorster 2011), it has been suggested that these two behaviourally and ecologically dominant native ant species may potentially provide biotic resistance to *L. humile* invasion in the Fynbos (Luruli 2007). However, this study shows that both these species are incapable of successfully competing for resources against *L. humile* and probably why these dominant ants do not co-occur with *L. humile* in the field (Vorster 2011; see Chapter 4). With the establishment of an *L. humile* colony, numeric abundance increases, so too does their ability to exploit resources and this together with their highly aggressive behaviour promotes interference competition (Holway 1999), and ultimately influences the distribution of native ants in the community (Andersen & Patel 1994; Human & Gordon 1997; Holway *et al.* 2002). Thus the lack of comparatively competitive ant species within the Fynbos possibly offers a niche opportunity for *L. humile* to successfully establish (Davidson 1998; Hierro 2005).

Overall, our study shows no support for biotic resistance to *L. humile* by native ant species of the Fynbos. *Linepithema humile* uses physical aggression to overcome these native ant species, and based on its ability to raid the nests of other ants, it shows a high intolerance for epigaiec species (De Kock 1990; Human & Gordon 1999; Zee & Holway 2006). The foraging behaviour of *L. humile* observed in this study is consistent with previous findings found in field studies in terms of recruitment intensity and aggression to other ants present at a shared resource (Human & Gordon 1996; Holway 1999; Walters 2006). *Linepithema humile* does not fulfil the key ecological role performed by the native ant species it displaces in the Fynbos (Bond & Slingsby 1983, 1984; Christian 2001; Witt & Giliomee 2004). The native species that are able to survive or co-exist with *L. humile* are those that are adapted to high temperatures and those that have developed behavioural mechanisms that allow them to persist (De Kock & Giliomee 1990; Witt & Giliomee 1999). This may ultimately lead to the disassembly of the native ant community which will eventually lead to significant losses in native ant species richness and plant biodiversity through reduced seed dispersal and pollination.

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CHAPTER 3: Resource competition assays between the African big-headed ant, *Pheidole megacephala* (Fabricius) and the invasive Argentine ant, *Linepithema humile* (Mayr): mechanisms of interspecific displacement"

ABSTRACT

The successful establishment and spread of the Argentine ant, *Linepithema humile*, in introduced areas is mainly attributed to their colony structure as well as their ability to displace native ant species through high interspecific competition. In South Africa, *L. humile* has not established in the climatically suitable Eastern and Northern escarpments, dominated by the endemic African big headed ant, *Pheidole megacephala*. It is possible that local biotic resistance from *P. megacephala* is a factor limiting the spread of *L. humile* into these areas. We evaluated interspecific competition between these two ant species at the individual and colony levels, assessing their ability to defend their nests and to compete for a shared resource under laboratory conditions. Aggression between the two ant species was very high in all of the assays. However, mortality between the two ant species was not significantly different in the one-on-one aggression assay, suggesting that both ant species are proficient at defending themselves. Mortality in the symmetrical group assay was significantly higher for *L. humile* workers, while in the unequal group assay both ant species killed more of the other ant species when they had numeric advantage. Both ant species were equally fast in locating a shared resource, however, once *P. megacephala* discovered the bait, they displaced *L. humile* from the bait through high interspecific aggression, thereafter dominating the bait for the remainder of the trial. We demonstrate the potential of *P. megacephala* to prevent the establishment and survival of incipient *L. humile* colonies through enhanced resource competition and high interspecific aggression. This is the first study to indicate potential biotic resistance to the spread of *L. humile* in South Africa.

Keywords: *Pheidole megacephala*, *Linepithema humile*, interspecific competition, foraging efficiency, biotic resistance.

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INTRODUCTION

Interspecific competition is considered an important factor in shaping ant community structure (Hölldobler & Wilson 1990). Ecologically dominant species shape the ecosystem function through their interactions with other ant species over resources and space, which consequently affects the distribution and the activity patterns of other ant species within the community (Hoffman & Andersen 2003; Sanders & Gordon 2003). These competitive asymmetries structure the ant community resulting in a competitive hierarchy with few ecologically dominant species which are very abundant within the habitat, a few subdominant species as well as several species that are inferior, cryptic or specialised (Andersen 1995; Davison 1998; Le Brun *et al.* 2007). Ecologically dominant ant species can be described as those ant species that make up a large proportion of the ant community biomass and can be found in high abundances at resources (Davidson 1997). Ecologically dominant species can also be behaviourally dominant exhibiting high levels of interspecific aggression which allows them to control resources and influence ant community assemblage structures (Davidson 1998). The combination of these two aspects of dominance may allow a species to structure a whole community, with detrimental impacts if the species is invasive or non-native (O'Dowd *et al.* 2003).

Invasive species often arrive as small propagules that must survive a wide range of abiotic and biotic barriers in the recipient environment to successfully colonise, establish, persist, spread and naturalise (Richardson *et al.* 2000; Chapman & Bourke 2001; Walters & Mackay 2005). Suitable abiotic and biotic conditions define niche space and influence invasibility of an environment by colonising species (Richardson *et al.* 2000; Le Breton *et al.* 2005; Von Holle & Simberloff 2005; Menke *et al.* 2007). At each of these phases of the invasion process biotic factors such as propagule pressure and biotic interactions with biota already present in the recipient environment, determines the ability of the introduced species to successfully establish; while abiotic factors such as environmental suitability further affect the ability of the incipient populations of the introduced species to persist and spread (Moller 1996; Mack *et al.* 2000; Shea & Chesson 2002; Krushelnycky *et al.* 2005; Hartley *et al.* 2010). Invasive ant species are typically associated with human-influenced environments which present a niche opportunity for them (Hölldobler & Wilson 1990; Holway *et al.* 2002a; Torchin *et al.* 2003: King & Tschinkel 2006) since these areas often have high resource availability and offer limited biotic resistance because most native ant species have a low tolerance for highly disturbed environments (Elton 1958; Holway *et al.* 2002a; Colautti *et al.* 2006; King & Tschinkel 2008). This lack of competition and high resource availability combined with climatic suitability are factors that facilitate establishment and persistence of invasive ants (Chapman & Bourke, 2001; Holway *et al.* 2002a; Payne *et al.* 2004; Roura-Pascual *et al.* 2004; Abbott *et al.* 2007; Roura-Pascual *et al.* 2011). Many invasive ants share similar life-history traits that are thought to facilitate their ecological success such as unicoloniality, strong aggression, omnivory and a high affinity for carbohydrate rich resources (O'Dowd *et al.* 2003; Abbott *et al.* 2007; Sarty *et al.* 2007). Furthermore, invasive ants

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commonly excel at both resource exploitation (Davidson 1998), locating and retrieving resources faster than other ant species (Morrison 1996; Le Brun *et al.* 2007) and interference competition, through their ability to usurp resources from native ants and displace them, particularly those who share similar ecological requirements (Heterick *et al.* 2000; Holway *et al.* 2002b; Lach 2005; Le Breton *et al.* 2007). Colony level attributes such as worker size and number positively influence the proficiency to exploit available resource or effectively defend them from other ant species. This influences the ability of different ant species to co-exist within a community. Usually, ant species that co-exist have to trade-off between exploiting and defending a resource (Morrison 1996; Davidson 1998; Le Brun & Feener 2007), but invasive ant species appear to have broken this trade-off (Davidson 1998, Adler *et al.* 2007, but see Parr & Gibb 2012). Although invasive ants commonly reduce the abundance and species richness of native ant communities (Holway 1999; Carpintero *et al.* 2007), species rich native communities with ecologically dominant species may limit their spread into these natural communities through biotic resistance (Elton 1958; Hoffman *et al.* 1999; Wetterer *et al.* 2006; Walters & Mackay 2005; Blight *et al.* 2010).

The Argentine ant, *Linepithema humile* (Mayr), is one of the best studied invasive ants (Holway *et al.* 2002; Wetterer *et al.* 2009; Vogel *et al.* 2010). Originally native to Argentina and parts of Brazil (Suarez *et al.* 2001; Holway *et al.* 2002b; Wetterer *et al.* 2009), it now occupies a global distribution as a direct result of human movements (Suarez *et al.* 2001; Sunamura *et al.* 2009; van Wilgenburg *et al.* 2010; Vogel *et al.* 2010). *Linepithema humile* is successful globally owing to its highly aggressive behaviour and large colony sizes which allow this ant to rapidly colonise, establish and spread in new environments (Sunamura *et al. 2009*). In South Africa, *L. humile* was first recorded in the late 1800s in Stellenbosch (Skaife 1955; Prins *et al.* 1990), Western Cape, which is characterised by a Mediterranean climate similar to its native range in Argentina, and has since established in both urban and natural areas in six of the nine provinces (Luruli 2007). However, it has not established successfully in the Eastern and Northern parts of the country, dominated by the African big-headed ant, *Pheidole megacephala* (Prins *et al.*1990; Luruli 2007), even though these regions are climatically suitable for *L. humile* (Roura-Pascual *et al.* 2004; Luruli 2007; Roura-Pascual *et al.*2011). Thus, local biotic resistance has been proposed to explain the current distribution of *L. humile* in South Africa (Luruli 2007).

Pheidole megacephala is endemic to Africa, originating from tropical regions of sub-Saharan Africa (Wilson & Taylor 1967; Ross & Trager 1990; Bolton 1995; Dejean *et al.* 2005; Moreau 2008; Fournier *et al.* 2012; Wetterer 2012) and dominates the northern and eastern escarpment of South Africa (Prins *et al.* 1990; Majer & De Kock 1992; Luruli 2007). Although not well studied (Holway *et al.* 2002b), *P. megacephala* has been introduced in many parts of the world (Haskins & Haskins 1965; Heterick 1997; Hoffmann *et al.* 1999; Heterick *et al.* 2000; Lach 2005; Dejean *et al.* 2008; Fournier *et al.* 2009; Wetterer 2012), including on a number of tropical islands. It is aggressive to both native and

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invasive ants found in its territory and excludes them (Wetterer 1998; Wetterer & Wetterer 2004) through high interspecific aggression (Fluker & Beardsley 1970, Lieberburg *et al.* 1975; Jones *et al.* 2001; Kirschenbaum & Grace 2008), such as *L. humile* (Krushelnycky *et al.* 2005) and *Anoplolepis gracillipes* in Hawaii (Kirschenbaum & Grace 2008). Originally it was suggested that the mutually exclusive distribution pattern was influenced by climate and altitude, with *L. humile* showing a preference for the higher, cooler altitudes (Cole *et al.* 1992; Krushelnycky *et al.* 2005). More recent studies however have included biotic factors to further explain these distribution patterns (Kirschenbaum & Grace 2007, 2008; Lach 2008). From aggression bioassays, Kirschenbaum and Grace (2008) suggested that *P. megacephala* and *L. humile* maintain their mutually exclusive distribution through high levels of aggression, while Lach (2008) showed that their temporal foraging patterns and resources were similar which brought them into conflict and further contributed to their disparate distributions on tropical islands where they have both been introduced (Jones *et al.* 2001; Krushelnycky *et al.* 2005).

Empirical studies investigating the factors that facilitate successful range expansion in invasive ant species are lacking. Most studies have largely focussed on abiotic factors influencing range expansion of invasive ants (Roura-Pascual *et al.* 2004; Menke & Holway 2006, Menke *et al.* 2007; Roura-Pascual *et al.* 2011), with few studies investigating the importance of biotic factors in limiting or facilitating the spread of *L. humile* (Walters 2006). Many of these studies are largely based on bait sampling and generalisations with regards to biological characteristics of invasive ants as measures to predict invasion success (Holway 1999; Richardson *et al.* 2000; Holway *et al.* 2002a). However, detailed studies on the direct interactions between native ants and invasive ants are wanting, and are much needed to understand how invasive ant species colonise new environments and which of their biological traits are likely to enhance their invasion success (Holway 1999; Human & Gordon 1999; Blight *et al.* 2010; Vonshak *et al.* 2012). For *L. humile*, the combination between abiotic conditions and interspecific competition from native ant species may limit the extent to which these ants invade new environments as small propagules, largely because niche availability and suitability is determined by abiotic factors as well as the presence of competitive dominant ant species (Thomas & Holway 2005; Walters & Mackay 2005). Therefore, the aim of this study was to evaluate the interspecific competition between *L. humile* and *P. megacephala* using behavioural assays that evaluated individual and colony level aggression as well as competition for shared resources. This study tests the hypothesis that biotic resistance from *P. megacephala* is a plausible explanation for the current distribution patterns of *L. humile* in South Africa.

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METHODS

Ant colonies and laboratory maintenance

The distribution of the African big-headed ant, *Pheidole megacephala* (Fabricius), and the invasive Argentine ant, *Linepithema humile* (Mayr), in South Africa are mutually exclusive (Fig.1). We conducted the study in the laboratory and staged interactions between these two species that would normally occur under field conditions. Eighteen colonies of *P. megacephala* were collected (identification of all colonies collected was confirmed using the recently published key from Wetterer (2012)) along the eastern and northern escarpment of South Africa along its known distribution in the Kwazulu-Natal and Limpopo Provinces; and eighteen nests of *L. humile*, were collected from Stellenbosch and Jonkershoek in the Western Cape Province, South Africa (Figure 1). Intraspecific aggression was high between and not within *P. megacephala* colonies indicating multicoloniality (pers obs.). *Linepithema humile* forms two behaviourally, chemically and genetically distinct supercolonies with the large supercolony distributed throughout a large part of the country and the smaller colony limited to the Agulhas region of the Western Cape (Lado, 2008; Mothapo & Wossler, 2011). Ants were collected in a wide range of habitats including urban areas and in nature reserves. We located nests by laying small baits, consisting of cat food, directly on the ground and followed ants returning to the nests having fed on the bait. We also located the nests by overturning small rocks and looked for small soft mounds of soil which often were the nest entrances in the case of *P. megacephala*. Whole nests (consisting of workers, brood and queens) were dug out, using gardening trowles, and transferred into 4.5L plastic containers lined with Fluon™ (Fluoropolymer Dispersion, Whitford plastics LTD, England) on the sides to prevent ant escape and transported to the laboratory. All colonies were maintained under laboratory conditions at 25°C ± 2°C, 40% RH and a 12L: 12D light-dark cycle. The nest containers had a small amount of soil which was misted with water twice weekly to maintain soil moisture levels optimal for ants. The ants were provided with a diet of 0.25M sugar water daily, water *ad libitum* and were fed pin-head crickets twice weekly when experiments were not in progress.

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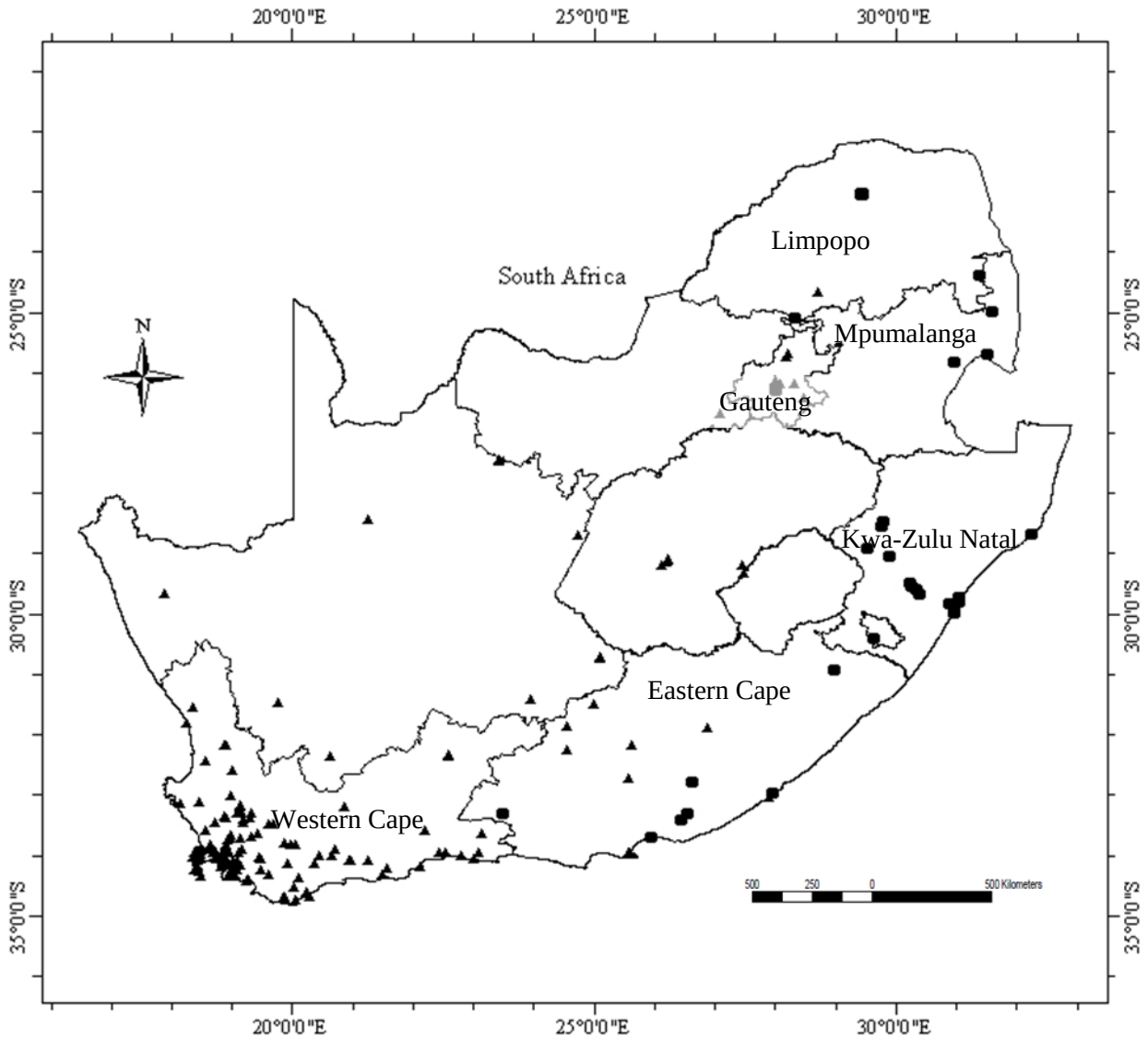

Figure 1. Distribution of *Linepithema humile* (▲) and *Pheidole megacephala* (■) in South Africa, showing the main provinces where the two ant species are found.

Aggression Bioassays

We conducted one-on-one aggression assays, symmetrical and asymmetrical group confrontations modified from Buczkowski and Bennett (2008) and Blight *et al.* (2010) to assess the fighting potential of both these species. Group competition at a resource using laboratory colonies of these two ant species was also assessed (see resource competition assay). Due to the differences in body size between *P. megacephala* majors (~3 mm) (Wetterer 2012) and *L. humile* (~2.6 mm) workers (Wild 2004), only the minors (~2-2.6mm) (Wetterer 2012) were used for all experiments with the exception of the resource competition assay where the whole colony was used. For the one-on-one aggression assays, two randomly selected workers from each colony of *P. megacephala* (n = 18 colonies) and *L. humile* (n = 18 nests) were paired in an 8 ml glass pill vial lined with Fluon 1 cm from the bottom.

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Ten trials were conducted per colony pair, and the ants were observed for two minutes (n = 180 trials). A maximum score, based on the most intense level of aggression recorded within the two minutes, was recorded for each trial. For the symmetrical group interactions, twenty workers per colony/nest were randomly selected (n = 17 colonies of *P. megacephala* and n = 17 nests of *L. humile*) and paired them together in a Fluon-lined glass petri dish (10 cm x 4cm). Similarly, for the asymmetrical group confrontations, 20 *P. megacephala* workers were paired against 10 *L. humile* workers (n = 12) and 10 *P. megacephala* workers were paired against 20 *L. humile* workers (n = 12) in a Fluon-lined glass petri dish (10 cm x 4cm). The workers were collected using an aspirator and transferred into an individual (for each species) Fluon-lined glass pill vial (8ml). Both groups of ants were allowed to settle for two minutes, after which both ant groups were simultaneously transferred to the glass petri dish. The ants were observed for five minutes and the behavioural interactions observed between the ants were recorded at one minute, three minutes and five minutes. The behavioural interactions between the two workers were scored according to the methods of Suarez *et al.* (1999) where 1- antennate with no aggressive response, 2 – avoidance (on contact, ants stay on opposite ends of vial), 3 – aggression (lunging, biting, pulling) and 4 – fighting (prolonged aggression or fight). The number of aggressive (categories 3 and 4) and non-aggressive (1 and 2) interactions observed during each trial (5 minutes) were counted. At the end of each trial we recorded the number of dead ants of each species.

Resource competition assay

We assessed the ability of these two ant species to compete for resources in a laboratory controlled experiment. We first assessed the ability of each species to exploit and assimilate a resource in the absence of a competitor (Baseline), then the potential of each species to interfere with the other during resource competition, pairing equally sized *L. humile* (nests) and *P. megacephala* colonies. We used the three measures associated with foraging success in ants: Discovery time- time taken to discover a resource, Recruitment intensity- the number of nestmates recruited to a resource over a given time period, Retrieval- the amount of resource removed by ants within a given time (Hölldobler & Wilson 1990; Davidson 1998; Morrison, 2000). The data for retrieval were not used in the analyses during interaction experiments as we did not mark or quantify the amount of food each species removed, only their presence on and around the bait.

Experiments were conducted in plastic arenas (120 x 60 x 15 cm) lined with Fluon™ (Fluoropolymer Dispersion, Whitford plastics LTD, England) to prevent ant escape. The floor of the arena was lined with Plaster of Paris which kept an even surface, and was regularly moistened to prevent dust and maintain a moist surface for ants to walk on. We established 15 experimental colonies, from original stock colonies, consisting of 500 workers, one queen and several brood pieces. Ants were starved for 48-72 hours prior to resource exploitation trials to maintain an equal state of hunger and ensure that the ants were sufficiently hungry to start foraging during the experiment

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(Buczkowski & Bennett 2008; Blight *et al.* 2010; Mathieson *et al.* 2012). We used bait consisting of a mixture of 3:1:2 parts tuna, honey and water. This bait is widely used in ant baiting studies and represents a clumped resource that is too large for an individual ant to move and requires the recruitment of colony members (Holway 1999; Human & Gordon 1999; Le Brun *et al.* 2007; Luruli 2007) to remove it efficiently.

Twenty four hours prior to the start of the baseline experiment (n = 15 colonies each of *L. humile* and *P. megacephala*), a nest container was placed against the edge of the arena, opened and a bridge made of cardbox was attached to the top of the container and the arena floor allowing the ants to move freely between the arena and nestbox. The ants were allowed to roam the arena to acclimatise. For the interaction experiment (n = 17 colonies each of *L. humile* and *P. megacephala*), the starved experimental colonies of both species were placed on opposite ends of the arena and the ants were not allowed to roam to prevent potential fighting. The bait was placed 60 cm from the nesting box in the centre of the arena. A circle with a 5 cm radius was measured around the bait location point and this was used as the observation area for all interactions. During the interaction experiment, behavioural interactions occurring between the two species within the given circumference around the resource were recorded. Scan sampling was used to randomly record the interactions between any interacting pair of ants within a given period. Every two minutes for 10 minutes at the 10, 20, 30, 60 and 90 minutes time interval of each trial a 10 s scan was done. The interactions were scored in the same way as described for the individual and group aggression bioassays. The number of aggressive (category 3 and 4) and non-aggressive (category 1 and 2) encounters were counted for the 90 minute trial. At the end of each trial, the numbers of dead ants within the given circumference of the bait were counted, as a result of the aggressive interactions that occurred around the resource.

One gram of the bait was weighed using a microbalance (Explorer-OHAUS with a weighing range of 0.001-410 g) and placed on a 2x2 cm weighing paper. Making sure that no ants were present within 30 cm of the bait location, the bait was placed on the demarcated point (60 cm from nesting box) within the circle centre. A second bait was placed next to the arena to measure weight loss by desiccation. The three measures associated with foraging success; discovery time, recruitment intensity and the amount of resource retrieved were recorded once the bait was placed in the demarcated area. Recruitment intensity was recorded by counting the number of ants present within the circle circumference around the bait for one minute every 10 minutes for a total of 90 minutes, after which the trial was ended. The experimental bait and the control bait were weighed to measure the amount of food retrieved by the ants and corrected for water loss.

Statistical analysis

The proportion of aggressive interactions was calculated from the total number of interactions for all the trials; only the proportion of aggressive interactions is presented. At the end of each trial in the

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one-on-one assay, mortality was recorded for each species. A score of 1 was given when a worker of either species was dead and 0 when the worker was still alive with no injury. A McNemar's test for paired dichotomous categorical data with continuity correction was used to compare the mortality of *L. humile* and *P. capensis* during the one-on-one assays. Worker mortality of each ant species during the group interactions were compared using either a Wilcoxon-signed ranks test or a Paired-samples T-test.

A Wilcoxon-signed ranks test or a Mann-Whitney U test was used to compare the time taken to discover the resource for each species during the assays. The recruitment intensity, number of ants at the bait per 10 minute interval, was averaged across all the trials for the two species and recruitment curves were generated. The amount of resource consumed by each ant species was assessed and compared using a Mann-Whitney U test for independent samples. Worker mortality of each ant species was compared using a Paired-samples T-test. To determine whether there was an effect of competitor presence on foraging efficiency; recruitment pattern (recruitment effort over time) and intensity without a competitor was compared to the recruitment effort and intensity when competing with a competitor for a shared resource, for each of the two species using two-way repeated measures ANOVA with Greenhouse-Geisser correction because sphericity was violated. Statistical significance was accepted at $p < 0.05$ and all analyses were conducted in SPSS 20.0 statistical software.

RESULTS

During one-on-one interactions, 83% of all interactions were aggressive (Fig. 2a) with a maximum score of four during most of the interactions resulting in a 52% mortality rate for *P. megacephala* (94 dead individuals from 180 trials) compared to a 46% mortality rate for *L. humile* (83 dead individuals from 180) (Fig. 2a); however this was not statistically significant (McNemar's $\chi 2 = 0.83$, $n = 180$, $P > 0.05$). Aggression between the two ant species during symmetrical group interactions was high (94%, Fig. 2b), with a significantly higher mortality of *L. humile* compared to *P. megacephala* ($Z = -1.99$, $P \leq 0.05$, Fig.2b). The higher mortality of *L. humile* workers during symmetrical group interactions suggests that *P. megacephala* dominated the fights, being more lethal than *L. humile*. Interactions remained highly aggressive between these two ant species in asymmetrical group interactions (Fig.2c and d), with both *P. megacephala* and *L. humile* showing superior fighting ability when they had numeric advantage as evident in the mortality rates. High levels of aggression (92%) were observed when *L. humile* had numeric advantage and resulted in significantly high levels of mortality for *P. megacephala* ($t_{(11)} = -8.01$, $P < 0.001$, Fig. 2c). When *P. megacephala* had numeric advantage, the level of aggression was only 56% yet *L. humile* still suffered high losses ($Z = -2.94$, $P < 0.001$, Fig.2d). Both species employed both physical and chemical aggression, which is common to *L. humile*.

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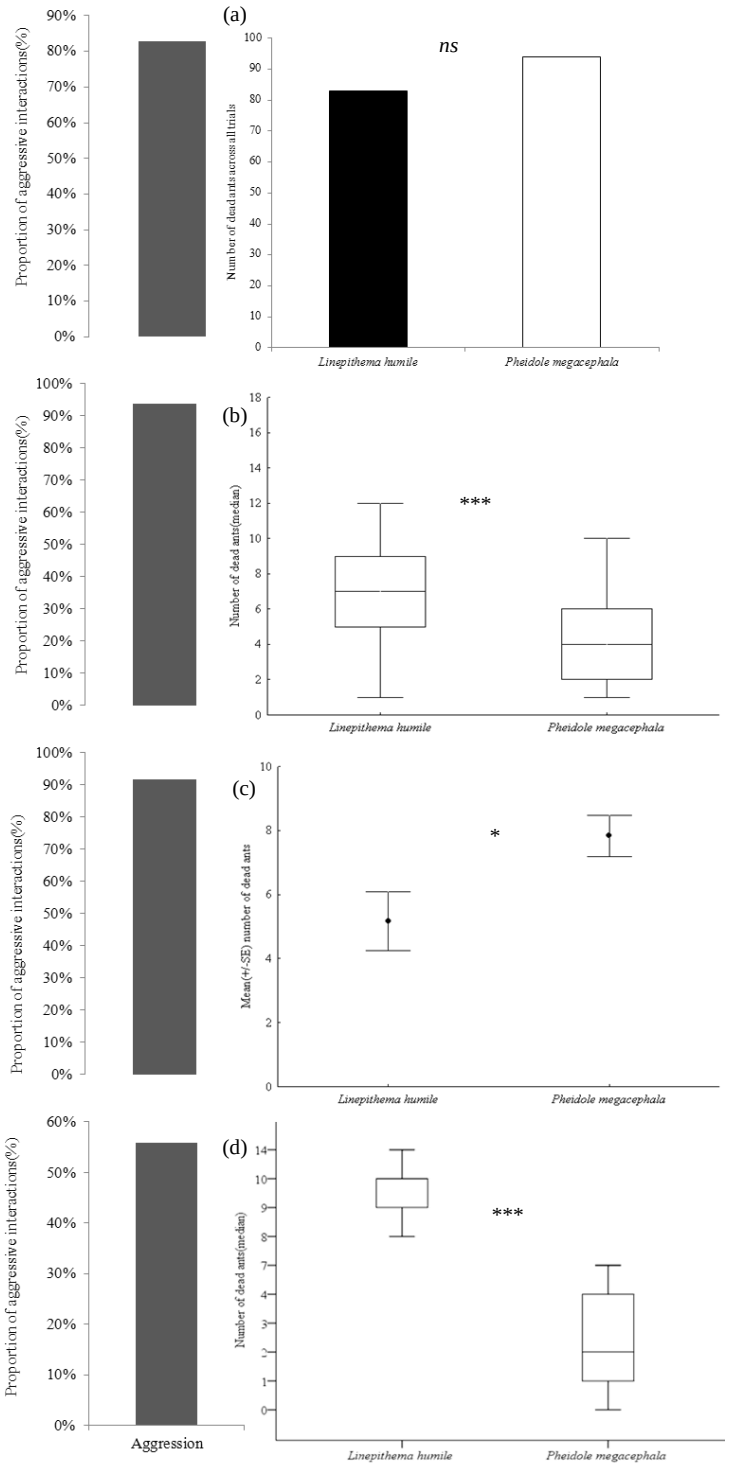

Figure 2. Proportion of aggression and mortality rates per trial of *Linepithema humile* and *Pheidole megacephala* during (a) one-on-one interactions (n=180 trials), McNemar's test (ns), (b) during interactions with equal sized groups (n=17 colonies of each ant species), Wilcoxon-signed ranks test(*** $p < 0.001$), (c) during asymmetrical group interactions with *L. humile* (n =20 workers per nest) and *P. megacephala* (n =10 workers per nest), for 12 trials, paired-samples T-test (* $p \leq 0.05$); (d) *L. humile* (n = 10 workers per nest) and *P. megacephala* (n=20 workers nest), for 12 trials, Wilcoxon-signed ranks test (*** $p < 0.001$). Box-plots b and d show Median, 25 and 75 percentiles, Min and Max.

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Resource competition

There was no significant difference in the time taken to discover food between *L. humile* and *P. megacephala* in the absence of a competitor (U = 70.5, Z= -1.74, n = 30, $P > 0.05$, Fig. 3a). Both species also showed similar levels of recruitment intensity (Fig. 3b), and even though *L. humile* retrieved more of the bait than *P. megacephala*, this was not statistically significant (U = 93, Z = -0.81, n = 30, $P > 0.05$, Fig. 3c). During the resource competition experiment, both species rapidly moved from the nest into the arena, and were equally fast in discovering the bait (Z = -0.91, p > 0.05; Fig. 4a). Having discovered the bait, *L. humile* rapidly recruited in high numbers within the first twenty minutes of the assay, thereafter however, the number of *P. megacephala* workers recruited increased with a concomitant decline in *L. humile* numbers, suggesting that they were displaced from the bait by *P. megacephala* (Fig. 4b) The recruitment effort changed significantly across time for both *L. humile* ($F_{(2.94, 82.19)} = 4.39$, $\varepsilon = 0.37$, $P = 0.007$), and *P. megacephala* ($F_{(2.92, 81.93)} = 16.02$, $\varepsilon = 0.37$, $P < 0.001$). However, *L. humile* recruited significantly less workers when competing for a resource with *P. megacephala* ($F_{(1)} = 17.77$, $P < 0.001$), while *P. megacephala*'s worker numbers were unaffected by *L. humile* presence ($F_{(1)} = 2.33$, $P = 0.14$). The high levels of aggression observed during the interactions of these two ant species (Fig. 4c), as well as the high mortality of *L. humile* (40.6 ± 6.1) in comparison to *P. megacephala* (22.3 ± 2.8) ($t_{(16)} = 4.46$, $P < 0.001$, Fig. 4d), suggests that *P. megacephala* outcompeted *L. humile* at the bait through very high levels of aggression.

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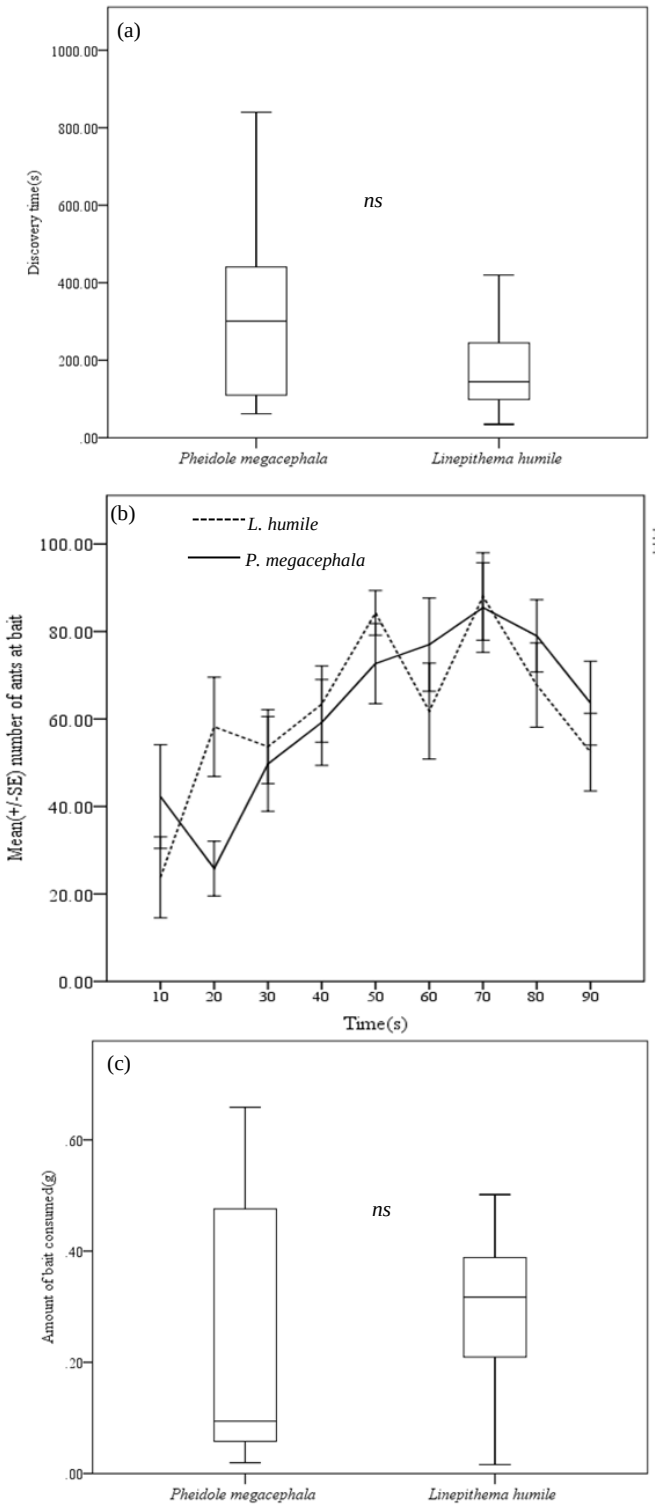

Figure 3a-c. Resource exploitation by each ant species in the absence of a competitor (Baseline): (a) Box-plot (Median, 25 and 75 percentiles, Min and Max) showing discovery time of a clumped resource for *Pheidole megacephala* and *Linepithema humile*, Mann-Whitney U-test (ns); (b) recruitment to a resource (Mean±SE over 90 minutes), and (c) retrieval of the resource after 90 minutes (Median, 25 and 75 percentiles, Min and Max), Mann-Whitney U test (ns).

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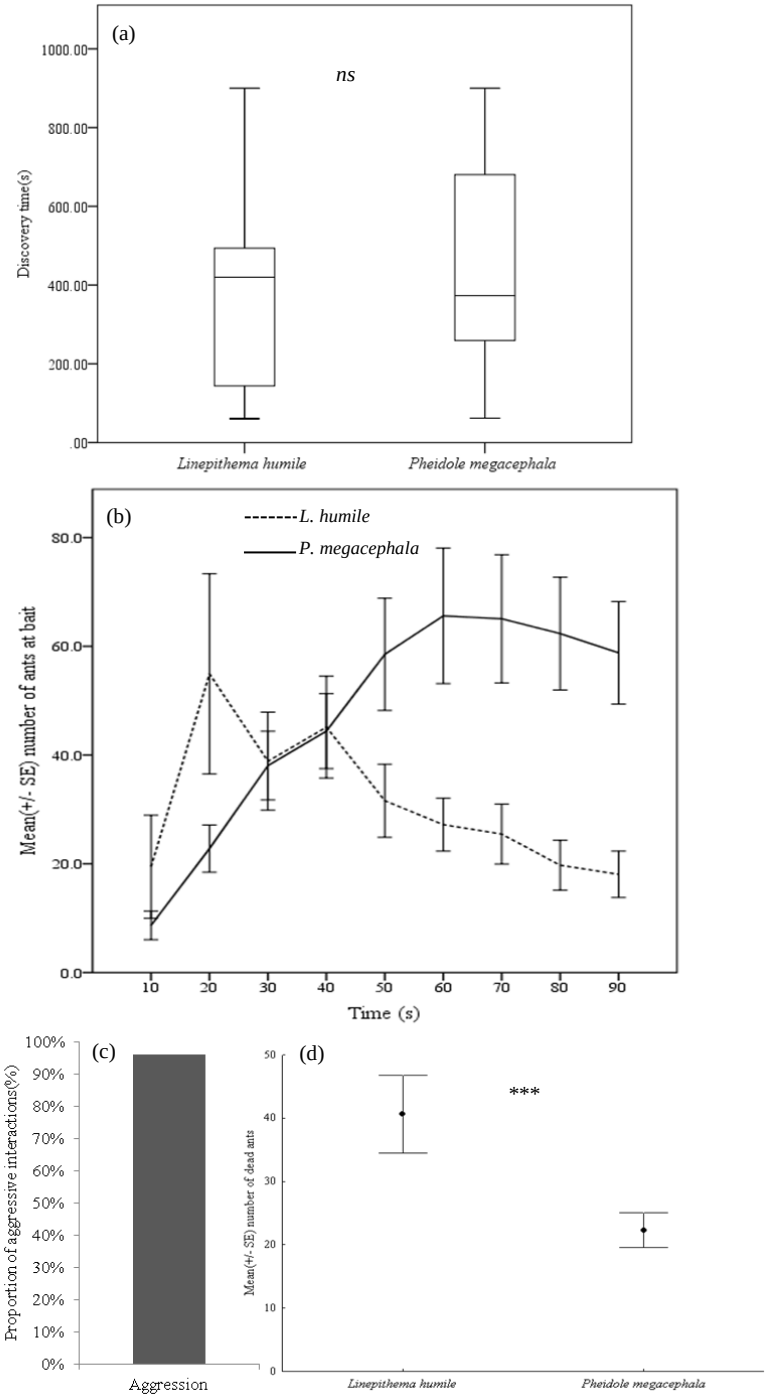

Figure 4a-d. Foraging parameters and interference during resource competition between *Linepithema humile* and *Pheidole megacephala* (a) Box-plot (Median, 25 and 75 percentiles, Min and Max) showing discovery time of a clumped shared resource for both species, Wilcoxon-signed ranks test (ns); (b) recruitment to a resource (Mean±SE over 90 minutes), *L. humile* recruitment patterns and number of workers recruited were significantly affected by *P. megacephala* presence (Two-way repeated measures ANOVA, $p < 0.001$); (c) aggression and (d) mortality of both species around the resource, Paired-samples T-test (***$p < 0.001$), n=17 colonies of each ant species.

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DISCUSSION

Both *L. humile* and *P. megacephala* are intrinsically aggressive and were highly antagonistic to each other from individual through to colony level interactions. High levels of aggression were evident during one-on-one bioassays which are contrary to previous studies which have suggested that aggression bioassays were context dependent and consequently one-on-one aggression bioassays do not reflect accurate aggression levels between species (Roulston *et al.* 2003; Buczkowski & Silverman 2005). Both *L. humile* and *P. megacephala* were able to rapidly discover, recruit to a resource with high intensity and retrieve a large amount of food with equal prowess in the absence of a competitor. Although this is a well known fact for Argentine ants (Davidson 1998; Human & Gordon 1996; Cerdá *et al.* 1998; Human & Gordon 1999; Holway *et al.* 2002b), evidence to support these characteristics for *P. megacephala* has not empirically been tested previously but rather inferred from the predictable behaviour exhibited by most invasive ant species and the fact that *P. megacephala* are populous where they are introduced (Majer & de Kock 1992; Hoffmann *et al.* 1999; Vanderwoude *et al.* 2000; Dejean *et al.* 2005). Foraging success is further determined by the ability of ants to defend a shared resource or prevent other ants from having access to it (Andersen & Patel 1994; Davidson 1998). *Pheidole megacephala* displaced *L. humile* from a shared resource through lethal aggression in this study. Interspecific competition is one of the key determinants of foraging success in ant communities, allowing for co-existence of different species adapted to different foraging strategies (Tilman 1982; Fellers 1987; Davidson 1997; Santini *et al.* 2007; Parr & Gibb 2010). The inability of introduced populations of *L. humile* and *P. megacephala* to co-exist as shown in previous studies is likely exacerbated by the fact that they have similar foraging strategies (Lach 2005; Kirschenbaum & Grace,2007; Lach 2008), nesting and food preferences that bring them into high conflict (Crowell 1968; Heterick 1997; Holway *et al.* 2002b). These findings suggest that *P. megacephala* potentially limit the establishment of incipient colonies of Argentine ant, into areas of South Africa where it dominates through high interference competition for shared resources.

Ants show heightened interspecific aggression during competition for resources or in nest defence (Reeve 1989; Holway 1999; Buczkowski & Silverman 2005; Velasquez *et al.* 2006). The level of aggression shown during these interactions are also context dependent (Tsutsui & Case 2001; Roulston *et al.* 2003; Tsutsui *et al.* 2003; Buczkowski & Silverman 2005) and fights between two ant species are more likely to occur between groups of ants rather than between individual foraging workers, since the presence of other nestmates can indicate proximity to the nest and the increased need to defend (Hölldobler & Wilson 1990; Roulston *et al.* 2003; Buczkowski & Silverman 2005), and potentially the increased likelihood to succeed. Individual workers may need to evaluate the costs and benefits of engaging in fights, facing a trade-off between initiating and winning a fight or the cost of injury and death (Roulston *et al.* 2003; Tsutsui *et al.* 2003; Corin *et al.* 2007; Blight *et al.* 2010). This cost of injury and death is significantly minimised when the fights are in groups of nestmates

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(Buczkowski & Silverman 2005). *Linepithema humile* often loses in individual worker aggressive encounters, but are more successful in group encounters using both physical and chemical defence (Holway 1999; Buczkowski & Bennett, 2008). This ability to fight better in large groups gives them a competitive edge over most native ant species (Holway 1999), who do not always fight in groups (e.g. *Tapinoma sessile*-Buczkowski & Bennett 2008). Our findings showed that both ant species were aggressive across a range of contexts: from one-on-one assays to the resource competition assay. The minors of *P. megacephala* (±2-2.6mm) are only slightly smaller than Argentine ant workers (±2.5-3.0 mm) (Wild 2004; Lach *et al.* 2009; Wetterer 2012), so the effect of body size on the outcome of the aggressive interactions in the one-on-one and group assays was minimal (Nowbahari *et al.* 1999) since majors were not included in these assays. Kirschenbaum and Grace (2008) found that during aggression bioassays when only the minor workers of *P. megacephala* were used, staged interactions with *L. humile* resulted in increased average mortality of *P. megacephala* but survival increased when the soldier caste was present. In this study, *P. megacephala* minor workers were able to defend themselves against equal sized groups of *L. humile* with approximately four *P. megacephala* killed per trial while *L. humile* suffered on average a loss of 8.5 workers per trial. In the resource competition assay, even when *P. megacephala* majors were observed cutting up *L. humile* workers during interactions around the bait (N. Mothapo, *pers. obs.*), still *L. humile* suffered twice the mortality rate compared to *P. megacephala*, even though both suffered much higher mortality rates. This behaviour of *P. megacephala* majors cutting up *L. humile* workers was also observed by J. K. Wetterer (stated in Wetterer 2012).

The recruitment of *L. humile* workers during the resource competition assay declined when *P. megacephala* increased worker recruitment, suggesting that they were deterred from foraging. Depressing the foraging success of native ant species is a mechanism by which *L. humile* typically displaces native ant species in the field (Human & Gordon 1996, 1999). Here we show that *P. megacephala* uses a similar strategy against *L. humile*, when colony size was controlled for. Blight and his colleagues (2010) showed that the native dominant ant *Tapinoma nigerrimum* on the French island of Corsica was a much stronger competitor compared to *L. humile* and was highly efficient at both resource exploitation and interference competition. In their study, *L. humile* even exhibited submissive behaviours when interacting with *T. nigerrimum* at the bait. Therefore, co-occurring ant species with resource preferences that overlap are likely to be highly competitive, and since most ecologically dominant ant species share similar characteristics they may exclude each other from areas where they co-occur through high competition and aggression (Kirschenbaum & Grace 2008).

Numeric dominance has been cited in numerous studies on invasive ants as the most important factor promoting the success of these ants (Holway *et al.* 2002b; O'Dowd *et al.* 2003; Abbott *et al.* 2007; Rowles & O'Dowd 2007; Vonshak *et al.* 2012). However, invasive ant species first arrive within a recipient environment as small propagules and do not have this numeric dominance (Hee *et*

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al. 2000; Aron 2001; Holway *et al.* 2002b; Tillberg *et al.* 2008). During the most critical stages of invasion, arrival and establishment, these small propagules must contend with all abiotic and biotic factors in the recipient environment (Richardson *et al.* 2000; Holway *et al.* 2002a; O'Dowd *et al.* 2003; Walters & Mackay 2005; Rowles & O'Dowd 2007). The factors that promote their proliferation and spread from these small populations, overcoming these pressures, to the supercolonies most invasive ants form are little understood. It is known however that the presence of ecologically similar species may prevent the establishment of invasive species as well as limit their distribution in areas where they have already invaded (Rowles & O'Dowd 2007; Blight *et al.* 2010). Our findings and those of Blight *et al.* (2010), show that ecologically dominant ant species, such as *Tapinoma nigerrimum* (a Dolichoderinae like *L. humile*) and *P. megacephala* (a Generalised Myrmecinae, sensu Andersen 1995 for functional group classification) can outcompete other ants through highly effective foraging strategies and the ability to defend themselves through high aggression (see also Walters & Mackay 2005). In Australian communities, areas dominated by the native meat ant species of the genus *Iridomyrmex*, are free from *L. humile* (Walters & Mackay 2005; Walters 2006), but, these areas also tended to be drier (Thomas & Holway 2005). Thus, the combination of environmental suitability and the presence of ecologically similar, dominant and highly competitive native ant species are crucial to limiting the establishment of invasive ant species (Menke & Holway 2006; Walters 2006; Menke *et al.* 2007)

Taking into account the similar ecological niche requirements of *P. megacephala* and *L. humile* (Holway *et al.* 2002), as well as the results observed from their interactions in this study, our findings support the hypothesis that *P. megacephala* may have the potential to limit the spread of *L. humile* along the eastern and northern escarpment of South Africa. Our study also provides further confirmation that competition from ecologically similar and dominant ant species has the potential to significantly affect the invasion success of introduced ant species by limiting their survival and establishment (Richardson *et al.* 2000). Moreover, our study highlights the importance of studying the biological traits of ants within a recipient environment and those of introduced ant species in order to understand the factors facilitating successful invasion of natural communities.

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CHAPTER 4: Patterns of floral resource use by the invasive Argentine ant, *Linepithema humile*, and a native ant, *Anoplolepis custodiens*, in a biodiversity hotspot

ABSTRACT

Invasive ants opportunistically form mutualistic associations with plants and arthropods that produce carbohydrate exudates such as nectar and honeydew. Most studies have focused on the importance of mutualistic associations with trophobionts on the invasion success of invasive ants, while fewer studies have investigated the importance of floral nectar. In this study, the ability of the invasive Argentine ant, *Linepithema humile*, to utilise temporarily available floral nectar was compared to that of the dominant native ant, *Anoplolepis custodiens*, within the Cape Floristic Region (CFR). The abundance and species composition of ground foraging ants as well as floral arthropod visitors in inflorescences of two Proteacea species were assessed. The foraging activity of these two ant species was compared during three flowering periods; early, peak, and post bloom on two Proteaceae species native to the CFR. Differences in the natural abundance ratios of stable isotopes of Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$), as well as the ratio of Carbon to Nitrogen (C:N) were used to investigate the foraging ecology of *L. humile* and *A. custodiens*, and how they respond to increased resource availability. The presence of *L. humile* in flower heads altered the composition of floral visitors by 29% while *A. custodiens* changed the floral visitor composition by 6%. *Linepithema humile* increased its foraging activity with increasing nectar availability showing a concomitant lowered trophic position similar to that of herbivorous insects. With the depletion of floral resources, *L. humile's* diet became more enriched with protein, with a trophic shift towards that of a secondary predator. However, the C:N ratios, in contrast, suggests that *L. humile* continued to utilise carbohydrate resources post bloom and not revert to a purely protein diet. In comparison, *A. custodiens* did not respond with the same intensity as *L. humile* to increasing floral nectar availability. This study showed that *L. humile* has the potential to expand their range into natural Fynbos communities through a more effective utilisation of temporarily available carbohydrate resources than a native ant species. Consequently, their high carbohydrate resource utilisation, in addition to their aggression, may exacerbate the negative impacts they have on the native ants of the Fynbos.

Keywords: Proteacea, *Linepithema humile*, *Anoplolepis custodiens*, floral nectar, Cape Floristic Region, foraging ecology, Stable Isotope Analysis

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INTRODUCTION

Invasive ants are widely recognised for the substantial negative direct ecological impacts they have on native communities through the displacement of native ants and indirectly through the alteration of community structure across multiple trophic levels (Hoffmann *et al.* 1999; Holway *et al.* 2002; Abbott 2006). Generally, invasive ants are often found in areas with high human influence where there is high resource availability and limited biotic resistance (Holway 1998; King & Tschinkel 2008). However, many invasive ants have successfully established in natural areas (Holway *et al.* 2002; Sanders *et al.* 2003; Abbott *et al.* 2007; Hoffmann *et al.* 2009). Apart from the abiotic and biotic characteristics of the recipient environment that can affect successful establishment, the success of invasive ant species has largely been attributed to their generalist diet and ability to form populous supercolonies, which are characterised by the absence of intraspecific aggression (Holway 1999; Holway *et al.* 2002; Abbott *et al.* 2007; Pearce-Duvet *et al.* 2008; Sunamura *et al.* 2009; Mothapo & Wossler 2011). However, the high levels of interspecific aggression shown by these invasive ants contribute to their ability to displace native ant species (Hoffman *et al.* 1999; Rowles & O'Dowd 2007), and outcompete them at resources through competitive exclusion (Ness & Bronstein 2004). In addition, invasive ants have a high affinity for sugar-rich carbohydrate resources (Lach 2003), and are able to exploit them more efficiently (Lach 2005; Rowles & Silverman 2007; Gibb & Cunningham 2009). Consequently, carbohydrate resources are thought to contribute substantially to invasion success (Lach 2007; Tillberg *et al.* 2007; Rowles & Silverman 2009; Helms 2013; Wilder *et al.* 2013).

Resource availability is one of the most underappreciated factors contributing to invasion success of non-native ants in natural communities (Davis *et al*. 2000). In recent years, this subject has received increasing attention (Human & Gordon 1996; Holway 1999; Holway *et al*. 2002; Ness & Bronstein 2004; Walters & Mackay 2005; Rowles & O'Dowd 2007; Blight *et al*. 2010; Hoffman & Saul 2010). Ants are known to rely heavily on carbohydrate based resources (Helms & Vinson, 2002; Lach 2003; Ness & Bronstein 2004; Le Breton *et al*. 2005; Kay *et al*. 2010), and often form strong mutualisms with a variety of myrmecophilic arthropods and plants (Hölldobler & Wilson 1990; Holway *et al*. 2002; Davidson *et al*. 2003). Carbohydrate resources provide a source of fuel for colony growth (Dussutour & Simpson 2008, 2012), and have been shown to increase ant foraging activity and aggression (Grover *et al*. 2007; Helms & Vinson 2008; Shik & Silverman 2012). Consequently, the supply of carbohydrate resources can be used by invasive ants to support their large colony sizes, as a source of energy to capture prey, and fuel aggression to dominate resources and compete with native ants **(**Davidson 1997; Grover *et al.* 2007; Tillberg *et al*. 2007). Plants provide carbohydrates in the form of nectar and extra floral nectar, while honeydew is provided by sap-sucking trophobionts (Helms & Vinson 2002; Lach 2003; Kaplan & Eubanks 2005; Helms & Vinson 2008; Lach 2008; Helms 2013). The association between ants and honeydew producing trophobionts is usually mutually beneficial (Helms & Vinson 2008; Kay *et al.* 2010), while plants, on the other hand, are usually

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negatively affected since ants often deter floral visitation by pollinators, do not partake in pollination services and increase the negative effects of sap-sucking insects on plant health (Kaplan & Eubanks 2005). Thus ants are considered nectar thieves (Lach 2008, 2013). Honeydew is thought to be a high quality, stable, predictable and defensible resource and is preferred by ants more than plant based nectar (Goldberg *et al*. 2001; Lach 2003; Blüthgen *et al*. 2004; Blüthgen & Fiedler 2004; Lach 2008). The ants protect the trophobionts from their parasites and predators (Davidson 1998; Lach 2008); move them to choice parts of the plants, while some ant species even provide shelter for them in small domatia on the plants (Hölldobler & Wilson 1990). These behaviours all suggest that these trophobionts provide a highly profitable resource (Kay *et al.* 2010). However, it is unclear whether native ants differ in their preferences for honeydew and other carbohydrate resources in comparison to invasive ants (Lach 2003).

The availability of carbohydrate resources in the form of floral nectar and honeydew affect ant community structure in tropical environments (Davidson *et al.* 2003, Lach 2003; Gibb & Cunningham 2009), where ecologically dominant species generally monopolise carbohydrate resources and aggressively defend them (Blüthgen & Fiedler 2004; Savage & Whitney 2011). These resources are largely partitioned in such a way that ecologically dominant ants typically utilise high quality carbohydrate rich sources such as honeydew but will include floral nectar in their diets when it becomes available (Gibb & Cunningham 2009). while subordinate ants are usually limited to floral nectar when it is available (Blüthgen & Fiedler 2004) but may switch to honeydew in the absence of dominant ant species (Davidson 1998). For example, in an experiment manipulating carbohydrate availability (Rowles & Silverman 2009), the invasive Argentine ant, *Linepithema humile*, responded to increased carbohydrate resource availability by expanding their colonies and were able to persist in those areas even after the removal of excess resources (Rowles & Silverman 2009). Moreover, it has been predicted that carbohydrate resources, mainly plant exudates and honeydew, are likely to be more important to invasive ants than native ants in many systems because of their potential to influence establishment success (Ness & Bronstein 2004; Styrsky & Eubanks 2007; Wilder *et al.* 2013). Therefore, natural, undisturbed recipient environments with high carbohydrate resource availability and reduced uptake by native ant species are likely to have high invasibility for introduced ant species (Dukes & Mooney 1999; Davis *et al.* 2000). Introduced species probably benefit by exploiting available resources more effectively than resident ant species (Davis *et al.* 2000; Lach 2007).

Carbohydrate supply may be a key limiting resource affecting invasive ant population growth and survival as indicated by previous studies (Grover *et al.* 2009; Kay *et al.* 2010; Shik & Silverman 2012). However, many of these studies are based on honeydew from trophobionts and very few on floral nectar (Lach 2008; Gibb & Cunningham 2009; Savage & Whitney 2011). Floral nectar, although widely abundant, is a temporarily available resource (Lach 2013), and much work is needed

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to understand how invasive ants respond or change their behaviour when this resource becomes available and whether it may fuel invasion success (Lach 2013). For example, some invasive plant species have higher trait plasticity by utilising fluctuating resources better, in terms of soil nutrients and tolerating a wide range of environmental conditions, than similar native species (Dukes & Mooney 1999; Funk 2008). This gives these invasive plants an advantage to establish and spread in the recipient community. Invasive ant species may also increase their chances of establishment by profitably responding to fluctuating resources, and utilising those resources not consumed by resident species (Tilman 2004), or being stronger competitors for those resources (Gibb & Cunningham 2009). An example of a fluctuating resource could be floral nectar (Lach 2013), which is highly abundant and so can be partitioned among resident species (Davis *et al.* 2000; Tilman 2004). However, if the uptake of this resource by resident species is slower than the supply then colonising species that respond quickly to this resource may improve their establishment (Dukes & Mooney 1999; Davis *et al.* 2000). Carbohydrate based resources are likely to be more important for colony growth and survival than protein based resources for invasive ant species (Wilder *et al.* 2011a,b; Dussutour & Simpson 2012; Shik & Silverman 2012; Wilder *et al.* 2013). Thus the abundant supply of floral nectar, although temporarily available, may allow invasive ants to fuel colony growth during flowering seasons, a period of high carbohydrate resource supply. Moreover, carbohydrates have been shown to fuel worker forgaing activity and aggression which may also be an important factor in facilitating invasive ant success (Grover et al. 2009).

It is important to understand how invasive species integrate into and the extent to which they alter established food webs once they successfully establish within a resident community (Shea & Chesson 2002; Feiner *et al.* 2013). Invasive species may alter the diets of native species with which they co-exist by forcing or limiting them to specific resources that the invasive species are not utilising (Mooney & Cleland 2001; Sanders *et al.* 2003; Gibb & Cunningham 2009; Ottonetti *et al.* 2008; Menke *et al.* 2010) or altering the way in which they utilise available shared resources (Shurin *et al.* 2004). Stable isotope analysis has been widely used in ant ecology studies to quantify the impacts of invasive species particularly on food webs (Mooney & Tillberg 2005; Le Brun *et al.* 2007; Tillberg *et al.* 2007; Lach *et al.* 2010; O' Grady *et al.* 2010). This technique has been used to follow the flow of nutrients in a food web and to quantify the impact of invasive species on the resource use of resident species in the recipient community (Sanders & Platner 2007).

The Argentine ant, *Linepithema humile*, is a widespread invasive species that has successfully established across the globe (Sunamura *et al.* 2009; Vogel *et al.* 2010). They are thought to have been introduced into South Africa in the late 1800s during the Anglo-Boer war and have since established in both urban and natural environments (Skaife 1955; de Kock & Giliomee 1989; Prins *et al.* 1990; Luruli 2007). This ant is emerging as a serious threat to the future of the plant species in the Cape Floristic Region (CFR), a globally renowned biodiversity hotspot (Cowling *et al.* 1996). The Fynbos

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biome is the largest of the CFR and is characterised by high plant endemism (Cowling *et al.* 1996). *Linepithema humile* has been shown to displace native ants important in seed dispersal of many Fynbos plants (Bond & Slingsby 1984; Christian 2001; Luruli 2007). Moreover, they negatively affect floral visitors within the Fynbos, such as honeybees, Lepidoptera and Coleoptera responsible for pollination (Buys 1987; Lach 2007, 2008). Nearly 83% of Fynbos plants rely on insects for pollination, thus the potential impacts of this ant in this system are serious (Visser 1992; Visser *et al.* 1996). In laboratory studies, *L. humile* outcompeted native ants in resource competition through intense aggression, invading their nests (chapter 2), and may be utilising these aggressive strategies to eliminate ground foraging ants (de Kock 1990). The potential invasibility of the CFR by *L. humile* is likely to be high due to its rich carbohydrate resource availability in terms of high floral species diversity and concomitant nectar-producing plants (Cowling *et al*. 1996). Proteacea species of the Fynbos contain fairly large amounts of floral nectar and attract a wide range of nectarivorous birds, such as Cape Sugarbirds, and insects which are important pollinators (Coetzee & Giliomee 1985; Visser *et al.* 1996; Rebelo 2001; Geerts & Pauw 2011). Recently, Lach (2013) found that *L. humile* were better at exploiting floral nectar from Proteacea species than the native dominant ant species, *Anoplolepis custodiens*, dominating most of the sampled inflorescences. However, in her study, Lach (2013) compared ant visitation to the inflorescences of several Proteacea species and did not quantify foraging patterns between the two ant species. In the current study, we aimed to examine foraging patterns and quantify the exploitation of a transient carbohydrate resource, floral nectar, by a dominant native ant species, *A. custodiens* and invasive *L. humile*. *Anoplolepis custodiens* is considered a keystone species and is also regarded as ecologically dominant in the Fynbos biome (Bond & Slingsby 1984; de Kock 1990). *Anoplolepis custodiens* plays an important role in the seed dispersal of most Fynbos plants (Bond & Slingsby 1984; Christian 2001), dispersing seeds of all sizes for a number of different species (Christian 2001). *Linepithema humile* and *A. custodiens* distributions are mutually exclusive as demonstrated by pitfall trap and baiting data (Luruli 2007; Vorster 2011; Lach 2013).

Foraging patterns can be influenced by the amount of resources available and distance to those resources (Martin & Vinson 2008). In this study, the number of open inflorescences was used to infer the proportion of available nectar. Nest density around the base of the trees was used to ascertain whether the two ant species studied are dispersed central place foragers. Dispersed central place foraging species move their nests closer to resources with increasing availability of those resources (Holway & Case 2000) and is associated with polydomous ant species (de Kock 1990; Holway & Case 2000). Since *L. humile* is potentially more efficient at using protea floral nectar (Lach 2007; Lach 2013), through high worker recruitment, and poses a threat to pollinators (Lach 2008), we further surveyed the species composition of insects in the flowers of the two protea species used in this study, namely *Protea repens* and *Protea nitida*, to ascertain the effect of ant presence on the insects associated with the inflorescences (see Coetzee & Giliomee 1985; Visser *et al.* 1996; Lach

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2008). Ant assemblages and abundances were compared between sites dominated by *L humile*, and those dominated by *A. custodiens*. The potential diets of Fynbos ants were inferred using stable isotope signatures, and the trophic relationship between *L. humile* and *A. custodiens* were compared across flowering periods to determine whether ants respond to floral nectar availability by switching their foraging behaviour. The importance of this temporarily available carbohydrate resource to other ant species sampled was also examined and whether the opportunistic association of *L. humile* with nectar rich Proteacea is likely to promote its spread into natural Fynbos communities. We hypothesised that with increasing floral nectar availability, the foraging activity of *L. humile* would also increase and that their trophic position would switch to reflect their carbohydrate rich diet during this time, indicating a behavioural response with respect to their foraging ecology, to this increased carbohydrate resource in comparison to the native ant, *A. custodiens*. If *L. humile* is better at exploiting this resource than the native ant and it relies heavily on it, then it is likely that the availability of this resource will drive the successful spread of *L. humile* and an increase in the negative impacts it already has on the Fynbos biome.

METHODS

Study sites and organisms

This study was conducted in two protected areas in the Boland Region of the Western Cape Province, South Africa, Helderberg Nature Reserve (HNR) (34°03' S, 18°52'E) and Jonkershoek Nature Reserve (JNR) (34°58' S, 18°56'E). Helderberg Nature Reserve is a 385ha nature reserve situated outside town of Somerset West with the dominant Fynbos fauna being Mesic Mountain Fynbos and small patches of Renosterveld vegetation (Van Wyk & Smith 2001; CapeNature 2010). Jonkershoek Nature Reserve is larger at 9800ha and is situated outside the town of Stellenbosch with the dominant Fynbos vegetation being Mesic Mountain Fynbos, Kogelberg Sandstone Fynbos and Boland Granite Fynbos (Van Wyk & Smith 2001; Mucina & Rutherford 2006; CapeNature 2011). The climate in both these reserves is similar to most of the Mediterranean type climate typical of the southern part of the Western Cape Region characterised by hot and dry summers (October to March) and, cold and wet winters (June to July) (De Kock 1990; Le Maitre *et al*. 1996; CapeNature 2010). Both reserves have perennial streams forming part of the large Eesterivier, have pine plantations as well as recreational areas which ensure daily visitors to the areas (http://www.helderbergnaturereserve.co.za; de Kock 1990). Both reserves are invaded by *L. humile* (Luruli 2007). Grid based and occupancy analysis methods showed that Argentine ants currently occupy 172ha out of a total of 272ha that were sampled in HNR and occupies 236ha out of the 920ha that were sampled in JNR (Luruli 2007). *Linepithema humile* dominates areas mutually exclusive of the native dominant ant, *Anoplolepis custodiens* (Luruli 2007). Thus in HNR, an area free of *L. humile* and dominated by the native ant *A. custodiens* was selected.

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In JNR, the selected study area was Swartboskloof which is known to be invaded and dominated by *L. humile*. This same site has been used in previous studies that investigated the impacts of Argentine ants in the Fynbos (Coetzee & Giliomee 1985; Donnelly & Giliomee 1985; De Kock 1990; Witt 1993; Visser *et al*. 1996; Witt & Giliomee 1999; Witt *et al*. 2004; Witt & Giliomee 2004; Lach 2007). The Swartboskloof site was dominated by *Protea nitida*, *Cliffortia ruscifolia*, Ericaceae species such as *Erica hispidula* and *Erica cerinthoides*, several species of *Aristea*, *Leucadendron* and *Oxalis* amongst others; while the study site in HNR was dominated by *Protea repens* and also had some similar species to JNR and in addition *Protea cynaroides*, *Protea laurifolia*, *Leucospermum conocarpodendron* along with some species observed from the families Poacea and Euphorbiaceae (see Mucina & Rutherford 2006), but only a few *P. nitida*. In each study site, the most abundantly occurring nectar producing protea species was selected for the study. *Protea nitida* (M.) was selected as the study plant in the study site dominated by *L. humile* and *Protea repens* (L.) was selected as the study plant in the study site dominated by the native ant *A. custodiens*. *Protea nitida* is pollinated by both insect and birds, flowers from March/April to August/September and may sometimes flower throughout the year (Coetzee & Giliomee 1985; Cowling *et al.* 1996; Rebelo 2001) with plants flowering in July and August during my study. *Protea repens* is largely bird pollinated, flowers from April/May through to September but may also flower year round (Cowling *et al.* 1996), with *P. repens* flowering in July and August as well during this study. Both plants produce floral nectar, although the nectar properties differ in terms of volume, sugar concentrations are more similar (Geerts 2011; Geerts & Pauw 2011). Both study sites had dense stands of the respective plant species which were of similar age (Cowling *et al.* 1996; Rebelo 2001).

Sampling

All sampling was conducted during three flowering stages, March/April-Early bloom, June/July-Peak bloom and September/October-Post bloom in 2011 and 2012. In 2012, the post bloom sampling was conducted in November for both sites due to the longer rainy season, as well as longer flowering season. At each site, 15 trees of *P. nitida* or *P. repens* were randomly selected within a 100m x 100m plot, marked with red tape on a branch and the GPS coordinates recorded. Each tree was treated as a sampling unit. The trees were at least 5-10 m apart and roughly the same height. The number of inflorescences that were open on each tree (floral density) was counted during the early, peak and post bloom sampling periods for both sites. The number of open inflorescences was used to infer the amount of nectar available, with an increase in the number of open flowers representing a concomitant increase in nectar availability (Geerts & Pauw 2011; Lach 2013).

Ground activity and composition of ants during the three flowering periods

Pitfall traps were used to assess the species composition and abundance of ground foraging ants at the two sites during each of the flowering periods. Pitfall trapping is a widely used method in ecological studies on ant diversity (Agosti *et al*. 2000). Four 100m parallel transects separated by 25m were laid

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within the 100m x100m plot at each site. Pitfall traps were 50ml plastic vials containing 25ml of ethylene-glycol and water as a killing agent. Each trap was dug flush to the soil every 10m and left open for seven consecutive days during March 2011, June 2011, October 2011, April 2012, July 2012 and November 2012. The traps were collected, brought back to the laboratory where they were washed, sorted and identified to species level where possible. The abundance of other epigaeic arthropod species was also quantified from pitfall traps because they may represent the potential prey items, as well as predators of ants. Voucher specimens were deposited at the Centre of Excellence for Invasion Biology's Iimbovane Centre, Stellenbosch University.

Composition of arthropods in *Protea nitida* and *Protea repens* inflorescences

Inflorescences of both *P. nitida* and *P. repens* were collected in 2012 (only because the plants first flowered in 2011 after the severe fire that occurred in both reserves in 2009 and we wanted to allow the plants to fully recover before harvesting any flowers). A total of 128 *P. nitida* and 142 *P. repens* inflorescences were randomly collected from trees within the site, bagged in labelled zip lock bags and brought back to the laboratory where they were frozen to kill all insects inside. The inflorescences were then dissected and all arthropod species (including coleopteran larvae) found were identified under a microscope. Ants were identified to species level while beetles and other arthropod species were identified to family level, then stored in 70% ethanol for preservation.

Foraging activity

Ant foraging activity (rates) was quantified by measuring ant traffic along a randomly selected branch on each of the 15 experimental trees, with a diameter greater than 10cm for better visibility, for both *P. nitida* and *P. repens* trees during each of the flowering periods. Only branches one metre from the ground with high ant traffic were selected to standardise counts between the sampling units. A 4cm line was drawn across the branch and ants moving across the line in one direction were counted for one minute at 09h00 and 11h00 in the morning, and 14h00 and 17h00 in the afternoon for five days using a clicker. The mean of two counts in the morning and two counts in the afternoon was used for data analysis.

Stable isotope analysis

Stable isotope analysis has received much attention in food web studies in the past decade and is considered a useful tool to assess dietary inputs and the trophic ecology in ants (Post 2002; Tillberg 2004; Mooney & Tillberg 2005; Tillberg *et al.* 2006, 2007; Menke *et al.* 2010). More importantly, stable isotope analysis has been used in studies on the ecological effects of biological invasions on the trophic ecology of native species (Tillberg *et al.* 2006; Lach *et al.* 2010; Menke *et al.* 2010; Wilder *et al.*2013). The isotopic composition of an organism can provide insights about the resources that have been assimilated over time (Post 2002). Carbon and nitrogen are biologically relevant elements which

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exist as multiple stable isotopes (Post 2002). Their isotope ratios are used to provide information about the basal source of carbon and nitrogen in the diet of organisms within a community, their food web structure as well as infer the relative trophic position of these organisms (DeNiro & Epstein 1981; Grey 2006; Layman *et al.* 2007). In stable isotope analysis, the ratio of heavy to light isotopes can be traced from primary producer to consumer (Tillberg *et al.* 2006; Menke *et al.* 2010). Typically, consumers are enriched relative to their prey i.e. carbon values in a consumer will reflect the carbon of the plant on which the organism depends in the food web while the nitrogen is accumulated across trophic levels with higher values representing higher trophic levels (Kay 2002; Post 2002: Tillberg *et al.* 2006; Menke *et al.* 2010). The carbon to nitrogen ratio (C:N) represents the relative amounts of carbohydrate or protein assimilated by the organism (Smith & Suarez 2010), with higher C:N values indicating a high carbohydrate component to the diet while low C:N values indicate a high protein component to the diet (Fry 2006). The sample ratios, heavy to light isotopes of C and N, are calculated using the following formula:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

The sample ratio is compared to the element specific standard, that is, the ratio of heavy to light isotopes of the sample to that of the standard. The standard for carbon is PeeDee Belemnite carbonate and atmospheric air for nitrogen (Post 2002; Tillberg 2002). Delta (δ) values are presented as per mil (‰) for both carbon and nitrogen isotopes.

Sample preparation

In each site, 15 nests each of *A. custodiens* and *L. humile* close to the experimental trees (sampling units) were located, disturbed, and 10 individuals each of *L. humile* and *A. custodiens* from each nest were collected in an 8ml glass vial (sample = 10ants/vial, n=15 samples), and killed in 95% ethanol. These killing methods have no effect on $\delta^{15}N$, but have an effect on $\delta^{13}C$ values (Tillberg *et al.* 2006). To overcome this effect, storage times were less than one day, after which there was immediate drying in order to minimise the effect on $\delta^{13}C$ (Feldhaar *et al.* 2010). Leaf samples of each plant species were also collected for analysis. We collected other ant species from the pitfall traps (a minimum of five individuals of each ant species per glass vial with more than three replicates). A range of non-ant arthropods were also collected randomly on the plants.

All abdomens were removed from all arthropods collected to avoid skewing the results due to recent feeding events (i.e. food consumed on the day of collection) obscuring the effect on long-term resource assimilation information (Tillberg *et al*. 2006). All samples were dried in an oven at 60°C for two days and then ground to a fine powder using a mortar and pestle and stored in glass containers with desiccant until processing. Caterpillars and larvae were processed and ground whole. The samples were sent to the University of Cape Town Stable Isotope Unit where 1,5ug of each sample was weighed into small tin capsules and the $\delta^{13}C$ and $\delta^{15}N$ signatures of all samples were determined

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using a continuous flow Isotope Ratio Mass Spectrometer (IRMS), after sample combustion in on-line Carlo-Erba preparation. Beet sugar and Merck gelatine were used as standards, calibrated against International Atomic Energy reference materials (PeeDee-Belemnite and air for $\delta^{13}C$ and $\delta^{15}N$ respectively). The results are expressed in standard delta notation, $\delta X = ([R_{sample}/R_{standard}] - 1) \times 1000$, where X is the element in question and R is the ratio of the heavy over the light isotope. Precision of replicate determinations for both carbon and nitrogen was ± 0.05‰.

Estimating trophic position of *L. humile* and *A. custodiens*

The trophic position of *L. humile* and *A. custodiens* was estimated relative to that of known herbivores, predators and study plants within each site, in order to overcome issues of spatial heterogeneity in resource acquisition between the two ant species (Post 2002; Feldhaar *et al.* 2010; Wilder *et al.* 2013). The trophic position of each ant species within a site was calculated using the following formula from Lach *et al*. (2010) as adapted from Post (2002).

$$\rho_1 = [\delta^{15}N_{ant} - \delta^{15}N_{(2)} - \Delta N''] / \{[\delta^{15}N_{ant} - \delta^{15}N_{(2)} - \Delta N''] + \delta^{15}N_{(1)} + \Delta N' - \delta^{15}N_{ant}\}$$

$$\rho_2 = 1 - \rho_1$$

ρ_1 and ρ_2 represent the proportions of dietary inputs from two main sources, herbivores and predators, $\delta^{15}N_{(1)}$ and $\delta^{15}N_{(2)}$ represent the trophic position of herbivores and those of predators respectively. The $\delta^{15}N$ values were used to calculate the trophic position for each replicate for all ant species. The most widely used fractionation factors from herbivores to predators is 2.2‰ to 3.4‰ (N) and from plants to herbivores is 1.3‰ (C) (Vander Zanden *et al.* 1999; McCutchan *et al.* 2003; Fry 2006). However, we calculated site specific enrichment factors (ΔN) by comparing mean fractionation/enrichment from plants to herbivores, and from herbivores to predators (spiders) for each site.

The trophic position (TP) of *L. humile* and *A. custodiens* was then calculated according to the formula below. The trophic position of plants was 1, and that of herbivores and predators was 2 and 3 respectively:

$$TP_{ant} = TP_{predator} + 1 - (TP_{predators} - TP_{plants})\ \rho_1$$

Statistical analysis

Ground activity and composition of ants during the three flowering periods

A Generalised linear Model (GLZ) assuming a Poisson distribution with a log-link function, and posthoc pairwise analyses with Least Square Difference (LSD) was used to determine whether there was a difference in the abundance of ground dwelling ant species across the three flowering periods. The estimation scale parameter used was Pearson Chi-Square to obtain more conservative variance

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estimates and statistical significance. The factors used in the model were (i) species abundance per trap as the dependent variable, (ii) ant species (all ant species sampled) and (iii) flowering period as predictors. A GLZ assuming a Binomial distribution with a log-link function was used to compare the proportion of pitfall traps occupied (a proxy of ground foraging activity) across the three flowering periods as well as between the ant species. The variables in this model were (i) proportion of pitfall traps occupied (species presence/absence) as a categorical dependent variable, (ii) ant species and (iii) flowering period as predictors. Differences in the proportion of pitfall traps occupied between flowering periods per ant species were quantified using a GLZ assuming a Binomial distribution with a log-link function with factors (i) ant presence/absence as the dependent variable and (ii) flowering period as the independent variable. A McNemars test was conducted for species caught only at two flowering periods. All GLZ analyses were performed in STATISTICA version 10 for Windows. Statistical significance was accepted at $p < 0.05$.

Species composition and assemblage structure in *P nitida* and *P. repens* inflorescences

Linepithema humile has been shown to negatively affect flower visiting insects of *P. nitida* and *P. repens* (Coetzee & Giliomee 1987; Visser *et al.* 1996; Lach 2008). Multivariate analyses in PRIMER (Plymouth Routines in Multivariate Ecological Research, version 6: Plymouth Marine Laboratory, UK), were used to ascertain the effects of ant presence on the species composition of *P. nitida* and *P. repens* inflorescences. The factors used in the comparison were (i) presence of all ant species and (ii) presence of either *L. humile* or *A. custodiens* in inflorescences. Bray-Curtis Similarity Index analyses were used to assess the similarity matrix (Clarke & Gorley 2006). The abundance data were square-root transformed prior to analysis to balance the weight of contribution by common and rare species (Clarke & Gorley 2006). Analysis of Similarity (ANOSIM), with the two abovementioned factors/status, was used to determine whether there was a statistically significant difference in the species composition of inflorescences of both plant species. The Global R statistic is a useful comparative measure of the degree of separation of groups and its value is at least as important as the p value, if not more so (Clarke & Gorley 2006). Global R values closer to 0 indicate high similarity while those closer to 1 indicate large differences (Clarke *et al.* 2006). A non-metric multi-dimensional scaling (NMDS) ordination plot based on ant presence or absence was used to provide visual presentation. The ordination was generated using centroids of the similarity matrix using a Kruskal Wallis and 50 random restarts. Stress values closer or equal to 0 indicate goodness of fit, with no prospect of a misleading interpretation (Clarke *et al.* 2006).

Foraging activity

The Generalized Least Square Model (GLS) with a poisson distribution was used to determine the effects of floral density (number of open inflorescences), flowering period, sampling year and ant species on the variation in foraging activity. The GLS is a mixed model that accounts for violations of

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normality, linearity, repeated measures and autocorrelation between predictors and the dependent variable, and unequal variances. In R, the dredge function was used to select the model that best explained foraging activity by the predictors. The best model was selected based on the lowest Akaike Information Criterion. The model was rerun with all the predictors selected in the best fit model. This analysis was run in RStudio.0.97.551 on the R platform.

A two way repeated measures ANOVA was used to compare nest density between the two ant species over the three flowering periods. Independent pairwise comparisons were done to compare nest density within each flowering period as the Tukey HSD pairwise test did not detect the main differences in nest density over the flowering period. Independent samples T-test or Mann-Whitney U tests were used to compare nest densities at each flowering period. These analyses were conducted in STATISTICA version 10 for Windows and statistical significance was accepted at $p < 0.05$.

Stable-Isotope analysis

The isotopic signature, $\delta^{13}C$ and $\delta^{15}N$, of all insects and the two respective plants under investigation were jointly compared using a Multivariate Analysis of Variance (MANOVA) to minimise chances of Type 1 error (Ottonetti *et al.* 2008). $\delta^{13}C$ and $\delta^{15}N$ values were both used as dependent variables, pairwise differences were compared using Tukey's HSD posthoc test. Preliminary assumptions testing were conducted to check for normality, linearity, equality of variance, outliers, multicollinearity and equality of covariance. Pillai's Trace lambda was used as the test statistic due to the violation of the assumption of equality of covariance (Box's M test was less than 0.05). The factors entered into the MANOVA were (i) $\delta^{13}C$ and $\delta^{15}N$ as dependent variables; and (ii) ant species and (iii) flowering period as the independent variables. Multivariate comparisons were conducted for within flowering period and $\delta^{13}C$ and $\delta^{15}N$ were entered as the two dependent variables and ant species as the independent variable.

The carbon to nitrogen ratio (C:N), which represents the ratio of the mass of carbon to nitrogen in the sample, was compared using a Generalised Linear Model assuming a gamma distribution and log-link function, with Least Square Difference posthoc tests. The factors entered into the model were (i) C:N ratio as the dependent variable, (ii) ant species and (iii) flowering period as independent variables. Independent pairwise comparisons were conducted to ascertain within species differences in C:N ratios over the flowering period using Repeated measures ANOVA or Friedman test and independent samples T-test when ant species only occurred during two of the flowering periods. High C:N ratio indicates high contribution of carbohydrate to the diet while low C:N indicates high contribution of protein to the diet (Ottonetti *et al.*2008).

The trophic position of *L. humile* and *A. custodiens* over the flowering period was compared using a two-way ANOVA. When Tukey posthoc analyses did not detect differences across flowering period, independent comparisons using independent samples T-test was used to compare differences

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within each flowering period. The C:N ratio of *L. humile* and *A. custodiens* was compared using a Generalised Linear Model with LSD posthoc tests and the factors entered into the model were (i) C:N ratio as the dependent variable, (ii) ant species and (iii) flowering period as independent variables. When the main effects of flowering period were not found to be significant and LSD pairwise comparisons did not detect differences in C:N ratio, independent pairwise comparisons were also conducted using a Mann-Whitney U test to ascertain differences in C:N ratio between the two ant species at each flowering period. All analyses were conducted in STATISTICA 10.0 for Windows. Statistical significance was accepted at $p < 0.05$.

RESULTS

Ground activity and composition of ants during the three flowering periods

Helderberg Nature Reserve

Thirteen ant species were caught in the pitfall traps, and the area was largely dominated by the native ants *Anoplolepis custodiens*, *Lepisiota capensis* and *Tetramorium quadrispinosum* (Figure 1a). The number of ants of each of the 13 species caught in pitfall traps varied with some species showing fluctuations in abundance subject to flowering season (GLZ, Wald $\chi^2_{(19)} = 71.35$ $p < 0.0001$), however, flowering period did not have an effect on the overall ant abundances (GLZ, Wald $\chi^2_{(2)} = 3.01$ $p > 0.05$, Figure 1a). The proportion of pitfall traps occupied by the different species however varied significantly over the flowering periods. (GLZ, Wald $\chi^2_{(19)} = 101.41$ $p < 0.0001$). The native ants *A. custodiens* and *C. niveosetosus* occupied the most number of pitfall traps during the peak flowering period when the most number of inflorescences of *P. repens* were open (Figure 1a and b). Some native ants were only caught in pitfall traps at certain times throughout the flowering periods (Figure 1a and b). The abundance of different species of ground foraging ants caught in pitfall traps differed significantly (GLZ, Wald $\chi^2_{(12)} = 414.45$ $p < 0.0001$, Figure 1a), but this difference was not driven by season. Flowering period did however have an effect on ant abundances of *A. custodiens* (GLZ Wald $\chi^2_{(2)} = 13.95$ $p < 0.01$) and *L. capensis* (GLZ Wald $\chi^2_{(2)} = 5.10$ $p \leq 0.05$) as caught in pitfall traps. *Anoplolepis custodiens* had highest abundances during the peak flowering period; while *L. capensis* had the highest abundances during the early flowering period (see Figure 1a).

The ground foraging activity of the ant species within the experimental plot, as measured by their occupancy of pitfall traps, differed significantly between ant species (GLZ, Wald $\chi^2_{(12)} = 330.43$ $p < 0.0001$, Figure 1b), and between flowering periods (GLZ, Wald $\chi^2_{(2)} = 7.44$ $p < 0.05$, Figure 1b). Thus, the 13 ant species that were caught in pitfall traps within HNR, varied significantly in their ground foraging activity, with some increasing their activity during the peak flowering period, while some only increased their activity in the early or post bloom period (Figure 1b). The ground foraging activity of the native ant, *A. custodiens*, did not differ significantly across the three flowering periods (GLZ Wald $\chi^2_{(2)} = 0.85$ $p > 0.05$). However, the native ants *A. steingroveri* (GLZ Wald $\chi^2_{(2)} = 13.25$ p

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≤ 0.01), *Crematogaster sp.2* (GLZ Wald $\chi^2_{(2)}$ = 28.72 p < 0.0001), *Camponotus sp.1* (GLZ Wald $\chi^2_{(2)}$ = 7.40 p <0.05), *M. peringueyi* (GLZ Wald $\chi^2_{(2)}$ = 5.97 p ≤ 0.5), *Monomorium sp.1* (GLZ Wald $\chi^2_{(2)}$ = 9.01 p <0.05) and *O. barbiger* (GLZ, Wald $\chi^2_{(2)}$ = 6.27 p < 0.05) did differ in their ground foraging activity across the three flowering periods (see Figure 1b). Some ant species increased their ground foraging activity during the early bloom, while others were more active in the peak bloom. *Camponotus sp.1* and *M. peringueyi* were most active at the post bloom period (Figure 1b).

Jonkershoek Nature Reserve

Thirteen ant species, although different in species composition from those found in HNR, were also caught at JNR, with the area largely dominated by *L. humile* (Figure 1c and d). Similarly, the number of ants of each of the species caught in the pitfall traps (GLZ, Wald $\chi^2_{(19)}$ = 85.19 p < 0.0001), as well as the proportion of pitfall traps occupied by ants (GLZ, Wald $\chi^2_{(19)}$ = 101.41 p < 0.0001), varied significantly subject to flowering periods, with the most number of ants and proportion of pitfall traps occupied during the peak and post bloom (Figures 1c and d). *Camponotus maculatus* and *Plagiolepis sp.1* occupied the most number of pitfall traps during the early bloom period (Figure 1d). As was the case in HNR, some ant species were only caught at certain times of the flowering period (Figure 1c and d). Both the abundance of ground foraging ants (GLZ, Wald $\chi^2_{(12)}$ = 2001.64 p < 0.0001, Figure 1c) as well as their activity, as measured by the proportion of pitfall traps occupied was significantly different for the 13 ant species (GLZ, Wald $\chi^2_{(12)}$ = 309.61 p < 0.0001, Figure 1d). Thus not only was the number of ants per species caught different but so too was the extent of their ground activity, however the total number of ants caught did not differ between the flowering periods (GLZ, Wald $\chi^2_{(2)}$ = 0.27 p > 0.05). *Linepithema humile* (GLZ, Wald $\chi^2_{(2)}$ = 22.32 p < 0.001) and *T. quadrispinosum* (GLZ, Wald $\chi^2_{(2)}$ = 6.11 p < 0.05) however are the two species that do change in ant abundance between flowering periods, with *L. humile* having increased numbers during the peak bloom period while *T. quadrispinosum* shows increased numbers post bloom (Figure 1c). Even though total ant abundances were not affected by flowering period, the activity of the ants, as measured by pitfall trap occupancy, was significantly affected by flowering periods (GLZ, Wald $\chi^2_{(2)}$ = 8.89 p < 0.05, Figure 1d). *Linepithema humile* occupied the most number of pitfall traps at the study area over the three flowering periods (Figure 1d), increasing their ground foraging activity significantly during the peak and post flowering periods (GLZ, Wald $\chi^2_{(2)}$ = 10.70 p < 0.05, Figure 1d). The native ant species *Crematogaster sp.1* (GLZ, Wald $\chi^2_{(2)}$ = 17.08 p < 0.0001), and *T. quadrispinosum* (GLZ, Wald $\chi^2_{(2)}$ = 39.64 p < 0.0001) increased their ground foraging activity significantly in the post bloom period, while *M. peringueyi* (GLZ, Wald $\chi^2_{(2)}$ = 15.02 p < 0.01) and *Plagiolepis sp.1* (GLZ, Wald $\chi^2_{(2)}$ = 7.27 p < 0.05) increased ground foraging activity in the peak and early flowering periods respectively. *Camponotus maculatus* (GLZ, Wald $\chi^2_{(2)}$ = 5.89 p ≤ 0.05) and *O. barbiger* (GLZ, Wald $\chi^2_{(2)}$ = 6.09 p ≤0.05) increased their ground activity during the early and post, and peak and post respectively (Figure 1d). The native ants *M. peringueyi*, *Monomorium sp.2* and *T. quadrispinosum* were the only ant species able to sustain relatively high abundances and utilise a large part of the experimental plot

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compared to other native ant species (Figure 1 c and d).

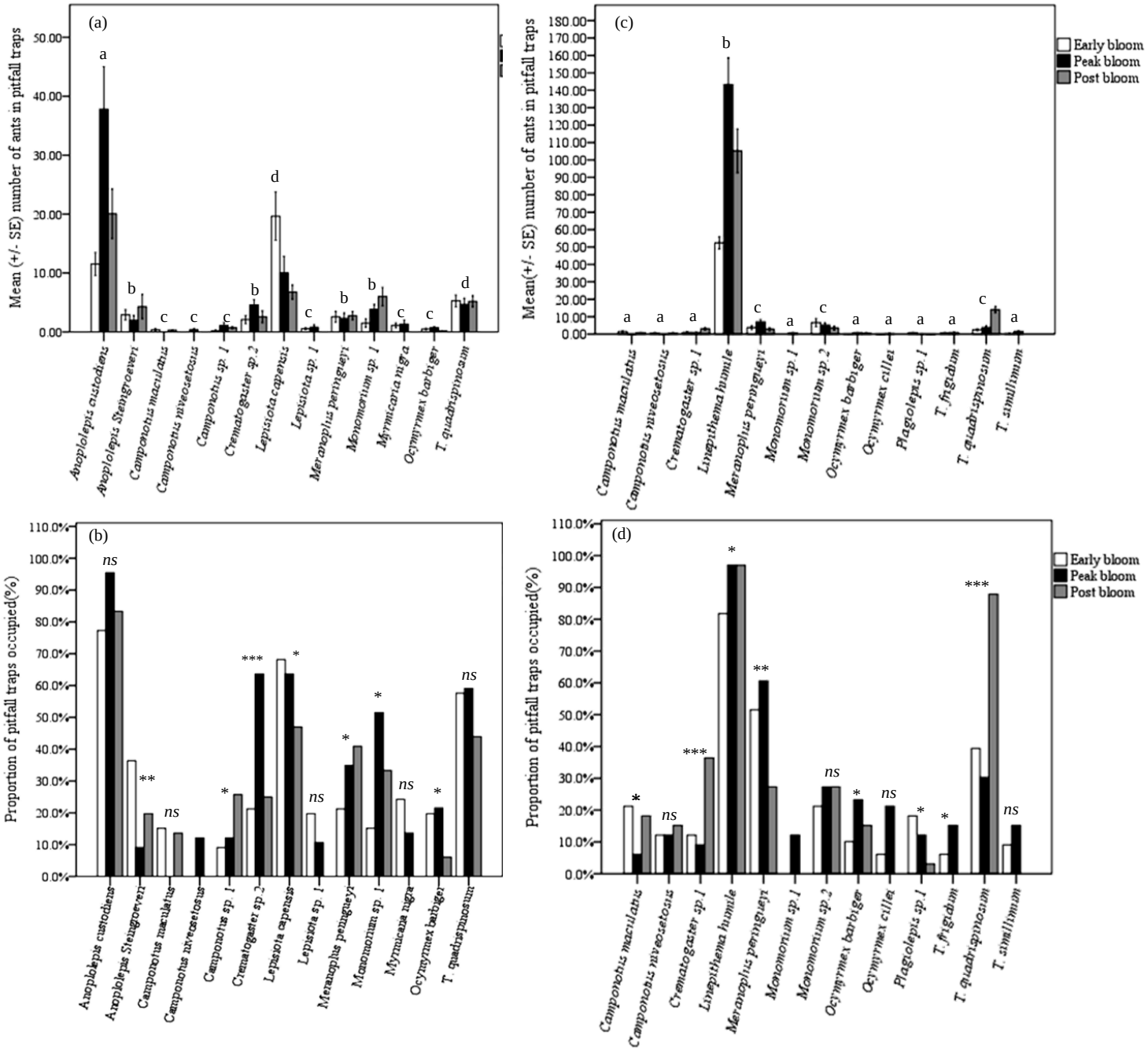

Figure 1a-d. Comparison of ant abundance in pitfall traps (a and b), and proportion of pitfall traps occupied(c and d), a measure of ground foraging activity, across three flowering periods combined for 2011 and 2012 sampling seasons in Helderberg Nature Reserve (a and b) and Jonkershoek Nature Reserve (c and d). Significant differences in abundances between ant species, based on GLZ LSD, are illustrated with different letters above bars (a and c). Significant difference in the proportion of pitfall traps occupied by each ant species between flowering periods based on GLZ and McNemars tests are indicated with an asterix (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.0001$; b and d). *Linepithema humile* exclusively dominated the study site in terms of numerical abundance during all the sampling periods at Swartboskloof, while *Anoplolepis custodiens* and *Lepisiota capensis* were both numerically dominant at Helderberg.

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Species composition and assemblage structure in inflorescences of *P nitida* and *P. repens*

Only 40% of *P. nitida* (52 of 128) and *P. repens* (57 of 142) inflorescences were occupied by ants (Table 1). *Protea nitida* inflorescences with ants were dominated by *L. humile*, while *P. repens* had *A. custodiens* followed by *Crematogaster sp*.1 and *L. capensis* (Table 1). Chrysomelidae beetles, Nitidulidae beetles and fruit flies (Diptera) comprised a large part of arthropods present in the inflorescences and were present in nearly all inflorescences collected (Table 1, *P .repens* and *P. nitida*). *Linepithema humile* had the highest overall ant abundance in inflorescences, while native ant species in general were found in higher abundances on *P. repens* than on *P. nitida*. *Linepithema humile* made up 95.5% of all Hymenopteran visitors to *P. nitida*, while *A. custodiens* made up 82.35% of Hymenopteran visitors to *P. repens*. Ants that were never found in inflorescences were assumed to be ground foragers only.

Table 1. Abundance and composition of arthropods found in the inflorescences of *Protea nitida* and *Protea repens*. Ants are identified to species level while beetles and other arthropods are identified to family level. The total number and the proportion (%) of each species of ants, beetles and other arthropods are given. Dashes denote when species were not found in inflorescences of a particular species.

	P. nitida (128)		*P. repens* (142)	
	n	proportion (%)	n	proportion (%)
Anoplolepis. custodiens	-		1583	37.52
Linepithema humile	4564	18.21	-	-
Camponotus sp.1	5	0.02	-	-
Camponotus niveosetosus	121	0.48	34	0.79
Crematogaster sp.1	90	0.36	132	3.13
Lepisiota capensis	-	-	101	2.39
Lepisiota sp. 1	-	-	42	1.00
Apis mellifera capensis	1	0.004	32	0.76
Anthocoridae	1	0.004	-	-
Chrysomelidae	19020	75.89	1367	32.40
Cucujoidea	153	0.61	157	3.72
Histeridae	-	-	129	3.06
Melolonthinae	8	0.03	4	0.09
Meloidae	13	0.05	3	0.07
Nitidulidae	646	2.58	-	-
Pentatomidae	12	0.05	-	-
Scarabaeidae	39	0.16	5	0.12
Curculionidae	2	0.01	-	-
Staphyinidae	25	0.10	290	6.87
Other arthropods				
Dermaptera (earwigs)	9	0.04	-	-
Thysanoptera(Thrips)	105	0.42	25	0.59
Diptera	244	0.97	309	7.32
Arachnida	5	0.02	6	0.14
Total	**25063**	**100.0**	**4219**	**100.0**

Footnote: The very high arthropod densities found in *P. nitida* compared to *P. repens* is probably due to *P. nitida's* reliance on insect pollinators (Cowling *et al.* 1996; Rebelo 2001)

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Protea repens inflorescences were less affected by the presence of ants. The similarity of the arthropod assemblage within flowers having ants to those not, was 88% (ANOSIM Global R = 0.12, $p < 0.001$) compared to *P. nitida* where ants altered the species composition by 34% (ANOSIM Global R = 0.34, $p < 0.001$). Inflorescences with *A. custodiens* were 94% similar in their arthropod assemblages to those without (ANOSIM Global R = 0.06, $p < 0.05$, Figure 2b), while *L. humile* presence altered arthropod assemblage by 29% (ANOSIM Global R =0.29, $p < 0.001$, Figure 2a), suggesting that *A. custodiens* had less of an effect on floral visitors than did *L. humile*.

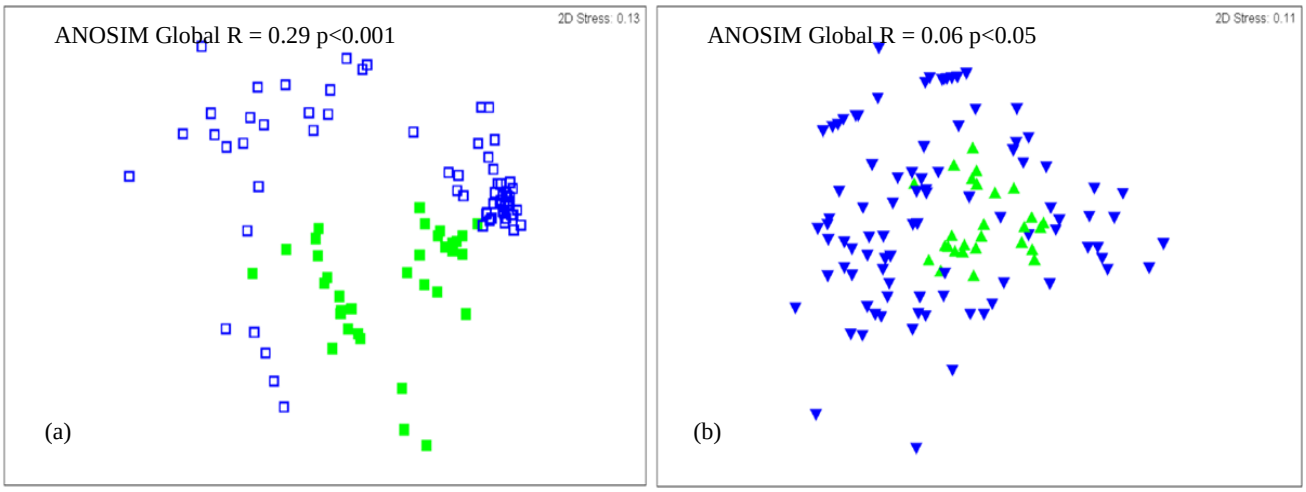

Figure 2. Non-metric Multidimensional Scaling plots showing species composition in (a) inflorescences of *Protea nitida* with *Linepithema humile* (■) and those without (□) , and (b) in inflorescences of *Protea repens* with *Anoplolepis custodiens* (▲) and those without (▼). The MDS shows that *L. humile* presence had a greater effect on floral arthropod species composition than *A. custodiens*.

Foraging activity

The foraging activity (rates) of *A. custodiens* and *L. humile* on Protea bushes, with respect to the flowering period, were compared. Foraging activity was significantly affected by flowering period ($F_{(2)} = 191.63$, $p < 0.0001$), floral density ($F_{(1)} = 39.38$ $p < 0.0001$), ant species ($F_{(1)} = 285.99$, $p < 0.0001$) and sampling year ($F_{(1)} = 53.53$, $p < 0.001$). Ant foraging increased significantly during the peak bloom ($\beta = 41.66$ $t = 15.57$ $p < 0.0001$) but was significantly reduced in the post bloom period ($\beta = -14.59$ $t = -12.56$ $p<0.0001$) in comparison to the early bloom period. Foraging activity also differed between the ant species, with *L. humile* showing increased foraging activity in comparison to the native ant *A. custodiens* ($\beta = 15.11$ $t = 16.94$ $p < 0.0001$). Foraging activity was significantly higher in 2012 than in 2011 ($\beta = 4.34$ $t = 7.33$ $p < 0.0001$), while foraging activity increased by a small but significant degree with increased floral density (number of open flowers) ($\beta = 0.50$ $t = 6.29$ $p < 0.0001$).

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The nest densities of both ant species differed over the three flowering periods (Wilks lambda = 0.77, $F_{(2,57)}$ = 9.17, p < 0.001, Figure 3). Overall, the nest densities of *L. humile* were significantly higher than that of *A. custodiens* ($F_{(1)}$ = 12.72, p < 0.01). However, pairwise comparisons within each flowering period show that the nest densities only differed significantly in the post bloom period (Figure 3), where *L. humile* had higher nest densities than *A. custodiens* ($t_{(58)}$ = -5.05, p < 0.0001) but not in the early (U = 418.00, Z = - 0.48, p > 0.05) or peak bloom (U = 351.50, Z = - 1.47, p > 0.05).

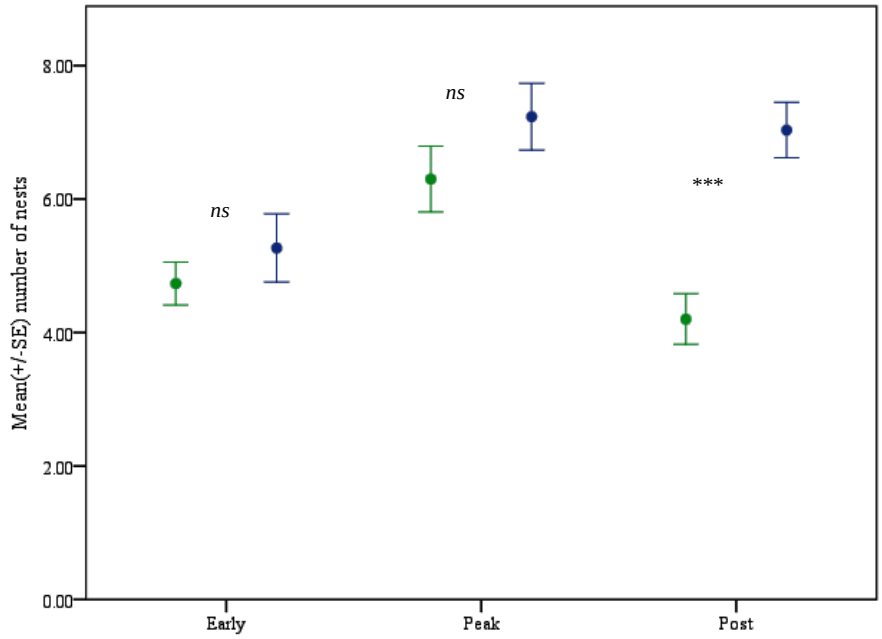

Figure 3. Mean(±SE) nest density for (●) *L. humile* and (●) *A. custodiens* over the three flowering periods. Two way repeated measure ANOVA showed that there is a significant difference in nest density between the two ant species over the three flowering periods. Pairwise differences are compared using the Mann-Whitney U test and the independent samples t-test (*** p < 0.001).

Stable-Isotope analysis

Helderberg Nature Reserve

The $\delta^{13}C$ values of most ant species and herbivorous arthropods were within the range of C3 plants (-24 to -34‰), suggesting that the basal source of carbohydrate in this community is the plant (Figure 3a-f). Moreover, herbivorous arthropods (coleopteran and lepidopteran larvae) were similar in their isotopic signature and matched that of *P. repens* (Figure 3a-c), indicating that arthropods in the same trophic groups clustered together. The two metric variables of trophic ecology, $\delta^{13}C$ and $\delta^{15}N$, varied significantly between the species sampled (Table 2). The isotopic signatures of all species sampled differed with some isotopic signatures being affected by flowering period (Pillai's lambda = 0.29, $F_{(54,630)}$ = 2.01, p < 0.0001), yet flowering period was not altogether responsible for generating the differences in isotopic signatures of the species sampled (Pillai's lambda = 0.02, $F_{(4,630)}$ = 1.70, p > 0.05). However all species sampled did differ in their isotopic signature (Pillai's lambda = 1.23

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$F_{(38,630)}$ = 26.58, p < 0.0001, Table 2). The interaction effect of flowering period and species sampled was significant for both $\delta^{13}C$ ($F_{(27,315)}$ = 1.57, p < 0.0001) and $\delta^{15}N$ ($F_{(27,315)}$ = 2.44, p < 0.0001), suggesting that these two isotopes vary significantly between the species sampled with some of this variation evident for only some species across flowering period. Both $\delta^{13}C$ ($F_{(19,315)}$ = 14.10 p < 0.0001) and $\delta^{15}N$ ($F_{(19,315)}$ = 72.28, p < 0.0001), differed significantly between species, however, flowering period did not generate the differences in the $\delta^{13}C$ and $\delta^{15}N$ isotopes of all species sampled (Table 2). In terms of trophic ecology, this suggests that the diets of all the animals do not vary significantly across the flowering periods, although individual species may show some variation. The differences in the isotopic signature was mainly influenced by $\delta^{15}N$, explaining 86.9% of the variation.

The isotopic signatures of all species sampled were significantly different in the early flowering period (Pillai's lambda = 1.07, $F_{(28,218)}$ = 8.97, p < 0.0001). Both $\delta^{13}C$ ($F_{(14,109)}$ = 6.01, p < 0.0001) and $\delta^{15}N$ ($F_{(14,109)}$ = 18.27, p < 0.0001) were significantly different between all species sampled, with $\delta^{15}N$ explaining 70.1% of the variation in isotopic signature. Multiple pairwise comparisons showed that ants differed in isotopic signatures to that of *P. repens*, the herbivorous arthropods, predatory arthropods (spiders), and detritivores (isopoda) (Figure 3a). Ant species with low $\delta^{15}N$ clustered closer to *P. repens* and herbivorous arthropods, while those with high $\delta^{15}N$ values cluster closer to the predator (arachnida) (Figure 3a-c).

In the peak bloom, the $\delta^{13}C$ values of all arthropod species shift towards *P. repens* (Figure 3b). Again, the isotopic signatures varied significantly between all the species sampled (Pillai's Trace lambda = 1.39, $F_{(40,254)}$ = 14.33, p < 0.0001, Table 2). $\delta^{13}C$ ($F_{(20,127)}$ = 7.75, p < 0.0001) and $\delta^{15}N$ ($F_{(20,127)}$ = 44.65, p < 0.0001) also differed among the species sampled with $\delta^{15}N$ values explaining 87.5% of the variation in isotopic signature (Figure 3b). *Camponotus niveosetosus* is a known nectarivorous ant and it clusters closer to *P. repens* and the herbivores than the more predatory ants such as *Ocymyrmex barbiger* (Figure 3b).

The isotopic signatures of all species sampled were also significantly different in the post bloom period (Pillai's trace lambda = 1.44, $F_{(30.182)}$ = 15.74, p < 0.0001, Figure 3c), and also differed in their $\delta^{13}C$ ($F_{(15,91)}$ = 8.44, p < 0.0001) and $\delta^{15}N$ ($F_{(15,91)}$ = 37.62, p < 0.0001), with $\delta^{15}N$ explaining 86% of the variation in isotopic signature. *Anoplolepis custodiens* clustered closer to the predator (arachnida) and predatory ant, *O. barbiger*, in the post bloom period with regards to $\delta^{15}N$. *Anoplolepis custodiens* shows variation in $\delta^{15}N$ values over the flowering period (Figure 3a-c). In the early bloom period, the isotopic signature of *A. custodiens* shows that it is more enriched in $\delta^{13}C$ (-24.36±0.44‰) relative to *P. repens* and depleted in $\delta^{15}N$ (4.55±0.35‰) relative to the spiders (arachnida). It then becomes more depleted in $\delta^{15}N$ (3.82±0.18‰) during the peak flowering period suggesting an increase in carbohydrate based food sources. In the post bloom period, it becomes more enriched in $\delta^{15}N$

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(6.09±0.26‰) and depleted in $\delta^{13}C$ (-25.84±0.27‰), suggesting an increase in the use of more protein based resources in its diet (Figure 3a-c).

Jonkershoek Nature Reserve (Figure 3d-f)

Similar to HNR, isotopic signatures differed significantly between all species sampled with these differences being affected by flowering period for some species (Pillai's Trace lambda = 0.65, $F_{(44,624)}$ = 6.82, $p < 0.0001$), however flowering period alone did not drive these differences between isotopic signatures of species sampled (Pillai's Trace lambda = 0.01, $F_{(4,624)} = 0.81$, $p > 0.05$). Even though isotopic signatures differed between the species sampled (Pillai's Trace lambda = 1.14, $F_{(30,624)}$ = 27.51, $p < 0.001$), the diet of a number of the species sampled does not change significantly with flowering period. Differences in both $\delta^{13}C$ ($F_{(22,312)} = 3.27$, $p < 0.0001$) and $\delta^{15}N$ ($F_{(22,312)} = 14.78$, $p < 0.0001$) between species sampled were more pronounced with the changing flowering period for some species, yet flowering period alone did not account for the differences in the $\delta^{13}C$ and $\delta^{15}N$ isotopes (Table 2). All species sampled differed in both their $\delta^{13}C$ ($F_{(15,312)} = 12.06$, $p < 0.0001$) and $\delta^{15}N$ isotopes ($F_{(15,312)} = 138.08$, $p < 0.001$). Ants that typically forage on floral nectar, such as *Crematogaster sp.1* and *Camponotus niveosetosus* clustered closer to the herbivorous arthropods and *P. nitida*, having the lowest $\delta^{15}N$ (Figure 3d-f).

The isotopic signature of all species sampled differed significantly in the early flowering period, (Pillai's Trace lambda = 1.23, $F_{(22,200)} = 14.46$, $p < 0.0001$). Both $\delta^{13}C$ ($F_{(11,100)} = 7.24$, $p < 0.0001$) and $\delta^{15}N$ ($F_{(11,100)} = 72.39$ $p < 0.0001$) also differed among the species sampled, with $\delta^{15}N$ explaining 88.8% of the variation in isotopic signature (Table 2).The isotopic signature of *L. humile* suggests that it is highly predatory during this time, clustering close to the arachnida and the predatory ant *O. barbiger* which is known to predominantly feed on other arthropods (Figure 3d).

In the peak flowering period, the isotopic signatures remained different between *P. nitida* and all arthropod species sampled (Pillai's Trace lambda = 1.35, $F_{(28,234)} = 17.15$, $p < 0.0001$). Both $\delta^{13}C$ ($F_{(14,117)} = 10.82$, $p < 0.0001$) and $\delta^{15}N$ ($F_{(14,117)} = 54.89$, $p < 0.0001$) also differed among the species sampled, with $\delta^{15}N$ explaining 86.8% of the variation in isotopic signature (Table 2). *Linepithema humile* clustered with *P. nitida*, nectarivorous ants and herbivorous arthropods (Figure 3e).

In the post flowering period, there are few open flowers and concomitant reduction in potentially available nectar. The isotopic signatures of all the species sampled are significantly different (Pillai's Trace lambda = 1.16, $F_{(24,184)} = 10.57$, $p < 0.0001$), and $\delta^{13}C$ ($F_{(12,92)} = 3.85$, $p < 0.0001$) and $\delta^{15}N$ ($F_{(12,92)} = 81.25$, $p < 0.0001$) also differed among the species sampled, with $\delta^{15}N$ explaining 91.4% of the variation in isotopic signature (Table 2). Once again, *Linepithema humile* clustered with the arachnida and the predatory ant O. *barbiger* (Figure 3f). The isotopic signature of *L. humile* shifted throughout the flowering period (Figure 3d-f). In the early bloom, *L. humile* is enriched in both $\delta^{13}C$ (-22.43±0.41‰) and $\delta^{15}N$ (6.02±0.22‰), relative to *P. nitida* and closer to the arachnida in $\delta^{15}N$ than

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other ants (Figure 3c). In the peak bloom the isotopic signature lowers to that of herbivores, $\delta^{13}C$ (-24.79±0.34‰) and $\delta^{15}N$ (2.30±0.23‰), which suggests that *L. humile* responded efficiently to available nectar by foraging almost exclusively on this temporarily available resource (Figure 3e). In the post bloom, *L. humile* becomes more enriched in both $\delta^{13}C$ (-21.88±0.54‰) and $\delta^{15}N$ (5.95±0.18‰) relative to *P. nitida* and the other ants (Figure 3f), suggesting that *L. humile* is feeding on a diet of both carbohydrate and protein sources.

Table 2. Manova results for multivariate and univariate ($\delta^{15}N$ and $\delta^{13}C$) comparison of stable isotope signatures of the plant and arthropod species for Helderberg Nature Reserve and Jonkershoek Nature Reserves across three flowering periods. The isotopic composition of the arthropod species did not change significantly with flowering period (FP), however, $\delta^{15}N$ and $\delta^{13}C$ varied significantly between all arthropod species within the three flowering periods at both study sites.

Helderberg Nature Reserve

		Overall			Early			Peak			Post		
Test	Effect	Pillai's λ	$F_{(df, n)}$	*P*	Pillai's λ	$F_{(df, n)}$	P	Pillai's λ	$F_{(df, n)}$	*P*	Pillai's λ	$F_{(df, n)}$	*P*
Multivariate													
	Species	1.23	$26.58_{(38,630)}$	<0.0001	1.07	$8.97_{(28,218)}$	<0.0001	1.39	$14.33_{(40,254)}$	<0.0001	1.44	$15.74_{(30,182)}$	<0.0001
	FP	0.02	$1.70_{(4,630)}$	>0.05									
	Species X FP	0.29	$2.01_{(54,630)}$	<0.0001									
$\delta^{15}N$													
	Species		$72.28_{(19,315)}$	<0.0001		$18.27_{(14,109)}$	<0.0001		$44.65_{(20,127)}$	<0.0001		$37.62_{(15,91)}$	<0.0001
	FP		$0.85_{(2,315)}$	>0.05									
	Species X FP		$2.44_{(27,315)}$	<0.0001									
$\delta^{13}C$													
	Species		$14.10_{(19,315)}$	<0.0001		$6.01_{(14,109)}$	<0.0001		$7.75_{(20,127)}$	<0.0001		$8.44_{(15,91)}$	<0.0001
	FP		$2.58_{(2,315)}$	>0.05									
	Species X FP		$1.57_{(27,315)}$	<0.0001									

Jonkershoek Nature Reserve

		Overall			Early			Peak			Post		
Test	Effect	Pillai's λ	$F_{(df, n)}$	*P*		$F_{(df, n)}$	*P*		$F_{(df, n)}$	*P*		$F_{(df, n)}$	*P*
Multivariate													
	Species	1.14	$27.51_{(30,624)}$	<0.0001	1.23	$14.46_{(22,200)}$	<0.0001	1.35	$17.15_{(28, 234)}$	<0.0001	1.16	$10.57_{(24,184)}$	<0.0001
	FP	0.01	$0.81_{(4,624)}$	>0.05									
	Species X FP	0.65	$6.82_{(44,624)}$	<0.0001									
$\delta^{15}N$													
	Species		$138.08_{(15,312)}$	<0.0001		$72.39_{(11,100)}$	<0.0001		$54.89_{(14,117)}$	<0.0001		$81.25_{(12,92)}$	<0.0001
	FP		$1.14_{(2,312)}$	>0.05									
	Species X FP		$14.78_{(22,312)}$	<0.0001									
$\delta^{13}C$													
	Species		$12.06_{(15,312)}$	<0.0001		$7.24_{(11,100)}$	<0.0001		$10.82_{(14,117)}$	<0.0001		$3.85_{(12,92)}$	<0.0001
	FP		$0.50_{(2,312)}$	>0.05									
	Species X FP		$3.27_{(22,312)}$	<0.0001									

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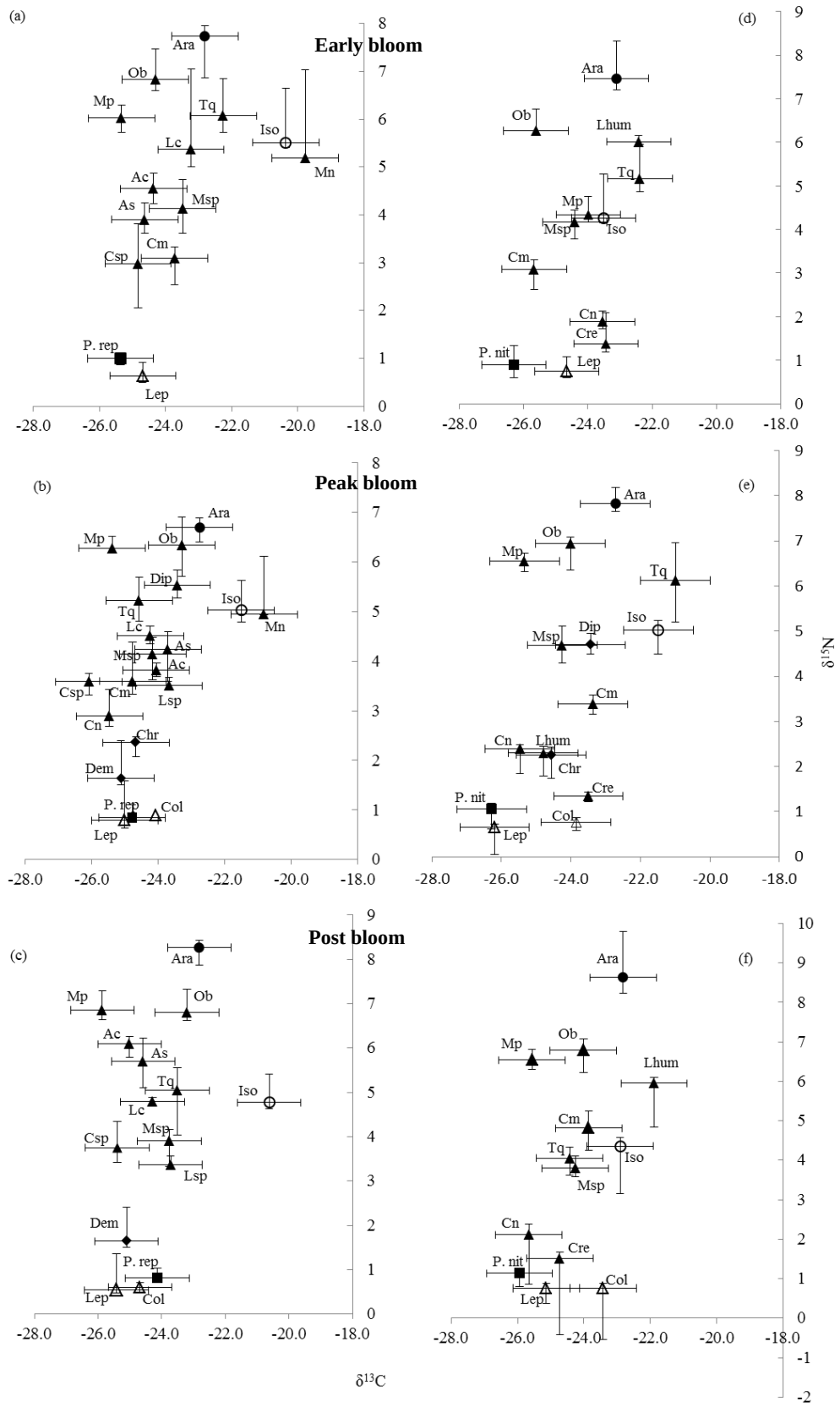

Figure 3a-f. Mean $\delta^{13}C$ and $\delta^{15}N$ values of ant species, herbivorous, detritivorous and predatory arthropod species during the three flowering periods in HNR (a-c) and JNR (d-f). Error bars indicate standard error of the mean. Abbreviations: Ara(arachnida),Ac(*Anoplolepis custodiens*), As(*Anoplolepis steingroeveri*), Chr(Chrysomelidae), Cm(*Camponotus maculatus*), Cn(*Camponotus niveosetosus*), Col(coleopteran larvae), Cre(*Crematogaster sp.1*), Csp(*Camponotus sp.1*), Dem(Demarptera), Dip(Diptera), Lc(*Lepisiota capensis*), Iso(Isopoda), Lhum(*Linepithema humile*), Lsp(*Lepisiota sp.1*), Mn(*Monomorium sp.1*), Mp(*Meranoplus peringueyi*), Msp(*Monomorium sp.2*), Tq(*Tetramorium quadrispinosum*), Ob(*Ocymyrmex barbiger*), P. nit(*Protea nitida*) and P. rep(*Protea repens*).

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Carbon-Nitrogen ratio

The C:N ratio indicates the relative contribution of carbohydrate or protein based dietary sources, with higher C:N ratios (>5‰) indicating a larger contribution of carbohydrate based foods while lower C:N values (<5‰) indicate a large contribution of protein to the diet (Ottonetti *et al.* 2008; Olsson *et al.* 2009; Smith & Suarez 2010; Jackson *et al.* 2012). The C:N ratio of herbivorous arthropods and nectarivorous ants, with the exception of the Chrysomelidae beetles, were relatively high compared to the remaining ant species and spiders at both study sites (Table 3).

Helderberg Nature Reserve

The C:N ratios differed significantly between species sampled, with these differences exacerbated by flowering period (GLZ, Wald $\chi^2_{(29)} = 98.44$ $p < 0.0001$). The C:N ratios were significantly different among all species sampled (GLZ, Wald $\chi^2_{(20)} = 2882.6$ $p < 0.0001$), but not across flowering periods (GLZ, Wald $\chi^2_{(2)} = 0.76$ $p > 0.69$), suggesting that the C:N ratio is not affected by flowering period alone, but rather varies between species sampled across the flowering periods. The difference in C:N ratios among species were highly significant within each flowering period, early (GLZ, Wald $\chi^2_{(14)} = 827.11$ $p < 0.0001$), peak (GLZ, Wald $\chi^2_{(20)} = 1252.66$ $p < 0.0001$) and post (GLZ, Wald $\chi^2_{(15)} = 2054.51$ $p < 0.0001$). Herbivorous arthropods and the nectarivorous ant, *C. niveosetosus*, had high C: N ratios reflecting their predominantly carbohydrate based diet. Most ant species had C:N ratios above 5‰ suggesting that they are also potentially utilising carbohydrate based food sources (Table 3a). *Lepisiota sp.1* and *Monomorium sp.1* had the lowest C:N ratios compared to all arthropod species, indicating similar foraging patterns to that of spiders, with a diet almost exclusively protein based. *Lepisiota capensis* showed an increase in C:N ratios in the peak flowering period suggesting an increased input of carbohydrate rich resources, similar to *C. niveosetosus* (a known nectarivorous ant). Strangely, *M. peringueyi* and *O. barbiger* showed an increase in C:N ratio values similar to that of coleopteran and lepidopteran larvae in the post flowering stage. *Ocymyrmex barbiger* is known to be an exclusively predatory ant (Witt & Giliomee 1999), while *M. peringueyi*'s foraging habits are not known. The C:N ratios of *A. custodiens* suggest that its diet is similar to that of detritivores, suggesting an equal contribution from both carbohydrate and protein based resources or a more omnivorous diet.

Jonkershoek Nature Reserve

The C:N ratio of species sampled in JNR differed significantly between species sampled depending on flowering period (GLZ, Wald $\chi^2_{(27)} = 49.89$ $p < 0.05$). The C:N ratios differed between the species sampled (GLZ, Wald $\chi^2_{(16)} = 2372.47$ $p < 0.0001$) and flowering period also had an effect on the differences in the C:N ratios between species (GLZ, Wald $\chi^2_{(2)} = 6.67$ $p < 0.05$). These differences in C:N ratios between species remained significant within the three flowering periods, early (GLZ, Wald $\chi^2_{(12)} = 827.31$ $p < 0.0001$), peak (GLZ, Wald $\chi^2_{(15)} = 876.38$ $p < 0.0001$) and post (GLZ, Wald $\chi^2_{(13)} =$

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668.78 p < 0.0001). Herbivorous arthropods, lepidopteran and coleopteran larvae, and the nectarivorous ants, *Crematogaster sp.1* and *C. niveosetosus*, had the highest C:N ratios next to *P. nitida*, which also reflect their carbohydrate based diet (Table 3b). Similarly, the spiders had the lowest C:N ratios, indicating the high contribution of protein based resources to their diet. The C:N ratios of *M. peringueyi* and *Camponotus maculatus* are similar to that of the arachnida during the early bloom period (Table 3b), which is in contrast to what is observed for *M. peringueyi* at HNR (Table 3a) and suggests that these ants are feeding exclusively on protein rich sources during this time. *Linepithema humile* showed an increase in their C:N ratio during the peak and post bloom period, suggesting that they are utilising the available carbohydrate resource and may be utilising other carbohydrate based resources when floral nectar is no longer available (Table 3b). Strangely, *T. quadrispinosum* showed the highest C:N ratio in the early flowering period (7.27±0.59), then lowered to a more protein based diet in the peak and post bloom period. However this ant was never found in the inflorescences of *P. nitida* (Table 1). Similarly high C:N ratios were observed for *M. peringueyi* (7.14±0.34‰) and *O. barbiger* (6.81±0.49‰) at HNR in the post bloom (Table 3a) and could suggest that these ants in HNR are potentially foraging on seeds that may be available during this time however we cannot explain the high carbohydrate input early bloom in JNR.

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Table 3. The Carbon: Nitrogen ratio of plant, ants and non-ant arthropods in Helderberg Nature Reserve (a) and Jonkershoek Nature Reserve (b). Small letters indicate significant difference between species within a flowering period based on Generalised Linear Model with LSD pairwise differences, while numbers indicate differences between flowering stages for each species, Repeated Measures ANOVA or Friedman test. Dashes (-) indicate that the particular species was not sampled during a given flowering period.

(a)	Helderberg Nature Reserve		
	C/N ‰		
Group	Early bloom	Peak bloom	Post bloom
Protea repens	23.51±1.18^{a}	22.84±0.97^{a}	22.41±0.72^{a}
Ants			
Anoplolepis custodiens	5.66±0.28^{b}	5.37±0.20^{b}	5.31±0.17^{b}
Anoplolepis Steingroeveri	5.53±0.34^{b}	4.48±0.37^{c}	4.71±0.29^{b,c}
Camponotus maculatus	4.82±0.27^{b,c,e}	5.56±0.5^{b,c,d}	-
Camponotus niveosetosus	-	6.30±0.39^{d,e}	-
Camponotus sp.1	5.04±0.44^{b,e}	5.52±0.34^{b,d}	4.55±0.28^{c}
Lepisiota capensis	5.53±0.32^{b}	7.35±0.46^{e}	5.07±0.22^{b,c,d}
Lepisiota sp.1	-	4.20±0.31^{c}	3.52±0.22^{e}
Meranoplus peringueyi	5.12±0.33^{b}	5.35±0.29^{b,c,d}	7.14±0.34^{f}
Monomorium sp.1	5.83±0.51^{b}	4.14±0.26^{c}	3.21±0.18^{e}
Myrmicaria nigra	5.45±0.61^{b,e}	4.90±0.40^{b,c,d}	-
Ocymyrmex barbiger	4.73±0.46^{b,c,e}	4.46±0.42^{b,c,d}	6.81±0.49^{f,g}
Tetramorium quadrispinosum	5.29±0.29^{b}	4.57±0.23^{c,d}	5.76±0.23^{b,g}
Non-ant arthropod			
Arachnida	4.00±0.29^{c}	4.10±0.24^{c}	4.12±0.19^{c,d}
Chrysomelidae	-	5.20±0.43^{b,c,d}	-
Coleopteran larvae	-	7.62±0.44^{e}	7.63±0.47^{f}
Diptera	-	3.61±0.27^{c}	-
Earwigs	-	5.01±0.48^{b,c,d}	5.00±0.34^{b,c}
Lepidopteran larvae	7.73±0.67^{d}	6.43±0.34^{d,e}	7.46±0.35^{c,f}
Isopoda	5.41±0.47^{a,e}	5.69±0.42^{b,d}	6.08±0.34^{g}
Scarabidae	4.37±0.38^{e}	4.58±0.38^{b,c,d}	4.76±0.24^{b,c}

(b)	Jonkershoek Nature Reserve		
	C/N ‰		
Group	Early bloom	Peak bloom	Post bloom
Protea nitida	20.36±0.94^{a}	22.71±1.13^{a}	22.41±0.72^{a}
Ants			
Camponotus maculatus	4.21±0.19^{b,1}	4.37±0.24^{b,1}	5.46±0.31^{b,2}
Camponotus niveosetosus	6.12±0.56^{c,1}	6.30±0.63^{c,1}	7.16±0.66^{c,e,2}
Crematogaster sp.1	6.47±0.37^{c,1}	7.34±0.40^{c,e,2}	7.56±0.53^{c,2}
Linepithema humile	5.65±0.20^{c,d,1}	6.50±0.36^{c,2}	6.03±0.50^{b,e,1}
Meranoplus peringueyi	3.97±0.29^{b,1}	5.91±0.40^{c,e,2}	6.10±0.48^{c,2}
Monomorium sp.2	4.90±0.28^{b,d,1}	4.70±0.32^{b,e,1}	4.31±0.32^{d,2}
Ocymyrmex barbiger	5.66±0.46^{c,d,1}	6.19±0.53^{c,1}	5.01±0.40^{b,d,2}
Tetramorium quadrispinosum	7.27±0.59^{c,d,1}	4.50±0.33^{b,f,2}	4.06±0.19^{d,3}
Non-ant arthropod			
Arachnida	4.00±0.29^{b}	3.96±0.29^{b}	4.06±0.35^{d}
Chrysomelidae	-	4.22±0.40^{b,e}	-
Coleopteran larvae	-	7.48±0.46^{c,d}	7.81±0.58^{c}
Diptera	-	3.70±0.32^{b}	-
-	-	-	-
Lepidopteran larvae	6.39±0.36^{c,1}	8.41±0.47^{d,1}	6.71±0.50^{b,c,1}
Isopoda	5.05±0.37^{b,d,1}	5.69±0.50^{c,e,2}	5.24±0.39^{b,d,1}
Scarabidae	5.98±0.32^{b,c,1}	3.61±0.27^{b,c,e,f,2}	4.57±0.37^{b,d,3}

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Trophic position and C:N ratio of *A. custodiens* and *L. humile*

The site specific enrichment factors for HNR between plants and herbivores were ΔN= 0.8, 1.0 and 0.8‰ for early, peak and post flowering periods respectively; while those between herbivores and predators were ΔN= 4.5, 2.4 and 2.8‰. In JNR, the enrichment factors for plants and herbivores were ΔN= 0.8, 1.0 and 1.0‰, and between herbivores and predators they were ΔN= 4.1, 2.2 and 2.6‰ for early, peak and post flowering periods respectively. The trophic position of *L. humile* and *A. custodiens* were calculated using these site specific enrichment factors and then compared using a relative scale of trophic position where 1 = primary producer (plant), 2 = herbivore, 3 = primary predator, 4= secondary predator (Fry 2006). The analysis of $\delta^{15}N$ values shows that the inferred trophic positions of these two ant species are primary predators (Figure 4).

The trophic positions of *L. humile* and *A. custodiens* differ significantly depending on flowering period ($F_{(2, 90)} = 19.02$, $p < 0.0001$). However, the overall trophic positions of these two ant species are similar ($F_{(2, 90)} = 1.64$, $p > 0.05$). In the early period, *L. humile* (3.44±0.02), occupies a higher trophic position than *A. custodiens* (3.32±0.03) ($t_{(28)} = 3.37$, $p < 0.01$), but lower in the peak bloom period [*L. humile* (3.05±0.02), *A. custodiens* (3.24±0.03); $t_{(33)}= -7.05$ $p < 0.001$)]. They do not differ in the post bloom [*L. humile* (3.58±0.02), *A. custodiens* (3.58±0.03), $t_{(25)} = -0.04$ $p > 0.05$), Figure 4a]. This suggests that although both species are primary predators, the trophic position of *L. humile* shows more variation over the flowering period, with a lower trophic position at the peak flowering period matching the changes in the isotopic signature (Figure 3e) and the C:N ratio (Table 3b). This implies that *L. humile* is potentially responding to the increased floral nectar availability by foraging predominantly on this resource.

Linepithema humile and *A. custodiens* differ in the C:N ratios (GLZ, Wald $\chi^2_{(1)} = 4.80$, $p < 0.05$), with *L. humile* having higher C:N ratio than *A. custodiens* overall (Figure 4b). This suggests that *L. humile* utilises more carbohydrate based resources than *A. custodiens* overall. The carbohydrate resource intake of *L. humile* (median = 6.14) was significantly higher in the peak flowering period in comparison to that of *A. custodiens* (median = 4.50, Mann Whitney-U test, U = 87.00 Z = -2.15 $p < 0.05$). *Linepithema humile* maintains a largely carbohydrate based diet in the post bloom, and thus may be feeding on another available carbohydrate resource at these times (Figure 4b). Thus, compared to *A. custodiens*, *L. humile* utilises available floral nectar more adequately than the native species.

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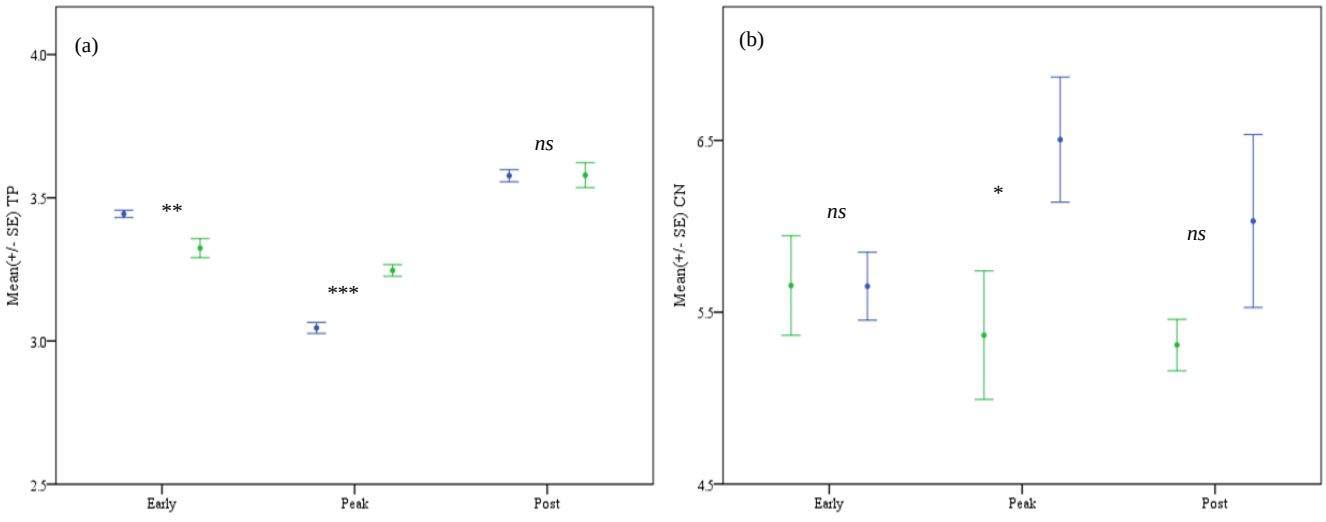

Figure 4. Mean(±SE) trophic position (4a) and C:N ratio (4b) for (●) *L. humile* and (●) *A. custodiens* over the three flowering periods. (a) Significant differences in trophic position based on two-way ANOVA with Tukey HSD posthoc test, (b) while differences in C:N ratios are based pairwise comparisons with Mann-Whitney U test. Statistical significance is shown as: *ns* $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

DISCUSSION

We found that the invasive Argentine ant, *Linepithema humile* utilised available floral nectar more actively and efficiently than the native dominant ant *Anoplolepis custodiens* by increasing its foraging rate when floral nectar became available, and switching its foraging range with its isotopic signature matching that of herbivorous arthropods and nectarivorous ants during this time. This evidence is supportive of the diet switching hypothesis (Tillberg *et al.* 2007) and reflects the trait flexibility of *L. humile* as an invasive species (Callaway *et al.* 2000; Wilder *et al.* 2013). Both *L. humile* and the native ant *A. custodiens* responded to the increased floral nectar availability by increasing their nest density at the base of the plant in line with dispersed central-place foraging (DCF) theory (Hölldobler & Lumsden 1980; McIver 1991; Davidson 1997; Buczkowski *et al.* 2006). To our knowledge, this is the first time that *A. custodiens* has been shown to use this foraging strategy, with only one record on the polydomous nest structure of this ant published to date (de Kock 1990). Very little work has been done on the importance of floral nectar to *L. humile* success in the Fynbos (Lach 2007, 2008 and 2013). The studies by Lach (2007, 2008, and 2013) suggested that *L. humile* is a threat to floral arthropods that visit inflorescences of protea plants. In a recent study, Lach (2013) further contended that ants in the Fynbos are not effectively utilising these abundant floral resources, with only a small proportion of the open inflorescences of many types of protea species occupied by native ants, and even by *L. humile*. We found a similar result in this study with ants only occupying a small proportion of inflorescences of both *P. nitida* (40.6%) and *P. repens* (40.1%). Although the abundance of *L. humile* (4564 individuals) was higher than that of *A. custodiens* (1583 individuals) in the inflorescences, *L. humile* occupied more inflorescences (25%) than *A. custodiens*

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(19%). These data, supported by recent finding of Lach (2013), suggest that native Fynbos ants are not utilising this available resource as effectively, providing a niche opportunity for *L. humile*, in terms of resource availability. Thus, the high availability of floral nectar and the apparent lack of competition for this resource may contribute further to the invasion success of *L. humile* in undisturbed Fynbos, and may explain the current distribution patterns of this ant in relatively undisturbed Fynbos (de Kock & Giliomee 1989; Lach 2007, 2008, 2013).

Anoplolepis custodiens is an ecologically important ant in the Fynbos, being the main seed disperser of most Fynbos plant species (Bond & Slingsby 1984; Christian 2001). It is a behaviourally and ecologically dominant native ant species that does not co-exist with *L. humile* (Luruli 2007). *Linepithema humile* does not fulfil the ecosystem services provided by *A. custodiens*, and thus indirectly poses a significant threat to Fynbos plant communities which rely on the native ant species for seed survival (Christian 2001). *Linepithema humile* was the most dominant ant species caught in Jonkershoek Nature Reserve, and never co-occurred with *A. custodiens*, *L. capensis* or *Lepisiota sp.* 1, with native species contributing only 5% to all ground dwelling ants caught in JNR. Conversely, *A. custodiens* in Helderberg Nature Reserve co-existed with four other native species which contributed 16% to all ground dwelling ants caught. This difference in ant assemblages in areas with and without *L. humile* has been shown in previous studies in the Fynbos (Donnelly & Giliomee 1985; Witt *et al*. 2004; Luruli 2007). Not only was *L. humile* the most dominant ground dwelling foragers but they were also found in high abundances in the inflorescences of *Protea nitida* and had a much larger negative effect on the species composition of floral arthropod visitors than did *A. custodiens.* This evidence is further supportive of the potential indirect negative effects of *L. humile* in the Fynbos through the displacement of floral visitors that are important in pollination (Lach 2007, 2008) and ground foraging ants important in seed dispersal (Christian 2001).

Elemental stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ showed that the isotopic signature of *L. humile* was similar to that of predatory spiders and ants (*O. barbiger*) in the early and post period with high $\delta^{15}N$ values, but was similar to that of leaf chewing beetles, and ants that are known to be nectarivorous (*Camponotus niveosetosus* and *Crematogaster sp.1*) in the peak flowering period. While that of *A. custodiens* did not change as drastically over the flowering period, showing a more equal contribution of carbohydrate and protein in their diet, which reflects a more omnivorous diet (see Le Brun *et al.* 2007). The trophic position of *L. humile* and *A. custodiens* indicate that they are both primary predators, level 3, however, both lower their trophic positions at the peak flowering period when there is increased nectar availability, suggesting a significant diet switch. However, the trophic position of *L. humile* does lower substantially more than *A. custodiens* in the peak flowering period (Figure 4b), showing that it is likely more efficient at diet switching in response to fluctuating resources (Tillberg *et al.* 2007; Wilder *et al.* 2013). Trait plasticity has been shown in studies of invasive plants, where these plants were able to

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outperform native plant species at utilising fluctuating resources such as an influx of soil nutrients due to environmental perturbations within the environment (Funk 2008), as well as being better able to tolerate changes in environmental conditions (Dukes & Mooney 1999). Thus, this flexibility may benefit invasive species establishment success. Introduced species may benefit by being better able to respond more efficiently to changes in resource availability than resident species when competing for shared resources (Dukes & Mooney 1999; Davis *et al.* 2000; Funk 2008; Mata *et al.* 2013). Thus, this result implies that *L. humile* can potentially increase its invasion success in the Fynbos by being more effective at utilising these periodically available carbohydrate resources, which are essential for colony survival (Wilder *et al.* 2011; Shik & Silverman 2012; Wilder *et al.* 2013).

The ability of *L. humile* to switch trophic position has been shown in recent studies on the foraging ecology of this ant (Tillberg *et al.* 2007). Both short term and long term studies show that *L. humile* is able to rapidly switch its trophic position in response to available carbohydrate resources, particularly in response to honeydew (Tillberg *et al.* 2007; Menke *et al.* 2010; Wilder *et al.* 2013). This ability to switch diets is thought to be an important factor contributing to the success of *L. humile*, allowing it to utilise a wide range of resources as they become available in the recipient environment (Tillberg *et al.* 2007); and is considered an effective mechanism that allows these ants to maintain and grow their large supercolonies once they have successfully established (Tillberg 2007). A similar result was found in areas invaded by the Yellow crazy ant, *Anoplolepis gracilipes*, where experimentally increased nectar resources resulted in higher forager recruitment as well as aggressive behaviours (Savage & Whitney 2011). Thus, the phenotypic plasticity shown by these two invasive ants, *L. humile* in this study and *A. gracilillpes* in Savage and Whitney's study (2011), in response to changing resource availability is possibly a trait common to invasive ant species (Kaplan & Eubanks 2005).The trophic positions of the two ant species studied here did not differ, with both species mainly foraging as primary predators in their respective ant community. However, our findings suggest that *L. humile* is utilising more carbohydrate based resources than *A. custodiens* and is potentially exclusively foraging on floral nectar during the peak flowering period. These outcomes support our hypothesis that the carbohydrate rich floral resources available within the Fynbos are likely to fuel the spread of *L. humile* within this region.

The C:N ratio represents the relative contribution of carbohydrate and protein to the diet of an organism (Smith & Suarez 2010). High C:N ratios indicate a high contribution of carbohydrate to the diet, while low C:N ratios indicate high contribution of protein to the diet (Ottonetti *et al.* 2008). The C:N ratio of *L. humile* remains higher and indicates a more substantial contribution of carbohydrates to the diet throughout the flowering periods than that of *A. custodiens* (Figure 4b). Both the C:N ratio and trophic position (Figure 4a and b) indicate that *A. custodiens* is not responding as effectively to this floral nectar availability as *L. humile*. In the early and post flowering period, floral nectar availability is expected to be

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minimal and thus ants' C:N ratios and trophic positions should reflect this. However, based on C:N ratios, *L. humile* appears to be foraging on carbohydrate resources outside of the flowering period of *P. nitida*. The C:N ratio in *L. humile* diets changed with the flowering period of *P. nitida* with their diet being high in carbohydrates during peak bloom, with less carbohydrates making up the diet early and post bloom. Even though this suggests there is a decrease in carbohydrate intake post bloom when floral nectar was depleted, the C:N ratio remained relatively high suggesting that the ants still utilised carbohydrate resources. We did not quantify floral nectar availability from other Fynbos plant species, such as the Ericacea (Cowling *et al.* 1996), which could have been flowering at this time and may have provided an additional carbohydrate supply. However, a previous study by Lach (2007) found that a treehopper species, *Beaufortiana sp*., occurred on *P. nitida* at JNR, which she thought facilitated *L. humile*'s success in this area. However, we did not find this treehopper on any of the *P. nitida* sampled, and it is likely that they may not have successfully recolonized after the fire that occurred in this area in 2009; although the ants may still have access to honeydew in areas outside our study plot.

The ability of *L. humile* to increase forager number and foraging activity with an increase in short term resource availability can be attributed to its foraging strategy. *Linepithema humile*, like most polydomous invasive ant species, uses dispersed central-place foraging (DCF), where ant species will move nests closer to resources to minimise costs associated with long distance travel and improve foraging efficiency (Hölldobler & Lumsden 1980; McIver 1991; Davidson 1997; Holway & Case 2000; Buczkowski & Bennett 2006; Martin & Vinson 2008). The nest densities of both ant species studied changed across the flowering period which is indicative of their ability to move nests closer to a resource to minimise travel costs. Both species had similar nest densities except for post bloom when *A. custodiens* nest densities around the protea bushes declined significantly. A factor that we cannot account for is worker densities within nests and so even though nest densities were equal it does not necessarily mean that the species had an equal work force which could have affected foraging rates. The nesting patterns of *A. custodiens* have not been well studied in the Fynbos, or recorded in the literature as far as we are aware. Only a single observation study by de Kock (1990) showed that *L. humile* raided the nests of *A. custodiens*, and therein the author briefly describes the nest structure of *A. custodiens* to be large and polydomous. We found that this ant showed similar nesting patterns to *L. humile*, and increased nest numbers around the base of *P. repens* with increasing floral density. A correlation analysis showed that there was a moderate positive association found between the number of nests at the base of the trees and the number of open flowers on the trees for *A. custodiens* ($r = 0.249$; $p < 0.001$) and a significantly strong association was found for *L. humile* ($r = 0.480$; $p < 0.001$). Increasing nest numbers closer to the resource, is an effective way of taking advantage of the resource while it is still abundant. Nests which are in close proximity to resources make for more efficient retrieval of those resources (McIver 1991; Davidson 1997; Martin & Vinson 2008). However, *A.custodiens* are most likely moving their nests to an alternative food resource

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when the flowering period ends, which could potentially explain why they do not maintain high nest numbers as observed for *L. humile* in this study during the post bloom period.

Overall, this study shows that *L. humile* may further increase its spread in the Fynbos during periods of high resource availability. The ants effectively exploited this temporarily available resource better than the dominant native ant species, *A. custodiens* (see also Lach 2007). In this way, *L. humile* is able to increase and sustain its population sizes with concomitant negative impacts on ant fauna and pollinators. However, a number of the native species that co-exist with *L. humile* have different foraging strategies that do not bring them into conflict with the invader (Witt & Giliomee 1999; Witt *et al.* 2004). In summary, *Linepithema humile* is more efficient at utilising floral nectar than the native dominant ant. Favourable abiotic conditions and the availability of floral resources from Proteacea, as well as the ants' effective foraging strategy may give *L. humile* the added potential to further spread into more pristine areas and speed up their rate of invasion. The lack of resource competition from native ants, and the negative impacts on native arthropods associated with Proteacea by *L. humile* may indirectly threaten the future of this biodiversity hotspot.]

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CHAPTER 5: Trophic relationships among Fynbos ants in invaded and uninvaded sites: effects of *Linepithema humile* presence on the diet of native ants

ABSTRACT

Food web studies in ant ecology have recently focused on the use of stable-isotope analysis and diet-manipulation experiments to investigate patterns of resource assimilation in ant colonies. In studies of invasive ant species, this technique has been used to study the degree to which invasive ants utilise resources, particularly carbohydrate resources, which have been linked to invasion success, in recipient environments. *Linepithema humile* shows dietary flexibility and shifts in trophic position in invaded areas, and alters the resource use of native ant species in recipient environments through competition for shared resources. This ecological plasticity is thought to enhance the invasion success of *L. humile*. In this study, we investigated isotopic signatures of ants across seasons to reflect differential use of available resources and in particular to assess the effect *L. humile* had on the diets of native Fynbos ant species. . We assessed species composition of ants along an invasion continuum using pitfall trap surveys and used stable-isotope analysis to investigate the isotopic signatures of ants across four seasons and different stages of invasion. We found that *L. humile* presence negatively affected ant species composition, with fewer ant species found in the invaded sites. The isotopic signatures and C:N ratios of *L. humile* showed strong diet switching across the seasons compared to all native ant species, particularly during the winter flowering period. Native ant species do not appear to be carbohydrate limited, with their isotopic signature indicating a predominantly protein based diet. *Linepithema humile* altered the diets of three native ant species with which it commonly occurs, supporting the hypothesis that the presence of invasive ants results in diet switching among co-occuring species that manage to persist with the invaders. This plasticity in diet by some native ant species is possibly a mechanism that allows them to persist in invaded environments. Our study shows that the dietary flexibility of *L. humile*, coupled with its ability to effectively utilise available carbohydrate resources and alter ant assemblages within the Fynbos can facilitate its successful spread in this region. The Fynbos is rich in floral resources, providing ample opportunity for *L. humile* to spread further in this biodiversity hotspot.

Keywords: Trophic ecology, Fynbos, *Linepithema humile*, native ant species, carbohydrate resources, dietary flexibility

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INTRODUCTION

Introduced species often form novel interactions in ecosystems on arrival which facilitate their ability to establish, spread and become invasive (Richardson *et al.* 2000; Keane & Crawley 2002; Shea & Chesson 2002). These introduced species act as novel consumers and competitors, utilising a wide range of resources available within the recipient environment (Elton 1958; Vitousek *et al.* 1996, Mack *et al.* 2000; Holway *et al.* 2002; Sanders *et al.* 2003), which results in the alteration of resource use among resident species (Dawson *et al*. 2002). Resident species may however exclude the introduced species from resources through competitive exclusion and efficient uptake of available resources within the community (Davis et al. 2000; Mooney & Cleland 2001; Shea & Chesson 2002; Gurnell *et al.* 2004). On the other hand, introduced species may overcome resident species through superior competition and prevent those species from having access to certain resources within the community thereby altering their diets as well as interactions among them, ultimately transforming community composition (Sanders *et al.* 2003). Consequently, it is important to understand the influence of introduced species on the trophic structure of a community in terms of resource partitioning among resident species following invasion (Ehleringer *et al.* 1986, Gannes *et al.* 1997). The ensuing community level impacts observed once an introduced species has become invasive are largely dependent on its trophic level and how it interacts with resident species (Levin *et al.* 2002).

Resources are typically partitioned among co-occurring species within a community, and maintained through competition (Davis et al. 2000). This partitioning of resources among species is important in maintaining species co-existence, including competing species (Tillman 1994). The presence of an introduced species may alter this resource partitioning and disrupt co-existence patterns of resident species (Raimundo *et al.* 2009). Co-existence is possible only when competing species have different foraging strategies for similar resources (Vepsäläinen & Savolainen 1988, Cerdá *et al.* 1997, Lessard *et al.* 2009). Most successful invaders are generalists (Andersen 2008), foraging on a wide range of resources (Feiner *et al.* 2013), and thus can commonly adapt to new environments very quickly. This flexibility in diet potentially facilitates the displacement of native species through resource competition, where introduced species have successfully established (Mack *et al.* 2000; Vitousek *et al.* 1996).

Linepithema humile is considered among the most harmful of invasive ant species (Skaife 1955; Vega & Rust 2001; Pyšek *et al*. 2008; GISP 2013), associated with the displacement of native fauna and negative impacts on ecosystem function (Bond & Slingby 1984; Cerda *et al*. 1992; Human & Gordon 1996; Holway 1998; Human & Gordon 1999; Blancaford & Gomez 2005). *Linepithema humile* have been shown to switch trophic positions once they have successfully established in a new environment (Tillberg *et al*. 2007). At the onset of the invasion, they are highly carnivorous, actively predating on ground-dwelling ants and arthropods (De Kock & Giliomee 1989; Cole *et al*. 1992;

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Human & Gordon 1999; Zee & Holway 2006; Tillberg *et al*. 2007), and once they have established, they switch to an herbivorous diet utilising a wide range of plant and animal exudates. The protein is important for queen production and larval growth (Aron 2001; Dussutour & Simpson 2009; Wilder & Eubanks 2010), while the carbohydrate is thought to sustain these extremely large colonies and fuel worker activity (Bristow 1991; DiGirolamo & Fox 2006; Grover *et al.* 2007; Helms & Vinson 2008). However, more recent studies suggest that carbohydrate resources may also be critical for colony growth, and may play a more important role in the successful establishment of exotic ant species (Kay *et al.* 2010; Wilder *et al.* 2011a, b; Shik & Silverman 2012).

Linepithema humile has successfully established in the Cape Floristic Region and is having negative impacts on the native ant fauna as well as indirect effects on the plant community structure of this biodiversity hotspot (Bond & Slingsby 1984; de Kock & Giliomee 1989; Christian 2001; Lach 2008, 2013). *Linepithema humile* displaces three native species *Pheidole capensis* (Mayr), *Anoplolepis custodiens* (Smith) (see chapter 2) and *Anoplolepis steingroeveri* (Forel) which play a significant role as seed dispersers within this ecosystem (Bond & Slingby 1984; Christian 2001; Luruli 2007). Their distribution within natural or protected areas is mutually exclusive of *L. humile* (Luruli 2007; Vorster 2011; see chapter 4). This is largely due to these native species sharing similar biological characteristics with *L. humile*; such as foraging ability, nesting preferences, omnivory and high affinity for trophobiont (and plant) exudates (Samways 1983; Addison & Samways 2009, see Chapter 4). Although many Fynbos ant species are eliminated from invaded areas, a few ant species such as *Ocymyrmex barbiger* and *Tetramorium quadrispinosum* co-exist with *L. humile* owing to these ant species having different thermal tolerances to *L. humile* (Witt & Giliomee 1999) and therefore forage at different temperatures (Witt & Giliomee 1999; Christian 2001). *Monomorium Sp. 8* and *Meranoplus peringueyi* are also found in high abundances in sites invaded by *L. humile*, and this is most likely due to their inconspicuous behaviour (Luruli 2007). However, very little is known about the trophic ecology of native ant species within the Cape Floristic Region. Further, the impact of *L. humile* presence on the trophic ecology, in terms of diet, of resident ant species has not been quantified.

Recently, studies on community wide impacts of biological invasions, particularly of invasive ants, have increased the focus on trophic ecology (Mooney & Tillberg 2005; Le Brun *et al.* 2007; Tillberg *et al.* 2007; Lach *et al.* 2010; Wilder *et al.* 2013). An organism's diet over time can be ascertained from its isotopic signature (Fry 2006), which can reflect the diet over short and long-term periods depending on the growth rate of the particular organism (Hesslein *et al.* 1993). Moreover, information about the organism's relative trophic position, as well as the resource base (whether carbohydrate or protein) can also be ascertained from the isotopic signature using stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (Post 2002, Sanders & Platner 2007, Schmidt *et al.* 2007). Although the advantages of applying stable-isotope analysis in invasion biology are numerous, few studies have used this tool to

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investigate the influence invasive ants have on the diet of co-occurring resident native ant species (Sanders *et al.* 2003). The main focus has been on the competitive interactions between invasive and native ants in terms of access to shared resources (Human & Gordon 1999; Holway *et al.* 2002).

Ants show seasonal shifts in resource preferences due to colony requirements (Helms & Vinson 2002), even though some ants may be specialists foraging on specific resources, most invasive ants act as both predators and herbivores (Holway *et al.* 2002; Mooney & Tillberg 2005; Wilson et al. 2009). Spatio-temporal variation in isotopic signatures of an invasive ant have been shown in several studies to reflect the variations in resource assimilation in both field and laboratory studies (Mooney & Tillberg 2005; Schmidt *et al.* 2007; Tillberg & Breed 2004; Menke *et al.* 2010). Therefore, stable-isotope analysis can be used to study changes imposed by species introductions on native fauna, particularly on the resource use of native competitors, and food webs in general (Vander Zanden et al. 1999). Moreover, more studies are needed to reaffirm the notion that diet switches, as reflected by isotopic signatures and carbon to nitrogen ratios (C:N), are a common feature of *L. humile* invasions (see Tillberg *et al.* 2007; Wilder *et al.* 2013). Some studies have considered seasonal variability in dietary changes in ant studies using stable isotope analysis (Mooney & Tillberg 2005; Tillberg *et al.* 2007; Wilder *et al.* 2013; but see Menke *et al.* 2010).

This study aimed to investigate whether *L. humile* presence resulted in diet switching of co-occurring native ant species, and whether there was seasonal variation in the isotopic signature of *L. humile* and native ant species. The effects of *L. humile* presence on the trophic relationships among ants in invaded and uninvaded sites were determined. The isotopic signatures were also used to infer niche separation among ant species in invaded and uninvaded sites in terms of resource use. The carbon to nitrogen ratio (C:N) was used to determine the contribution of carbohydrates and protein to the diet of ants. The nutritional requirements of a colony differs seasonally and thus the C:N ratio can elucidate changes in diet over time. The ability of *L. humile* to switch diets on an invasion continuum (see Tillberg *et al.* 2007) is inferred from the isotopic signatures of this ant in uninvaded Fynbos, invaded Fynbos and Pine forest in a protected area of the Cape Floristic Region. This study will supplement the information on *L. humile* foraging ecology in this region (where there is currently a paucity of studies on *L. humile* invasion), as well as globally.

METHODS

Sampling and study sites

This study was conducted in Jonkershoek Nature Reserve (34°58' S, 18°56'E) in the Boland region of the Western Cape between May 2011 and August 2012 (see Chapter 4 for reserve description). Sampling was conducted during end autumn (May 2011), beginning winter (June 2011), end winter (August 2011), beginning spring (September 2011), end spring (November 2011), beginning summer

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(December 2011) end summer (February 2012) and beginning autumn (April 2012). Two sites each of uninvaded Fynbos, invaded Fynbos adjacent to the Pine plantations (*Pinus radiata*) found in this reserve, and Pine plantations on the north and south side of the reserve were selected. The sites were grouped into three categories based on invasion status: i) uninvaded Fynbos, ii) invaded Fynbos and iii) Pine forest, the invaded Fynbos and the Pine forest sites represented invaded sites.

Species composition and abundance

In each site, two 50x 50 m plots, 30m apart were established. Three 50 m transects, 10 m apart were set in each plot to assess species composition and abundance using pitfall traps. At each sampling session, pitfall traps were placed at 10m intervals along the three 50m transects in each plot, dug flush to the soil and left opened for seven consecutive days. The pitfall traps were 50ml plastic vials containing 25ml of ethylene-glycol and water as a killing agent. The traps were collected and brought to the laboratory for washing, sorting and identification to species level. Coleopteran and lepidopteran larvae were collected live at random times within the sampling effort, including those caught by pitfall trapping. Only ant species, spiders, coleopteran and lepidopteran larvae were used in the analyses for this study. Voucher specimens were deposited at the Centre of Excellence for invasion Biology, University of Stellenbosch.

Stable-Isotope analysis

Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of *Linepithema humile* and native ants in the three invasion categories

Linepithema humile shifts between trophic positions, being highly carnivorous at the onset of invasion and then relying heavily on carbohydrate resources to maintain high worker populations (Tillberg *et al.* 2007). Moreover, during peak reproductive cycles ants are highly dependent on protein-rich food substances (Aron 2001; Dussutour & Simpson 2009; Wilder & Eubanks 2010; see Kay *et al.* 2010; Wilder *et al.* 2011a, b; and Shik & Silverman 2012 for alternative view) and we potentially expected to see increases in $\delta^{15}N$ enrichment during these periods. It has also been suggested that *L. humile* alters the diet of native ant species through competition for resources (Tillberg *et al.* 2006; Le Brun *et al.* 2007; Wilder *et al*. 2013).

During each sampling effort, ants were collected from the pitfall traps and prepared for stable isotope analysis to ascertain the effect of season on the isotopic signature and the diet (measured as the ratio of C:N) of ants. Only the most abundant ant species caught in the pitfall traps were used for the analysis. The sample was as follows: sample = 10ants/vial, n=5-10 sample replicates per species. To overcome storage effect and the effect of ethylene glycol and ethanol on $\delta^{13}C$, storage times were less than one day after collection, after which there was immediate drying and sample processing (see

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Tillberg *et al.* 2006; Feldhaar *et al.* 2010). Spiders and larvae of lepidoptera and coleoptera were used as representatives of the basal consumers and third level consumers (Fry 2006).

Sample preparation

All abdomens were removed from all ants and spiders to avoid skewing the results due to recent feeding events (Tillberg *et al*. 2006). Samples were dried in an oven at 60°C for two days and then ground to a fine powder using a mortar and pestle and stored in glass containers with desiccant until processing. As in Chapter 4, caterpillars and larvae were processed and ground whole. The samples were sent to the University of Cape Town Stable Isotope Unit where 1,5µg of each sample was weighed into small tin capsules and the $\delta^{13}C$ and $\delta^{15}N$ signatures of all samples were determined using a continuous flow Isotope Ratio Mass Spectrometer (IRMS), after sample combustion in on-line Carlo-Erba preparation. Beet sugar and Merck gelatine were used as standards, calibrated against International Atomic Energy reference materials (PeeDee Belemnite and air for $\delta^{13}C$ and $\delta^{15}N$ respectively). The results are expressed in standard delta notation, $\delta X = ([R_{sample}/R_{standard}] - 1) \times 1000$, where X is the element in question and R is the ratio of the heavy over the light isotope. Delta (δ) values are presented as per mil (‰) for both carbon and nitrogen isotopes. Precision of replicate determinations for both carbon and nitrogen was ± 0.05‰.

Statistical analysis

Species composition and abundance

To compare ant species composition and abundances between the three invasion categories (uninvaded Fynbos; invaded Fynbos; invaded Pine forest), permutation multivariate analyses (PERMANOVA) followed by multidimensional scaling analyses (MDS) were used. Ant abundance data contained many zero counts (since many species were not found in all the traps), which is accounted for in PERMANOVA, which uses permutational procedures to obtain p values (Pseudo-p) (Clarke & Gorley 2006). The data were square root transformed to balance the weight of the contribution of the most abundant and most rare species (Clarke & Gorley 2001). Bray-Curtis Similarity Index analyses were used to compute the similarity matrix (Clarke & Gorley 2001). The PERMANOVA design was a two-way crossed design with two factors (i) invasion category (3 levels) and (ii) season (4 levels). The early and late season data were combined. The analyses were conducted under a reduced model to obtain the best fit (Clarke & Gorley 2006), with 999 permutations. A non-metric multi-dimensional scaling (NMDS) ordination plot based on invasion category as a factor was used to compile a visual plot of the species assemblage differences between the three invasion categories. This was followed by Analysis of Similarity (ANOSIM), with invasion category as a factor/status to determine whether the differences between the groups clustered by the MDS were significantly different. The ordination was generated using centroids of the Bray-Curtis

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similarity matrix using a Kruskal Wallis and 50 random restarts. Stress values closer or equal to 0 indicate goodness of fit, with no prospect of a misleading interpretation (Clarke & Warwick, 2001). The species richness (S), Shannon-Wiener's diversity index (H′) and Pielou's species evenness (J) (Kent & Coker 2002) were computed to measure the changes in species composition associated with *L. humile* invasion. These parameters of species composition were compared between the three invasion categories using a Kruskal-Wallis ANOVA, followed by pairwise comparisons using Mann-Whitney U test. A Bonferroni correction was applied (0.05/3 = 0.02). All analyses were conducted in PRIMER V6 with PERMANOVA+ (Plymouth Routines in Multivariate Ecological Research, version 6: Plymouth Marine Laboratory, UK) and SPSS version 20 statistical software. Statistical significance was accepted at $p < 0.05$ unless corrected for using Bonferroni correction.

A Generalised linear Model (GLZ) assuming a Poisson distribution with a log-link function, and posthoc pairwise analyses with Least Square Difference (LSD) was used to determine whether there was a difference in the abundance of ground dwelling ant species across the four seasons at each invasion category. The data were analysed for each invasion category with the factors in the model being (i) ant species and (ii) season. The estimation scale parameter used was Pearson Chi-Square to obtain more conservative variance estimates and statistical significance. Independent GLZs were also conducted to determine whether a given species differed in abundance across the four seasons. All GLZ analyses were performed in SPSS version 20 statistical software. Statistical significance was accepted at $p < 0.05$.

Stable-Isotope analysis

Isotopic signature comparison

The isotopic signature $\delta^{13}C$ and $\delta^{15}N$ of ants, larvae and spiders were jointly compared using a Multivariate Analysis of Variance (MANOVA) to minimise chances of Type 1 error (Ottonetti *et al.* 2008). The uninvaded Fynbos sites were dominated by the native ants *A. custodiens* and *P. capensis* which were mutually exclusive of each other and occurred only on one of the sites in the Fynbos. Therefore, the analyses were conducted independently for each invasion category (with the uninvaded Fynbos separated into Fynbos 1 and Fynbos 2), while the two sites each of the invaded Fynbos and the Pine forest were combined. $\delta^{13}C$ and $\delta^{15}N$ values were both used as dependent variables, with ant species and season as factors. Pairwise differences were compared using Tukey's HSD posthoc test. Preliminary assumptions testing were conducted to check for normality, linearity, equality of variance, outliers, multicollinearity and equality of covariance. Pillai's Trace lambda was used as the test statistic due to the violation of the assumption of equality of covariance (Box's M test was less than 0.05). The MANOVA was performed in SPSS version 20 statistical software.

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Comparison of C:N ratio - relative contribution of carbohydrate and proteins to ant diets

The carbon to nitrogen ratio (C:N) represents the ratio of the mass of carbon to nitrogen in the sample and the relative contribution of carbohydrates and/or protein to the diet of ants (Smith & Suarez 2012). High C:N ratios indicate a high contribution of carbohydrates to the diet while low C:N ratios indicate high contribution of protein to the diet (Ottonetti *et al.* 2008). C:N ratios of all species sampled were compared for each invasion category (with the uninvaded Fynbos separated into Fynbos 1 and Fynbos 2) using a Generalised Linear Model assuming a gamma distribution and log-link function, and posthoc pairwise comparison using Least Square Difference. The dependent variable was the C:N ratios while the two factors entered into the model were (i) ant species and (ii) season. The C:N ratio of each species was compared across the four seasons using Generalised Linear Models. To ascertain the effect of *L. humile* on the diet of ant species in invaded Fynbos and Pine forest and uninvaded Fynbos, the C:N ratios of commonly occurring ant species in invaded and uninvaded sites were compared using a Mann-Whitney U test when an ant species occurred in only two of the invasion categories and Kruskall-Wallis ANOVA, if an ant species occurred in all the invasion categories, followed by Mann-Whitney U tests for pairwise comparisons with Bonferroni correction applied depending on the number of pairwise comparisons. The GLZ and subsequent tests were conducted in SPSS version 20 statistical software and statistical significance was accepted at $p < 0.05$.

RESULTS

Species composition and abundance

Ant species composition and abundance differed between invasion categories and were dependent on season ($F_{(6)} = 14.32$, $p < 0.001$, Table 1). The ant assemblages and abundance differed significantly between the different invasion categories ($F_{(2)} = 258.72$, $p < 0.001$) with these differences being strongly affected by season ($F_{(3)} = 14.31$, $p < 0.001$, Table 1). Ant assemblages within the uninvaded Fynbos, invaded Fynbos and Pine forest sites were distinct from each other (Global $R = 0.97$, $p < 0.001$, Figure 1). Pairwise comparisons in PERMANOVA showed that ant assemblages were significantly different between pristine Fynbos and invaded Fynbos ($t = 15.15$, $p < 0.001$), pristine Fynbos and Pine forest ($t = 15.94$ $p < 0.001$), and invaded Fynbos and Pine forest ($t = 17.28$, $p < 0.001$).

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Table 1. Results of Permanova analysis performed on ant species abundance between invasion categories (Fynbos, Invaded Fynbos and Pine Forest) and across seasons; as well as comparison of Species richnss (S), Shannon diversity index (H´) and Pielou's evenness (J) based on ant species abundance in all study sites. Pairwise differences in S, H´ and J between invasion categories are compared using non-parametric Mann-Whitney U tests with significant diffferences between pairs shown with letter superscripts.

Test	*df*	*seudo-F*	*P*
Invasion category	2	258.72	0.001
Season	3	14.31	0.001
Invasion category X Season	6	14.32	0.001

	Fynbos	Invaded Fynbos	Pine Forest
Species richness (*S*)	8.90±0.3[a]	6.38±0.20[b]	6.31±0.25[b]
Shannon-Wiener diversity *(H')*	2.01±0.03[a]	1.69±0.34[b]	1.65±0.34[b]
Pielou's evenness (*J*)	0.96±0.00[a]	0.94±0.01[a]	0.94±0.00[a]

Linepithema humile presence was associated with a significant change in ant species composition between the invaded and uninvaded sites (Table 1). Ant assemblages between the three invasion categories differed in both species richness (S) (Kruskal Wallis $\chi^2_{(2)} = 47.03$, $p < 0.001$) and diversity (H´)(Kruskal Wallis $\chi^2_{(2)} = 45.17$, $p < 0.001$) but not evenness (J) (Kruskal Wallis $\chi^2_{(2)} = 11.56$, $p > 0.05$). Only the pristine Fynbos site was significantly different from the invaded Fynbos site and the Pine forest in terms of species richness and diversity (Table 1). This result is supportive of evidence from previous studies showing the impact of *L. humile* on native ant species composition in the Fynbos (Lach 2013).

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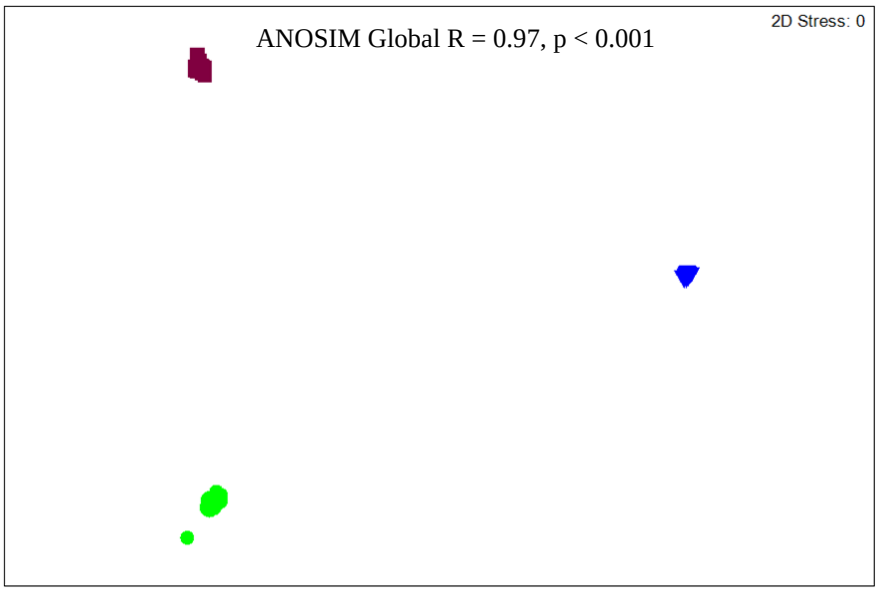

Figure 1. Non-metric multidimensional ordination analysis comparing ant assemblages between three sites, Fynbos (●), Invaded Fynbos (▼) and Pine forest (■). The three sites are distinct from each other in their ant species assemblages.

Ground foraging ant abundance across four seasons in three invasion categories

A total of 21 ant species were captured in pitfall traps at the uninvaded Fynbos site (Figure 2a). The abundances of ants differed between ant species sampled with these differences being amplified by season for three of the species (GLZ Wald $\chi^2_{(56)} = 327.19$, $p < 0.0001$). The abundances of ant species were significantly different (GLZ Wald $\chi^2_{(20)} = 120.76$, $p < 0.0001$), yet season was not completely responsible for these differences evident in ant abundances between species (Wald $\chi^2_{(3)} = 1.48$, $p > 0.05$). Still the abundances of *Anoplolepis custodiens* (GLZ Wald $\chi^2_{(3)} = 46.01$, $p < 0.0001$), *Meranoplus peringueyi* (GLZ Wald $\chi^2_{(3)} = 51.33$, $p < 0.0001$) and *Pheidole capensis* (GLZ Wald $\chi^2_{(3)} = 38.43$, $p < 0.0001$) differred significantly across season. *Anoplolepis custodiens* had the highest abundaces in the autumn and spring, while *M. peringueyi* and *P. capensis* had the highest abundances in winter and summer respectively.

Sixteen ant species were caught in the invaded sites (see Figure 2b and c for species identification). Ant abundances differed significantly between ant species sampled with these differences largely dependent on season at the invaded Fynbos site (GLZ Wald $\chi^2_{(26)} = 89.86$, $p < 0.0001$), but this significant interaction is largely driven by *L. humile* and to a lesser extent by *T. quadrispinosum* who show large fluctuations in abundance across seasons. Ant abundances differed significantly between ant species (GLZ Wald $\chi^2_{(15)} = 1496.33$, $p < 0.0001$) with these differences showing a strong seasonal effect (GLZ Wald $\chi^2_{(3)} = 11.31$, $p < 0.05$, Figure 2b). Ant abundance and season interact significantly in the Pine forest, (GLZ Wald $\chi^2_{(40)} = 138.24$, $p < 0.0001$) implying that some species show variation across season but that season is not altering ant abundances (GLZ Wald

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$\chi^2_{(3)}$ = 0.99, p > 0.05). Discounting season, ant abundances between species varied significantly in the Pine forest (GLZ Wald $\chi^2_{(15)}$ = 1258.03, p < 0.001). In the invaded Fynbos sites, the ant abundances of *L. humile* (GLZ Wald $\chi^2_{(3)}$ = 56.57, p < 0.001), *M. peringueyi* (GLZ Wald $\chi^2_{(3)}$ = 17.33, p < 0.001) and *T. quadrispinosum* (GLZ Wald $\chi^2_{(3)}$ = 12.80, p < 0.001) differed significantly across season with *L. humile* having highest abundances in summer, autumn and winter, while *T. quadrispinosum* and *M. peringueyi* reached high densities in autum and winter, and summer respectively. In the Pine forest, the ant abundances of *L. humile* (GLZ Wald $\chi^2_{(3)}$ = 28.84, p < 0.001), *M. schultzei* (GLZ Wald $\chi^2_{(3)}$ = 48.28, p < 0.001), *T. frigidum* (GLZ Wald $\chi^2_{(3)}$ = 17.91, p < 0.001) and *T. quadrispinosum* (GLZ Wald $\chi^2_{(3)}$ = 10.87, p < 0.05) differed significantly across season. *Linepithema humile* abundances were highest in summer, autumn and spring, *M. schultzei* and *T. quadrispinosum* were highest in summer and autumn, while *T. frigidum* reached abundances in spring.

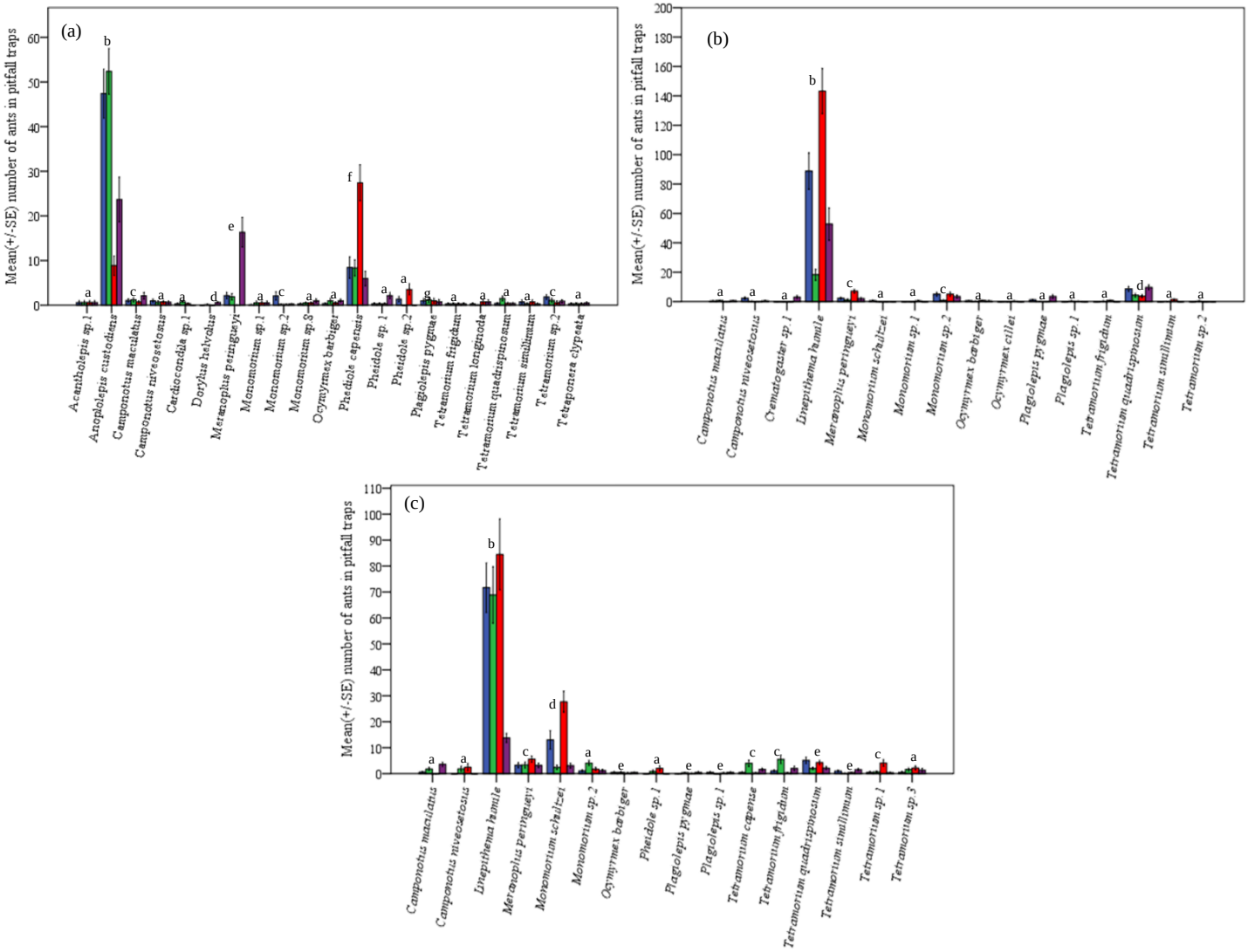

Figure 2a-c. Comparison of ant abundance in pitfall traps in the three invasion categories; uninvaded Fynbos, (b) invaded Fynbos (c) Pine forest, across four seasons. Data is combined for all the sites. Different seasons are denoted with Autumn (■), Spring (■), Summer (■) and Winter (■).There was a significant difference in ant abundances between species but not always between season at all three invasion categories. Pairwise differences between species abundances are indicated with letters based on GLZ Least Square Difference. Uninvaded Fynbos sites (a) were largely dominated by *Anoplolepis custodiens* and *Pheidole capensis*, while invaded Fynbos (b) and Pine forest (c) were largely dominated by *Linepithema humile* and *Monomorium schultzei*. Independent GLZs were conducted to ascertain differences in ant abundance per invasion category across season for each species and are reported in text.

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Stable-Isotope analysis

Seasonal shift in isotopic signature

Ants encompassed a broad range of $\delta^{15}N$ values (Figure 3a-d), suggesting that ants occupy several trophic levels at the uninvaded Fynbos (Fynbos 1 dominated by *P. capensis* and Fynbos 2 dominated by *A. custodiens*), invaded Fynbos and Pine forest (both dominated by *L. humile*). In uninvaded Fynbos (Figure 3a and b), the isotope signatures of the native ants *O barbiger* and *Camponotus niveoseotus*, are similar to those of predacious and herbivorous arthropods respectively throughout all the four seasons. *Ocymyrmex barbiger* is an obligate predator of arthropod species (Witt & Giliomee 1999; 2004; see chapter 4 for alternative results), while *Camponotus niveosetosus* is an obligate nectar feeding ant (Visser *et al.* 1996). The $\delta^{15}N$ values of the majority of co-occuring ant species in uninvaded Fynbos dominated by *P. capensis*, and invaded Fynbos and Pine forest dominated by *L. humile* are within the same range (4-6‰, Figure 3a, c and d), which suggests there is some overlap in resource use between these ants. The isotopic signatures between ant species were significantly different and dependent on season at the Fynbos site 1 (Pillai's trace lambda $\lambda = 0.54$, $F_{(66,464)} = 2.50$, $p < 0.0001$), invaded Fynbos (Pillai's trace lambda $\lambda = 0.23$, $F_{(52,644)} = 1.59$, $p < 0.05$) and Pine forest (Pillai's trace lambda $\lambda = 0.31$, $F_{(50,720)} = 2.67$, $p < 0.0001$), while these differences were not completely affected by season at Fynbos site 2 (Pillai's trace lambda $\lambda = 0.47$, $F_{(58,414)} = 2.17$, $p < 0.0001$), indicating that there was significant variation in isotopic signatures between ant species reliant on season, except for within Fynbos site 2 (Figure 3b). The isotopic signatures differed significantly between the ant species sampled in all the sites (Table 2), however, only the isotopic signatures of ants in the Fynbos 1 (Pillai's trace lambda $\lambda = 0.06$, $F_{(6,464)} = 2.18$, $p< 0.05$), invaded Fynbos (Pillai's trace lambda $\lambda = 0.02$, $F_{(6,644)} = 0.80$, $p < 0.0001$) and Pine forest sites (Pillai's trace lambda $\lambda = 0.04$, $F_{(6,720)} = 2.53$, $p < 0.05$) varied significantly across the four seasons (Table 2). The $\delta^{13}C$ and $\delta^{15}N$ values between ant species sampled at all sites were significantly different, with these differences exacerbated by season for the $\delta^{15}N$ isotope at all sites (Table 2), and the $\delta^{13}C$ isotope at both uninvaded Fynbos sites and Pine forest. However, the differences in the $\delta^{13}C$ isotope were dependent on season at the invaded Fynbos site ($F_{(3,322)} = 0.68$, $p < 0.05$). The difference in isotopic signature of ant species sampled was largely explained by $\delta^{15}N$, which explained between 80-91% of the variation in isotopic signature.

The isotopic signatures of the native dominant ants *P. capensis* and *A custodiens* do not show a strong seasonal shift, both remaining in the $\delta^{15}N$ (4 and 6‰) and $\delta^{13}C$ (-22 and -25‰) ranges through all four seasons. The isotopic signature of the native ant *Pheidole sp.1* show low $\delta^{15}N$ values and are similar to those of nectarivorous ants and cluster closer to the herbivorous arthropods at the site dominated by *A. custodiens* compared to that dominated by *P. capensis* during summer, autumn and winter. *Tetramorium quadrispinosum* also shows a similar pattern in winter, with a lower $\delta^{15}N$ value

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of the isotopic signature in areas dominated by *A. custodiens* than *P. capensis*. This suggests some dietary niche partitioning when certain native ants co-occur with either of the two dominant native species. It is known that dominant species control access to resources of subordinate ants. Moreover, *Pheidole* species are generalised myrmecinae and are able to co-exist with some dominant ant species through the partitioning of resources and space, as this observation suggests. The isotopic signature of *L. capensis* is variable across the season (Figure 3a), with lower $\delta^{15}N$ values in the autumn and winter during the peak flowering season in the Fynbos. This ant may be utilising carbohydrate resources at this time, similarly for *Pheidole sp.1*, in the site dominated by *A. custodiens* (Figure 3b), which may be feeding on these resources during the early bloom period which is in autumn for most Fynbos plants.

The isotopic signatures of *L. humile* and native ants in invaded Fynbos and Pine forest suggest strong overlap in resource use between all ants, with all ant species clustering tightly together (Figure 3c and d). However, close inspection shows more variation in the isotopic signatures between native ant species co-occurring with *L. humile* in the invaded Fynbos site, particularly in the spring and winter (Figure 3c), than in the neighbouring Pine forests (Figure 3d). The isotopic signature of *L. humile* in invaded Fynbos suggests that it has a more predatory diet in the spring and summer, and clusters closer to herbivorous arthropods in the autumn and winter, suggesting a more carbohydrate based diet. This observation is similar for *L. capensis* in areas dominated by *P. capensis*. In the Pine forest the overlap in $\delta^{15}N$ values did not fluctuate with season (Figure 3d), however, *L. humile* clusters closer to predatory spiders during spring, summer, and autumn and the $\delta^{15}N$ lowers marginally in the winter (Figure 3d). This suggests a highly predacious diet for *L. humile* at this site, and the lower $\delta^{15}N$ value in winter suggests that it may also be utilising some of the periodically available floral nectar accessible in neighbouring Fynbos. This result supports the diet switching hypothesis of Tillberg and colleagues (2007).

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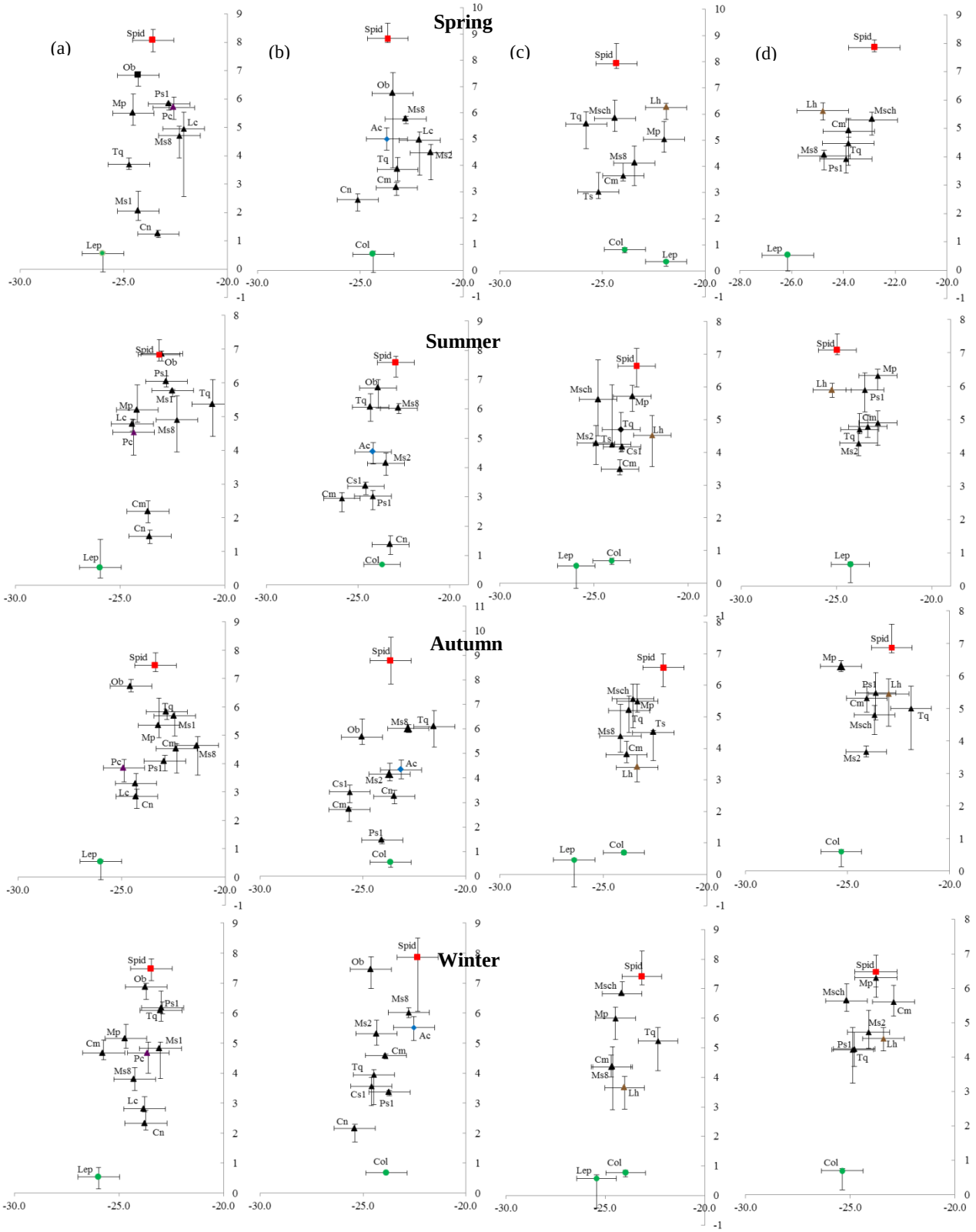

Figure 3a-d. Mean (±) $\delta^{13}C$ and $\delta^{15}N$ values of ants (▲), herbivorous (●) and predacious arthropods (■) at the Fynbos 1(a), Fynbos 2 (b), invaded Fynbos (c) and Pine forest (d). Abbreviations:Ac(*Anoplolepis custodiens*), Cm(*Camponotus maculatus*), Cn(*Camponotus niveosetosus*), Cs1(*Camponotus sp.1*), Col(Coleopteran larvae), Lep(Lepidopteran larvae), Lc(*Lepisiota capensis*), Lh(*Linepithema humile*), Mp(*Meranoplus peringueyi*), Ms1(*Monomorium sp.1*), Ms2(*Monomorium sp.2*), Ms8(*Monomorium sp.8*), Msch(*Monomorium schultzei*), Ob(*Ocymyrmex barbiger*), Pc(*Pheidole capensis*), Ps1(*Pheidole sp.1*), Spid(spider), Ts(*Tetramorium simillimum*).Tq(*Tetramorium quadrispinosum*).

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Table 2. Manova results for multivariate and univariate ($\delta^{15}N$ and $\delta^{13}C$) comparison of stable isotope signatures for ant species, herbivorous and predacious arthropods sampled at each of the sampling localities (invasion categories) at Jonkershoek Nature Reserves across four seasons. The isotopic signatures of ant species sampled were significantly affected by season, except for within the Fynbos 2 site, and differed significantly amongst the ant species sampled. Both $\delta^{15}N$ and $\delta^{13}C$ varied significantly between all ant species but not over the seasons, except for $\delta^{13}C$ in the invaded Fynbos, at all sampling localities.

		Fynbos 1 (with *P. capensis*)			Fynbos 2 (with *A. custodiens*)			Invaded Fynbos			Pine forest		
Test	Effect	Pillai's λ	$F_{(df, n)}$	*P*	Pillai's λ	$F_{(df, n)}$	*P*	Pillai's λ	$F_{(df, n)}$	*P*	Pillai's λ	$F_{(df, n)}$	*P*
Multivariate													
	Species	0.92	$18.12_{(28,464)}$	<0.0001	0.90	$16.98_{(20,414)}$	<0.0001	0.65	$15.57_{(20,644)}$	<0.0001	0.77	$25.10_{(18,720)}$	<0.0001
	Season	0.06	$2.18_{(6,464)}$	<0.05	0.02	$0.63_{(6,414)}$	>0.05	0.02	$0.80_{(6,644)}$	<0.0001	0.04	$2.53_{(6,720)}$	<0.05
	Spp. X Season	0.54	$2.50_{(66,464)}$	<0.0001	0.47	$2.17_{(58,414)}$	<0.0001	0.23	$1.59_{(52,644)}$	<0.05	0.31	$2.67_{(50,720)}$	<0.0001
$\delta^{15}N$													
	Species		$61.31_{(11,232)}$	<0.0001		$49.63_{(10,207)}$	<0.0001		$36.53_{(10,322)}$	<0.0001		$63.90_{(9,360(}$	<0.0001
	Season		$2.07_{(3,232)}$	>0.05		$0.58_{(3,207)}$	>0.05		$0.79_{(3,322)}$	>0.05		$1.38_{(3,360)}$	>0.05
	Spp. X Season		$3.40_{(33,232)}$	<0.0001		$2.48_{(29,207)}$	<0.0001		$1.90_{(26,322)}$	<0.0001		$2.47_{(23,360)}$	<0.0001
$\delta^{13}C$													
	Species		$6.83_{(11,232)}$	<0.0001		$7.30_{(10,207)}$	<0.0001		$6.09_{(10,322)}$	<0.0001		$9.42_{(9,360)}$	<0.0001
	Season		$1.92(_{3,232)}$	>0.05		$0.45(_{3,207)}$	>0.05		$0.68_{(3,322)}$	<0.05		$4.55_{(3,360)}$	>0.05
	Spp. X Season		$2.14_{(33,232)}$	<0.0001		$1.82_{(29,207)}$	<0.05		$1.56_{(26,322)}$	<0.05		$2.78_{(29,353)}$	<0.0001

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Comparison of C:N ratios among ant species in the three invasion categories

The C:N ratios of all ant species sampled suggest that many of the ants forage on both protein and carbohydrate based resources. The C:N ratios ranged between 4 and 6‰ for most ant species (Figure 4a-d), with the known nectarivorous ant, namely *C. niveosetosus*, having high C:N ratios similar to that of herbivores for Fynbos site 2, while this is true only for spring and summer in Fynbos site 1 (Figure 4a and b). The C:N ratios of all ant species sampled in all the sampling localities varied significantly with significant interactions with season: Fynbos 1 (GLZ Wald $\chi^2_{(33)} = 94.99$, $p < 0.0001$), Fynbos 2 (GLZ Wald $\chi^2_{(30)} = 53.95$, $p < 0.01$), invaded Fynbos (GLZ Wald $\chi^2_{(26)} = 64.85$, $p < 0.001$) and Pine forest (GLZ Wald $\chi^2_{(25)} = 80.85$, $p < 0.0001$), however season did not drive these changes in C:N ratios of ants for most localities. Thus, all species sampled within each of the localities showed variation in their C:N ratios: Fynbos 1 (GLZ Wald $\chi^2_{(11)} = 232.50$, $p < 0.0001$), Fynbos 2 (GLZ Wald $\chi^2_{(10)} = 178.64$, $p < 0.001$), invaded Fynbos (GLZ Wald $\chi^2_{(10)} = 231.0$, $p < 0.001$) and Pine forest (GLZ Wald $\chi^2_{(8)} = 178.99$, $p < 0.0001$), but these differences were not owing to season, except for the Pine forest where C:N ratios of the ant species changed across season (GLZ Wald $\chi^2_{(3)} = 12.30$, $p < 0.01$, Figure 4d). In the Fynbos locality dominated by *P. capensis* (Figure 4a), only the C:N ratios of *C. niveosetous* (GLZ Wald $\chi^2_{(3)} = 26.97$, $p < 0.001$), *Pheidole sp.1* (GLZ Wald $\chi^2_{(3)} = 11.54$, $p < 0.01$) and *T. quadrispinosum* (GLZ Wald $\chi^2_{(3)} = 16.85$, $p < 0.01$) varied significantly across season, while in the locality dominated by *A. custodiens* (Figure 4b) the C:N ratios of *A. custodiens* (GLZ Wald $\chi^2_{(3)} = 13.17$, $p < 0.01$), *C. maculatus* (GLZ Wald $\chi^2_{(3)} = 16.62$, $p < 0.01$), *Monomorium sp.2* (GLZ Wald $\chi^2_{(3)} = 13.05$, $p < 0.01$) and *Pheidole sp.1* (GLZ Wald $\chi^2_{(3)} = 11.66$, $p < 0.05$) differed significantly across season. In the invaded Fynbos locality, only the C:N ratios of *M. schultzei* (GLZ Wald $\chi^2_{(3)} = 10.74$, $p < 0.05$) and *T. quadrispinosum* (GLZ Wald $\chi^2_{(3)} = 12.59$, $p < 0.01$) were significantly different across the seasons (Figure 4c). The C:N ratios of lepidopteran larvae (GLZ Wald $\chi^2_{(3)} = 11.73$, $p < 0.05$), *L. humile* (GLZ Wald $\chi^2_{(3)} = 12.88$, $p < 0.01$), *M. peringueyi* (GLZ Wald $\chi^2_{(3)} = 36.23$, $p < 0.0001$), *M. schultzei* (GLZ Wald $\chi^2_{(3)} = 8.57$, $p < 0.05$) and spiders (GLZ Wald $\chi^2_{(3)} = 20.64$, $p < 0.0001$) all varied significantly across the seasons in the Pine forest. The C:N ratio of *L. humile* is higher (5.21±0.41‰) in winter compared to the other seasons in the Pine forest (Wald $\chi^2_{(3)} = 7.96$, $p < 0.05$) likely due to the use of carbohydrates from the Fynbos site adjacent to the Pine forest that contains many winter flowering protea species. *Meranoplus peringueyi* also showed an increase in the C:N ratio during winter for both invaded localities (6.22±0.31‰) suggesting that it may also be utilising a more carbohydrate based resource at this time.

The C:N ratios of ant species that were sampled in both invaded and uninvaded areas were also compared to ascertain the potential effect of *L. humile* presence on their diets in localities where they co-exist with *L. humile*. The C:N ratios of *M. peringueyi*, *Monomorium sp.8*, *Monomorium sp.2*, *Pheidole sp.1* and *T. quadrispinosum* which were the ant species found in both invaded and uninvaded sampling

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localities were compared between the invasion categories (Figure 2). Only the C:N ratios of *M. peringueyi* (Kruskal wallis H: $\chi^2_{(2)}$ = 16.54, p < 0.01), *Monomorium sp.8* (Mann-Whitney U test: U= 124.0, Z = -4.11, p < 0.0001) and *T. quadrispinosum* (Kruskal wallis H: $\chi^2_{(3)}$ = 8.66, p < 0.05) were significantly different between localities. *Monomorium sp.8* foraged more on carbohydrate resources in uninvaded Fynbos (5.71±0.21‰) than in invaded Fynbos (4.44±0.15‰); *M. peringueyi* foraged more on carbohydrate resources in the Pine forest (5.01±0.16‰) and the invaded Fynbos (5.67±0.15‰) than in the uninvaded Fynbos (4.33±0.16‰), and *T. quadrispinosum* foraged on protein resources in the invaded Fynbos (4.61±0.11‰) than in uninvaded Fynbos (5.24±0.15‰). These data suggest that *L. humile* presence does have some effect on the foraging ecology of certain native Fynbos ants as their diets are different in areas where *L. humile* is not present.

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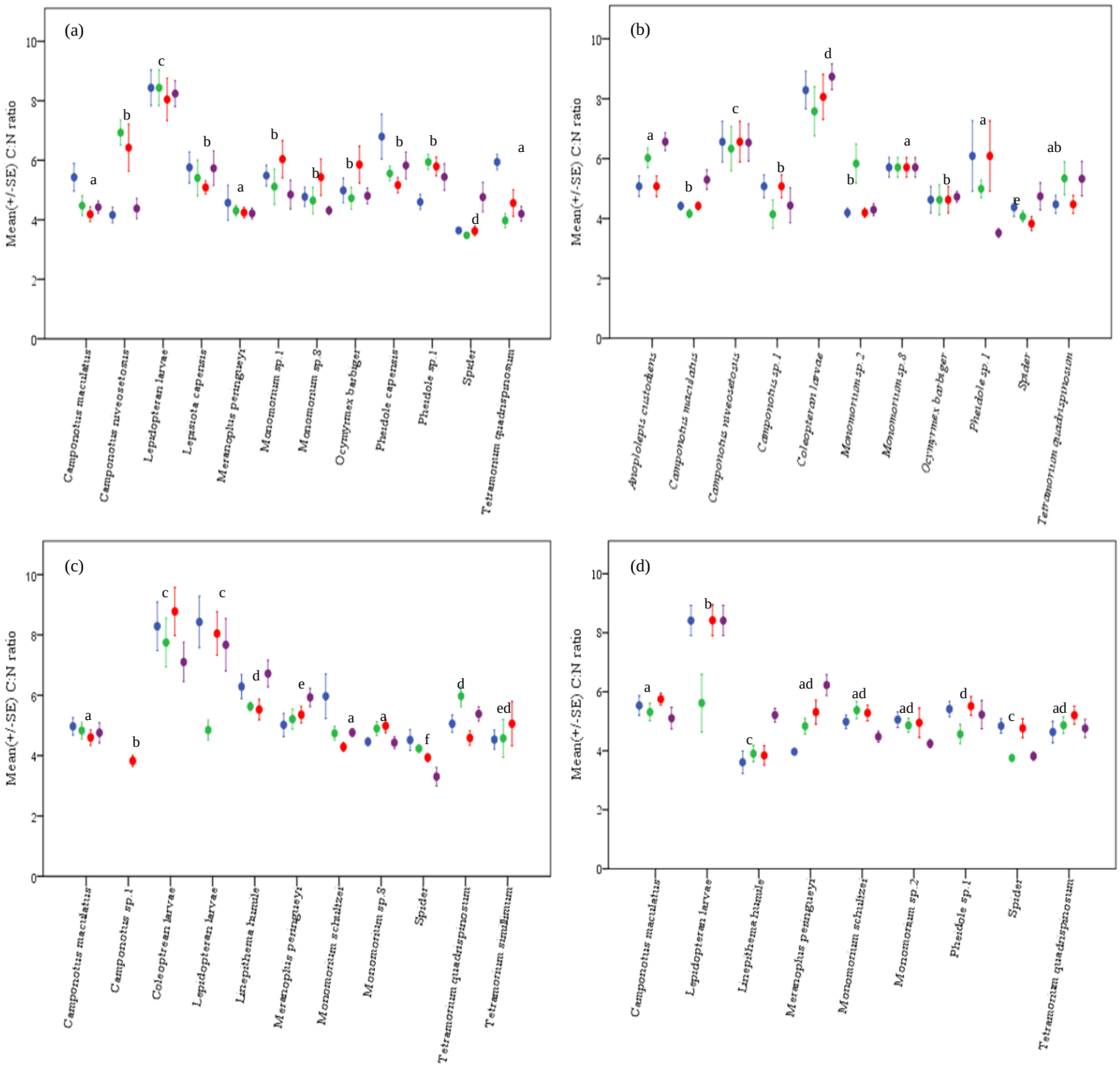

Figure 4a-d. The Mean (±SE) C:N ratios, across four seasons, of ant species sampled in the Fynbos 1 (a), Fynbos 2 (b), invaded Fynbos (c) and Pine forest (d). The C:N ratios of herbivorous arthropods are high, ranging between 7 and 10‰. *Linepithema humile* has low C:N ratios in the Pine forest similar to those of spiders, with similar C:N ratios to the dominant native ants, *Anoplolepis custodiens* and *Pheidole capensis*, in uninvaded Fynbos. Different seasons are denoted with Autumn (●), Spring (●), Summer (●) and Winter (●). Significant differences in C:N ratios between ant species, based on the GLZ Least Square Difference, are denoted with letters. Independent GLZs were conducted to compare the differences in C:N ratio within species over the seasons and are presented in text.

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DISCUSSION

The invasive Argentine ant, *Linepithema humile*, negatively affected the species richness and diversity of ground foraging native ants. Stable isotope analysis revealed that *L. humile*'s diet is highly predatory in the Pine plantation and more herbivorous in invaded Fynbos, suggesting a diet switch. This supports the hypothesis that *L. humile* can switch its diet along an invasion continuum (Tillberg *et al.* 2007). The C:N ratios for *L. humile* were very low in the Pine forest and the isotopic signature matched that of the spiders, implying that its nitrogen inputs were derived mainly from protein sources through predation on other arthropods. The C:N ratios were high during autumn and winter in both the Pine forest and invaded Fynbos, suggesting the ants utilised more carbohydrate-rich resources. However, the availability of carbohydrate resources was not assessed in the Pine forest but it is possible that *L humile* had access to seeds or some other carbohydrate source that may temporarily have been available in this environment or they accessed the adjacent Fynbos, since most Fynbos plants were flowering during winter (Cowling *et al.* 1996). *Linepithema humile* also significantly altered the diet of native ant species that commonly co-occurred with it (see Chapter 4). The C:N ratios showed that the native ant *M. peringueyi* foraged on more carbohydrate based resources in invaded areas and was more predacious in the uninvaded Fynbos (Figure 4a,c and d), while *Monomorium sp. 8* and *T. quadrispinosum* preferentially foraged on protein based resources in uninvaded Fynbos (Figure 4a-d). These data indicated that *L. humile* had an effect on the trophic ecology of some native Fynbos ant species and its presence induced diet switching in some native ant species within resident ant communities, likely due to competition for shared resources.

The availability of resources, as well as the type of resources available within an environment, has an effect on the diets of ants (Blüthgen & Feldhaar 2010), largely because the colony's nutritional needs change with season (Mooney & Tillberg 2005; Dussutour & Simpson 2009). The isotopic signatures of ant species sampled in this study suggest that many are omnivorous, feeding on a combination of carbohydrate and protein based resources (see Mooney & Tillberg 2005). Spring is typically a period of colony growth (Hölldobler & Wilson 1990; Mooney & Tillberg 2005; Menke *et al.* 2010) requiring high protein intake which coincides with high arthropod densities and should be reflected in their diet in terms of C:N ratios. This protein rich diet may continue into summer while arthropod prey densities remain high. In winter however, insect prey are few as most pupate and therefore a decrease in dietary protein is expected. However, the Fynbos is a winter flowering region inundated with pollinators, consequently protein is not a limiting resource in this biome during winter. Based on Tillberg and Mooney (2005) ants should be feeding on a high protein diet during reproductive seasons, while Wilder et al.'s (2013) study suggest a more balanced protein and carbohydrate intake as ants seem to require both macronutrients for colony growth (Wilder *et al.* 2011). Our findings are in agreement and showed that the C:N ratios of most ant species range between 4-6‰, indicating that there is some contribution of carbohydrates to their diets regardless of the season. Recently, Menke and colleagues (2010) cautioned the interpretation of seasonal

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variation in $\delta^{15}N$ values of field colonies of ants, since seasonal shifts can span up to one trophic step, as also observed in this study by up to 3‰ (Figure 3). They accounted for this lack of clarity, contending that the source of variation is likely due to i) shifts in seasonal changes owing to resource availability or ii) shifts in resource preferences owing to colony nutritional needs. In the case of ants in this study, the variability in $\delta^{15}N$ values for native ants is likely driven by colony nutritional needs, however, *L. humile* may be showing higher variability due to shifts in resource availability which may be happening when native ants are not requiring this specific macronutrient . *Linepithema humile* reproduces yearlong under the right conditions (Aron 2001; brood present all year round in Fynbos nests, *pers obs*), and their diet is thus less likely to be constrained by colony nutritional demands but rather by resource availability, which in a winter flowering biome may not result in resource limitations. Moreover their diet is flexible and thus they can utilise a wide range of resources as needed (Holway *et al.* 2002).

Linepithema humile is thought to rely on the availability of carbohydrate resources to increase invasion success (Holway *et al.* 2002; Kay *et al.* 2010; Wilder *et al.* 2013). However, the foraging ecology of native Fynbos ants is not well known or studied. The Fynbos is rich in floral carbohydrate resources that can potentially be exploited by ant species. However, the native ant species are not effectively utilising this abundant resource (Lach 2013, Chapter 4). *Linepithema humile* is known to thrive on carbohydrate rich resources and these are believed to be essential to its colony growth and survival (Grover *et al.* 2007; Tillberg *et al.* 2007; Shik & Silverman 2012; Wilder *et al.* 2013). Previous studies on ant nutritional ecology had suggested the importance of protein for colony growth in ants (Dussutour & Simpson 2009; Wilder & Eubanks 2010; Simpson & Raubenheimer 2012), however, recent studies showed that carbohydrates also play an essential role in colony growth (Wilder & Suarez 2011, 2013), if not more so than protein (Shik & Silverman 2012). The high protein diet of *L. humile* in the Pine forest is strange as these areas are the initial sites of introduction in this area (Donnelly 1983). Based on the hypothesis of Tillberg et al. (2007), *L. humile* should be foraging at a lower foraging level in these localities since colonies have long established. It is likely that ants in this area are carbohydrate limited due to the poor supply, although we did not quantify whether carbohydrate resources were available. The ability to attain high abundances in a resource poor environment such as the Pine forest bears testament to *L. humile*'s resilience and diet flexibility. Moreover, the C:N ratio data suggested that *L. humile*, and other native ant species found in the Pine forest, do periodically have access to carbohydrate resources during the winter flowering season. *Linepithema humile* may be utilising floral nectar from adjacent Fynbos sites to fuel their large colony sizes in the Pine forest during the winter (Rowles & Silverman 2009; Savage *et al.* 2009; see Chapter 4), as well as in the invaded Fynbos. The $\delta^{15}N$ values and C:N values of *L. humile* suggest that this ant responds more quickly to floral nectar availability in the Fynbos. The availability of Fynbos adjacent to Pine forest, as well as already invaded Fynbos may fuel the spread of this ant.

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Once an introduced species successfully establishes within a community, it may alter the way in which resident species utilise available resources by excluding them through competition (Gibb & Hotchuli 2004). Resident species can respond by altering their foraging behaviour and also changing their diet by switching to those resources for which there is low competition (Morrison 2000: Gibb & Hotchuli 2004). The environment may provide an opportunity for resource partitioning and niche adjustments for introduced species if there are unused resources within the environment (Mooney & Cleland 2001, Shurin *et al.* 2004; Gilbert *et al.* 2008). The mechanism used by most invasive ant species is to displace competing native species (Holway *et al.* 2002). *Linepithema humile* co-occurred with fewer species than the two native dominant ant species *A. custodiens* and *P. capensis*. Although there is some variation in the C:N of *A. custodiens* across season, and less so for *P. capensis* (Figure 4b), it appears that there is some resource partitioning among Fynbos native ants. *Linepithema humile* presence potentially changes resource partitioning and co-existence patterns among native ants and in so doing alters the foraging behaviour of co-existing native ant species. Fynbos native ants may be reliant on resource and niche partitioning to overcome the effects of competition for shared resources. The variation in available resources, particularly seeds and floral nectar, may be large in this environment due to the rich floral diversity of the Fynbos (Cowling *et al.* 1996). Species that can alter their foraging strategies in response to the changing availability in resources over the season need not compete with *L. humile* for shared resources and thus are able to persist with them. This might be a strategy applied by *M. peringueyi*, *T. qaudrispinosum* and *Monomorium sp.8*. Moreover, the aggressive nature of *L. humile* (Markin 1970; Holway *et al.* 2002; Chapter 2), coupled with efficient competitive foraging strategies contribute to its ability to displace Fynbos ant species which are not competitively strong in comparison to *L. humile* (Chapter 2). The consequences of these negative impacts on native ant species are substantial in the view of the important role indigenous ant species play in the Fynbos.

This study provides information on the foraging ecology of the poorly studied native Fynbos ants. Our results showed that *L. humile* has negative effects on the nutritional and foraging ecology of certain native ant species and is probably excluding a number of native ant species due to high resource competition. *Linepithema humile* is flexible in its dietary requirements and can readily switch its requirements in response to resource fluctuations. We found that *L. humile* altered the diets of the native ants that commonly co-exist with it, which may be a strategy of these ants that allows them to persist in invaded environments by avoiding competition with *L. humile*. Some seasonal variation in resource use was evident among some native ant species but not to the same extent as *L. humile*. The ability of *L. humile* to successfully establish in uninvaded Fynbos will likely be fuelled by this ant's ability to exploit available resources better than native ant species, as well as the ability to exclude native Fynbos ants from resources through competition.

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CHAPTER 6 - Perspective

The role of resource availability and biotic interactions in facilitating the invasion success of invasive ant species in natural communities

Studies on ant ecology have focused largely on competition and how it structures ant communities (Andersen 1992, Majer 1993, Andersen 1995, Davidson 1998; Gibb & Hotchuli 2004). Competition between ant species within a community arises due to the similar niche requirements between ant species in terms of resources, nesting space and foraging activity patterns (Davidson 1998). Consequently, competitive hierarchies arise through both direct and indirect competition between ant species (Andersen 1992). Due to this competitive hierarchy, ecologically dominant species structure ant assemblages and dictate the activity patterns of subordinate species through territorial behaviour (Hoffmann & Andersen 2003), since these ants are typically behaviourally and numerically dominant (Davidson 1998; Walters & Mackay 2005; Gibb & Cunningham 2009). Moreover, this competitive hierarchy has cascading effects on ant assemblages and promotes co-existence patterns among species through the partitioning of resources (Mooney & Cleland 2001). The co-existence between ant species within a community results mainly from resource and niche partitioning between those species (Morrison 1996; Gibb & Hotchuli 2004: Le Brun & Feener 2007), even when competition is not considered a main factor (Majer 1993), because ant species often have different biological, ecological and physiological requirements regarding resources present in the environment (Schmitt & Holbrook 2003; Abrams &Wilson 2004; Le Brun & Feener 2007). In addition, species with overlapping resource and niche requirements can co-exist in ecological communities if those resources vary over space and time (Tillman 1994; Davis *et al.* 2000; Schmitt & Holbrook 2003). Thus ant communities are often complex communities which are structured largely through dominance interactions (Andersen & Patel 1994), with species diversity thought to be one important factor in limiting community invasibility (Elton 1958).

Invasive ant species are associated with the loss of native ant biota and disruption of ecological processes where they are introduced (Bond & Slingsby 1994; Carpintero *et al.* 2000; Sanders *et al.* 2003). These ant species are typically competitive and become successful in recipient communities where they dominate native ants through competitive exclusion from resources (Porter & Savignano 1990, Holway 1998, Hoffmann *et al.* 1999, Morrison 2000; Holway *et al.* 2002). Two central questions in current studies of invasive ant species which largely focus on the interactions between the invader and the recipient community: (i) how does the recipient environment affect the extent to which an introduced species can successfully invade and (ii) what factors determine the impact of an invader on the recipient

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community. Most studies investigating these interactions are mainly observational (Holway 1999, Human & Gordon 1999) with few empirical studies that examine competition between native ant species and exotic ants introduced into these resident communities (Morrison 2000; Walters & Mackay 2005; Walters 2006). The results of these observational studies make inferences that the ability of invasive ants to dominate native ants at resources leads to the population level changes in the ant assemblage and ecological function in those invaded communities (Andersen & Patel 1994; Thomas & Holway 2005; Luruli 2007; Vorster 2011). To date, very few experimental studies have examined interference competition and resource exploitation between native and invasive ant species to causally show efficiency in food retrieval and changes in population dynamics (mortality rates) (see Buczkowski & Bennett 2008; Blight *et al.* 2010; Vonshak *et al.* 2012). This is often difficult to explore under field conditions where native ants have already been displaced by invasive ants and thus the opportunity to study interspecific interactions in existing ant communities is limited and one is often forced to conduct laboratory-based experiments (see Gibb & Hotchuli 2004). This can be problematic on the one hand, since laboratory environments are manipulated and may not always reflect interference competition accurately, particularly for resources that are not naturally occurring (see Kay 2002). On the other hand though, abiotic determinants are controlled within a laboratory environment and remove any bias that environmental fluctuations might play.

The Argentine ant, *Linepithema humile*, is one of the most well studied invasive ant species and introduced into South Africa in the late 1800's (Skaife 1955, Prins *et al.* 1990), yet even with its potential negative effects on the CFR, only a mere 2-3% of all published work (Web of Science) on this ant originates from South African based studies. Even less is known about the indigenous ants to the CFR highlighting the paucity in our knowledge of resident ant community structure and the threat of *L. humile* on our native ants and ultimately the CFR biodiversity hotspot. We studied the biotic interactions between *L. humile* and native Fynbos ants, as well as *Pheidole megacephala* which are found along the eastern escarpment of the country. The mutually exclusive distributions of the native Fynbos ants *Anoplolepis custodiens*, *Lepisiota capensis*, *Pheidole capensis* and *Tetramorium quadrispinosum*, as well as the African big headed ant, *Pheidole megacephala*, have largely been inferred from bait and pitfall trap surveys. We investigated resource exploitation and interspecific interactions between resident ants and *L. humile* to ascertain whether these resident ants were able to pose any biotic resistance to the spread of *L. humile*. We controlled for propagule size and standardised worker numbers in all nests/colonies reducing any numeric advantage, a benefit for unicolonial invasive social ants that usually allows them to overwhelm native ant species when they compete at shared resources. We feel therefore that all ant species tested had an equal footing. The Fynbos native ant species were not competitive towards *L. humile*, with *L. humile* recruiting far more workers to shared resources and interfering with the recruitment intensities of the native Fynbos ant *P. capensis* through high levels of aggression. Similarly,

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two other native ants were also similarly affected, however, *T quadrispinosum*, which commonly occurs with *L. humile* (Luruli 2007; Vorster 2011; see chapter 4 and 5) was not lethally affected by *L. humile* during interactions at a shared resource. *Tetramorium quadrispinosum* employs two defence mechanisms when interacting with *L. humile*, the submissive behaviour of thanatosis and also sealing their nests when threatened (Witt & Giliomee 1999; Witt & Giliomee 2004). These laboratory results if extrapolated to the field show that the superior competitive ability of *L. humile* may be contributing to the successful establishment and spread of this ant in the Fynbos. On the contrary, *L. humile* was unable to compete successfully against *P. megacephala*, with poor recruitment intensity and suffered high mortality rates during interactions at a shared resource. These results suggest *P. megacephala* may be offering some biotic resistance to *L. humil*e and may potentially be preventing its spread into areas where *P. megacephala* currently dominates.

The interactions between *L. humile* and *P. megacephala* are of great interest. Both these ant species are serious invaders with significant negative impacts on native biota (Hoffmann *et al.* 1999; Heterick *et al.* 2000; Krushelnycky *et al.* 2005; Lach 2005; Dejean *et al.* 2008; Fournier *et al.* 2009; Wetterer 2012), especially on tropical islands (Cole *et al.* 1992; Krushelnycky *et al.* 2005; Kirschenbaum & Grace 2008). There is a lack of consensus on the origins of P. *megacephala* with recent studies suggesting a Malagasy origin (Fournier *et al.* 2012; Wetterer 2012) while previous studies suggested a central African origin (Dejean *et al.* 2005; Fournier *et al.* 2009). This debate aside, *P. megacephala* is considered native to South Africa with preliminary aggression bioassays done during our sampling effort supporting a multicolonial colony structure which fits the pattern of a native species and not that of an invasive ant species which typically form supercolonies (Holway *et al.* 2002; Abbott *et al.* 2007; Sunamura *et al.* 2009). On the other hand, their numeric dominance across the landscape is more typical of a successful invasive species but could also be indicative of a highly competitive native species. Thus further behavioural studies on *Pheidole megacephala* are necessary, as well as phylogenetic and cuticular hydrocarbon chemical studies to elucidate population, as well as colony structure of this species. An integrative approach is necessary to fully understand the structure of the *Pheidole sp*. group in South Africa.

There is a strong link between resource availability and supply in recipient communities and the successful biological invasions by both plants and animals (Davis *et al.* 2000; Beggs 2001). The ability to utilise available resources within the recipient environment is critical to the successful establishment of propagules of introduced species (Helms *et al.* 2011; Wilder *et al.* 2011). Recipient environments with high species richness and low resource availability due to the uptake from resident species have low invasibility (Davis *et al*. 2000), compared to those with high resource availability and low uptake from resident species. Introduced species can gain a foothold through the uptake of unused resources within an

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environment or by strongly competing for resources with resident species (Dukes & Mooney 1999). In ant communities, carbohydrate resources are thought to be limiting resources that are essential for colony growth and survival (Shik & Silverman 2012; Wilder *et al.* 2013). Thus, ants strongly compete for these resources (Hölldobler & Wilson 1990). Moreover, a strong relationship between carbohydrate availability and behavioural dominance in ants exists (Davidson *et al.* 2003; Gibb & Cunningham 2009), with carbohydrate supply fuelling aggressive behaviour and foraging activity (Grover *et al.* 2007). The reliance of invasive ant species on carbohydrate resources is well-established (Abbott 2006; Rowles & Silverman 2009; Tillberg *et al.* 2007; Wilder *et al.* 2011, Wilder *et al.* 2013) and the availability of this resource can be directly linked to high abundances within the areas where these species have invaded (Kaplan & Eubanks 2005). However, many of these studies are based on honeydew from hemiptera in agricultural systems (Helms & Vinson 2008), and few are based on naturally occurring floral nectar (Lach 2013).

Floral nectar is an abundant carbohydrate resource due to the large number of flowering plants (Lach 2013), yet it is a fluctuating resource due to its seasonality (Gibb & Cunningham 2009). This makes the Fynbos biome (about 7500 plant species are found in this biome (http://www.ekapa.ioisa.org.za/biomes/Fynbos.htm) an ideal system to investigate utilisation of floral nectar as a carbohydrate resource by ants. With this high floral species diversity and high resource availability together with the low biotic resistance from resident ants, the invasibilty of this biome increases, which threatens plant germination through displacement of the native ant seed dispersers by *L. humile* (Bond & Slingsby 1984; Christian 2001). In an attempt to gain some understanding on whether invasive species potentially alter the diets of native ant species, we used stable-isotope analysis to trace the flow of nutrients in various ant species occurring within the Fynbos. *L. humile* switches diets/ trophic position in introduced environments, enabling it to survive in the recipient community by utilising a wide range of resources (Tillberg *et al.* 2007). This phenotypic plasticity is thought to be critical to its invasion success (Wilder *et al.* 2013). Floral nectar contributes in potentially facilitating the spread of *L. humile* into more pristine Fynbos environments since *L. humile* effectively exploited Fynbos floral resources, showed diet flexibility and also altered the diets of some native ant species. This suggests that *L. humile* not only changes ant species assemblages in this region, but also responds more efficiently to fluctuating resources provided by floral nectar than native Fynbos ants. It is surprising that the native Fynbos ants are not utilising this resource as efficiently as *L. humile* (Chapter 4 and 5). Effective use of this carbohydrate resource may increase *L. humile* colony growth (see Grover *et al.* 2007; Wilder *et al.* 2011 and Shik & Silverman 2012) and also facilitate its ability to spread into more pristine areas. *Linepithema humile* does not solely feed on carbohydrates but supplements its diets with protein resources likely from predation and scavenging on native arthropods. In terms of community invasibility, the Fynbos has increased vulnerability to *L. humile* due to climatic suitability and the lack of competition from native ant species.

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Linepithema humile actively displaces native ant species which are unable to compete with it (see Chapter 2; Luruli 2007; Vorster 2011).

Facilitation is another factor that can contribute to invasion success of introduced species (Shea & Chesson 2002). Invasive species can form food for protection mutualisms with honeydew producing insects, including invasive ones, which can speed up the invasion process due to the persistent resource supply (Helms & Vinson 2008; Helms *et al.* 2011; Shik & Silverman 2012). *Linepithema humile* has previously been found to associate with a treehopper species that occurs on *Protea nitida* bushes in the CFR (see Lach 2007). The combination of both floral nectar and honeydew can facilitate *L. humile* invasion success and exacerbate its negative impacts. We found that *L. humile* continued to utilise carbohydrate resources even after the flowering period, either due to mutualisms with honeydew producing insects, such as treehopper species or nectar thieving from Ericaceae species that may be flowering at different times. Honeydew would be a more stable, predictable and persistent resource while floral nectar a fluctuating resource (see Gibb & Cunningham 2009), with *L. humile* utilising both resources as they become available (Gibb & Cunningham 2009), especially since competition for these resources is low (see chapter 4 and 5, Lach 2013). However, it appears that both these resources are unpredictable in the CFR due to the reliance of Fynbos plants on fire for germination (de Kock *et al.* 1992; Cowling *et al.* 1996). We did not find the treehopper species studied by Lach (2007) and attributed this to the major fire that occurred in 2009 prior to our sampling effort in 2011 and 2012. After this fire, Vorster (2011) found that *L. humile* were very slow in re-establishing into burnt Fynbos areas while native ant species re-colonised within one week. Based on these observations, fires may limit the invasion of *L. humile* and play an important role in controlling the population dynamics of *L. humile*, and consequently be a potential mechanism that prevents the establishment and spread of *L. humile* into pristine Fynbos environments. Furthermore, the current invasion front within the Jonkershoek Nature Reserve stops in an area where there is a marked decline in nectar producing *P. nitida* and tall shrubs which leaves the ground more exposed (Vorster 2011), the soil is drier and since *L. humile* cannot tolerate dry environments (Thomas & Holway 2005; Menke & Holway 2006), its further spread may be limited in these areas. More studies are needed to understand the role of fire in regulating invasion success of introduced species, especially in the CFR where the vegetation is dependent on fires. Thus the role of abiotic factors cannot be dismissed and must be taken together with the findings of this study. A way forward is linking the incidence of fire in an ecosystem and the ecological effects of fire on the expansion of *L. humile* across the landscape.

In conclusion, carbohydrate resources available within the Fynbos, coupled with the lack of competition from native ant species are facilitating the spread of *L. humile* in pristine Fynbos

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environments. The negative impacts this ant species has on both above ground foraging ants and floral visitors highlights the threat of *L. humile* to this biodiversity hotspot.

Limitations of the study

This study focused on the mechanisms of how Argentine ants interact with native ants and the factors facilitating their spread into these natural communities. During this research we were made aware of a number of shortcomings. Firstly, the advantages of laboratory bioassays with their controlled environments have a concomitant disadvantage in that the outcomes cannot always be indicative of the field situation. Thus, a parallel field study would strengthen and support the laboratory results and confirm the importance of the abiotic factors which were largely not included in these studies. Secondly, there is uncertainty in the origins of *Pheidole megacephala* and this leads to confusion in the endemism of this species. Therefore, further studies on the aggression patterns and nest networks of this ant along the eastern escarpment of South Africa needs elucidation to clarify whether this ant is endemic or invasive to this country. Thirdly, to substantiate the exclusion of *L. humile* by *P. megacephala*, a contact zone of these two species needs to be established and their competitive ability ascertained in the field. The argument for the possibility of biotic resistance needs to be built up more comprehensively, indicating that the current distribution pattern is not simply due to a sampling gap or unsuitable abiotic conditions but rather that Argentine ants have not been able to invade, given the opportunity to invade and that *P. megacephala* covers the areas that Argentine ants have not invaded, but do not occur where Argentine ants do occur. Further, another possibility is that *L.humile* has excluded *P. megacephala* from area previously occupied by the Big headed ant and thus attaining historical data on distribution patterns of *Pheidole megacephala* within the Western Cape will begin shedding light on the historical distribution pattern of this ant species. Fourthly, one important limitation is the replication of experimental sites within natural reserves. This is unfortunately as a result of the fires that occurred in the reserves in and around Stellenbosch in 2009-2010. Having had more time available, sites further afield and more sites with the same plant species would have been located. This aside, we feel that the results presented do give an indication of how native ants and Argentine ants utilize the abundant floral resources within the Cape Floristic Region. Linking patterns of ant abundance and resource use enabled us to determine the role of carbohydrate resource availability in facilitating Argentine ant success. Fifthly, foraging rate was correlated to nectar abundance only and not to other variables, such as temperature and humidity and these parameters, together with carbohydrate availability, may have affected the rates at which ants forage. Lastly, the comparison of resource use within different communities, and thus different vegetations, did not include all possible communities which meant that direct comparisons between invaded Pine forests and uninvaded Pine forests were not possible. This was because all Pine forests in

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Nature Reserves within the Western Cape have Argentine ants present since they were introduced with the saplings (Richardson *et al*. 1983).

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