

**Mutualistic association between Num-num (*Carissa bispinosa*) and
mounds of Snouted harvester termites (*Trinervitermes trinervoides*) in a
semi-arid savanna.**

by

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A dissertation submitted in fulfilment of the requirements in respect of the degree

Master of Science

in the

Department of Zoology and Entomology

Faculty of Natural and Agricultural Sciences

at the

University of the Free State

Bloemfontein

January 2019

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DECLARATION

I, Gosego Nampa declare that the Master's research dissertation that I herewith submit at the University of the Free State, is my independent work and that I have not previously submitted it for qualification at another institution of higher education.



(Signature of candidate)

January 2019

Date

At Kuruman, South Africa

ACKNOWLEDGEMENTS

I'd like to thank my supervisor, Dr Mduduzi Ndlovu, who gave me guidance and support throughout my MSc study. I highly appreciate all that you have taught me.

Thanks to the late Prof. Schalk Louw for all comments and suggestions during proposal writing. Many thanks to Dr Antón Pérez Rodríguez who helped me with GAMM statistics and for useful suggestions given in Chapter 3. I'd like to thank Maliki Wardjomto who helped me with collection of soil samples. I am also grateful for the tireless logistical support the project received from Burton Maasdorp.

Thank you to management at Nylsvley nature reserve for their fieldwork logistical support. The Limpopo Provincial Government issued the required permit for me to perform fieldwork at the reserve.

Funding for this project came from the University of the Free State (UFS) and the National Research Foundation. I was awarded the UFS scholarship for my studies in 2017 and the DST-NRF Innovative Master's Scholarship (Award No: 112278) in 2018. Fieldwork running costs came from the NRF incentive funding for rated researchers awarded to my supervisor Dr Ndlovu.

My gratitude goes to my family whose encouragement, financial and moral support kept me going.

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GENERAL ABSTRACT

1
2 In many ecosystems, one individual, or species, may often alter the environmental conditions
3 in such a way that a stressful habitat becomes more hospitable for other individuals. Mutualism
4 is a relationship between two organisms of either the same species or different species that
5 enhances their survival or growth. Mutualism drives selection for traits through evolution,
6 leading to diversity.. However, very few of these mutual associations have been documented
7 in the semi-arid savanna region. Due to their close association yet no report of mutualism, I
8 studied whether there was a mutualistic association between *Carissa bispinosa*, a fast-growing
9 medium sized evergreen shrub, and *Trinervitermes trinervoides*, a mostly nocturnal termite
10 species, at Nylsvley Nature Reserve, Limpopo province. There was a significant benefit
11 accrued to both species from the plant-insect association. Plants on mounds were larger,
12 greener and fruited more in the dry season compared to stand-alone plants. Mounds under
13 shrubs were significantly less damaged compared to exposed mounds. Certain soil macro- and
14 micronutrients that contribute to plant growth and health were enriched in mounds relative to
15 the matrix. Overall, internal temperature changes were fairly constant in active mounds during
16 the 24-hour period in both seasons, while temperature changes in inactive mounds varied more.
17 Activity and season (including their interactions), were important in determining the internal
18 mound temperature profiles. Shade and mound size did not have any significant effect in
19 determining the internal temperatures profiles of mounds. To my best knowledge, my study
20 has revealed a previously undocumented survival mechanism that this species of termites uses
21 to escape predation in semi-arid savannas.

22

23 *Keywords:* Animal-plant association, Mounds, Mutualism, Nylsvley Nature Reserve,
24 Thermoregulation

25

CHAPTER ONE

26

27 **Introduction**

28 Mutualism is one of the key drivers of biodiversity evolution in many terrestrial ecosystems
29 and in interspecific co-evolution processes (Boucher 1988, Bronstein 2015). Mutualism is a
30 type of symbiosis that describes a relationship between two organisms, where both benefit in
31 some way from this association (Trefil 2001). The relationship can either be intra- or
32 interspecific (Suweis 2013, Bronstein 2015). Mutualistic relationships are also responsible for
33 the success of productivity in several savanna ecosystems (Loreau *et al.* 2002). Approximately
34 half of terrestrial plants rely on mycorrhizal relationships with fungi to provide them with
35 inorganic compounds and trace elements as nutrients (Johnson *et al.* 1997, Van Der Heijden
36 2008). However, mutualism has received little attention compared to other ecological
37 interactions such as predation and parasitism (Bronstein 1994, Begon *et al.* 1996).

38 Determining the exact fitness benefit to individuals in a mutualistic relationship is
39 challenging, particularly when the individuals receive benefits from several other sources
40 (Leung and Poulin 2008). Therefore, most mutualistic relationships are usually determined
41 according to the closeness of the association, which can either be facultative or obligate
42 (Ollerton 2006). The concept of "closeness" can also refer to mutual dependency, meaning the
43 species cannot live without one another, or the biological intimacy of the relationship in relation
44 to physical closeness (Leung and Poulin 2008).

45 In most mutualistic associations an organism provides a benefit to another and in turn
46 that organism also derives benefits from the processes of the species it serves (Boucher 1988,
47 Bronstein 1994, Ollerton 2006). Strict service-service mutual associations are rare in nature
48 and rarely investigated in a terrestrial savanna setting. A well-documented service-service
49 interaction in a terrestrial setting is that of the relationship between *Pseudomyrmex* ants
50 (*Pseudomyrmex ferruginea*) and Acacia trees (Janzen 1966, Eubanks *et al.* 1997, González-

51 Teuber *et al.* 2014). Through obligate interactions, specialised ants inhabit myrmecophytes
52 during major parts of their life span and the ants are entirely dependent on the food and nesting
53 space provided by the host Acacia tree. These ants, in return, defend their host efficiently and
54 aggressively against herbivores, encroaching vegetation (Heil and McKey 2003) and
55 phytopathogens (González-Teuber and Heil 2010).

56 Symbiotic relationships also play an essential role in termite evolution and involve a
57 range of intestinal microorganisms, including protists, archaea, and bacteria (Bignell 2000).
58 Macrotermitinae is, however, the only Termitidae subfamily that has evolved a mutualistic
59 ectosymbiosis with fungi of the genus *Termitomyces*. The ecto-symbiosis with fungi helps the
60 termites to break down the fibrous plant-derived material (Mueller 2002).

61

62 **Termites and plants**

63 In many ecosystems, an individual, or species, may alter the environmental conditions in such
64 a way that a stressful habitat becomes more hospitable for other individuals (Stachowicz 2001).
65 Soil fertility is generally low in some savannas, but may show marked small-scale variations
66 (Jones *et al.* 2013). Dead leaves and other tree litter drop to the soil surface near the tree, where
67 they decompose and release nutrients. A large proportion of dead organic matter
68 (approximately 30 %) is decomposed through the feeding activities of termites (Badertscher *et*
69 *al.* 1983). Thus, a significant proportion of released mineral nutrients may be stored for long
70 periods in termite mounds where they are not readily available to plants (Rafferty 2010).
71 During mound construction, termites translocate large amounts of soils from various depths of
72 the soil profile to the surface (Jouquet *et al.* 2011, Joseph *et al.* 2014). Over time, termites
73 redistribute resources in the ecosystem. Mounds provide small, high-nutrient patches that
74 influence the diversity and productivity of ecosystems (Joseph *et al.* 2014). Hence termites are
75 largely referred to as ecosystem engineers.

76 A study done by Fleming and Loveridge (2002) shows that termite mounds have a
77 higher pH, moisture, organic matter and mineral (such as carbon, nitrogen, calcium,
78 magnesium, potassium and phosphorus) content. Additionally, termites use saliva and
79 excretion in mound construction which causes a lower C:N ratio, and in turn promote plant
80 growth (Laker *et al.* 1982). Ackerman *et al.* (2007) found termite mounds to be nutrient-rich
81 microsites for seedling establishment in the Venezuelan Amazonia, therefore, plant growth is
82 relatively vigorous and diversity is high in the vicinity of termite mounds, compared to the
83 surrounding matrix (Jouquet *et al.* 2011, Davies *et al.* 2014, Joseph *et al.* 2014). Although
84 studies report that termite mounds have fertile soils for plant growth, anecdotal reports suggest
85 that plants growth on active mounds is rarely viable and mostly absent (Glover *et al.* 1964, Lee
86 and Wood 1971, Gillman *et al.* 1972, Pomeroy 1983). The absence of plants growing on active
87 mounds may be partly because of foraging behaviour of termites, given that vegetation growth
88 may damage the structural integrity of a mound (Rogers *et al.* 1999).

89 Vegetation, including shrubs of the genus *Carissa* have been reported to grow on
90 termite mounds in southern Africa (Sileshi *et al.* 2010, Spinage 2012). Although no research
91 has been done on how they establish on the mounds, the edible fruits of *Carissa* have been
92 reported to be eaten by birds (Mishra 2005, Yilangai *et al.* 2014), which may be the carriers of
93 the seed to the termite mounds. In southern Africa, plants with edible fruits favoured by birds
94 are common and widespread, even in dry areas (Milewski 1982). Once a seed is deposited on
95 or near the moisture- and nutrient-rich microsites created by termites, germination will be
96 favoured (Crawley 2009, Browdy *et al.* 2010, Joseph *et al.* 2014).

97

98 **Study site**

99 This study was conducted at a semi-arid savanna area in Nylsvley nature reserve in Limpopo
100 province. Nylsvley nature reserve (24° 39' 17.28" S, 28° 41' 27.6" E) is a 3120 ha protected

101 area, lying on the seasonally inundated floodplain of the Nyl river (Scholes and Walker 2004).
102 The area lies at the intersection of three different geological formations, leading to five distinct
103 soil groups (Scholes and Walker 2004). Seven discrete plant communities occupy the area
104 (Scholes & Walker 2004, Mistry and Beradi 2014). *Acacia spp*, *Burkea africana*, *Carissa*
105 *bispinosa* and *Commiphora spp*. trees dominate the surrounding tree vegetation. *Eragostea*
106 and *Panicum spp*. were more common in the grass layer. The reserve has a variety of native
107 herbivores and burrowing animals. There are also several termite mounds of *Macrotermes spp*.
108 and *Trinervitermes trinervoides* scattered throughout the reserve. The area is characterised by
109 a hot summer rainfall period (mean 28.4 °C and 584 mm from October to March) and cool dry
110 winters (mean 22.3 °C and 43 mm from April to September months) (Werner 2009).

111

112 **Study species**

113 *Carissa bispinosa*

114 The common num-num (*Carissa bispinosa*) is a fast-growing medium sized evergreen shrub
115 that can grow to heights of about five metres (Schmidt 2002). This multi-stemmed shrub has
116 prominent, sharp, green, y-shaped spines that stand out above the glossy leaves (Cooper and
117 Owen-Smith 1986, Grant and Thomas 2011). It bears deep red small conspicuous fruit berries
118 that grow in small clusters amongst the leaf rosettes. *C. bispinosa* is found in most frost free
119 and woodland areas of South Africa, Lesotho, Swaziland, Mozambique, and Zimbabwe
120 extending westwards to Botswana, Namibia and sporadically further north in Zambia, Tanzania
121 and Kenya (Schmidt 2002). The thorns and leaves of num-num show marked morphological
122 variation throughout its distribution range (Walisch *et al.* 2015).

123

124 *Trinervitermes trinervoides*

125 Snouted harvester termite (*Trinervitermes trinervoides*), recognised by a snout on the head, is

126 the only species of the family Termitidae, genus *Trinervitermes* found in the subtropical region
127 of South Africa (Richardson 1987, Adam 1993). It predominantly inhabits grasslands and
128 builds compact dome shaped mounds (Meyer 1997, Field 2012). Each mound houses a single
129 colony which consists of different castes that contribute in different ways towards the growth
130 and protection of the nest (Field 2012). Castes are organised according to their different tasks,
131 namely: workers, soldiers and those responsible for reproduction (Noirot 1985, Singer 1998).
132 The termites are nocturnal and emerge from small holes in the soil surface at distances of up to
133 20 m from the mounds and form dense foraging parties consisting of workers and the soldiers
134 that protect the workers by lining the foraging path and facing outwards (Richardson 1987).
135 Like most other termites, snouted harvester termites are preyed on by animals such as Aardvark
136 (*Orycteropus afer*), Aardwolf (*Proteles cristata*) and Pangolin (*Smutsia temminckii*). Aardvark
137 and Pangolin are known to break into the mounds and feed on colonies inside the termitarium
138 (Feldhamer *et al.* 2007). Most of these potential termite predator species are mainly nocturnal
139 and found in semi-arid savanna areas such as Nylsvley Nature Reserve (Skinner and Chimimba
140 2005, Kingdon 2015).

141

142 **Aim and objectives**

143 The aim of the study was to determine whether there is a mutualistic association between num-
144 num plants (*Carissa bispinosa*) and mounds of the Snouted harvester termite (*Trinervitermes*
145 *trinervoides*) in a semi-arid savanna. I attempted to understand whether the relationship
146 between *C. bispinosa* and the *T. trinervoides* mounds (1) benefits both species (mutualistic in
147 nature), or (2) only benefits one entity, and (3) determine to what extent the relationship
148 benefits the species involved. The study tested the hypothesised that termite mounds provide
149 favourable soil conditions for vegetation in the savanna areas due to nutrient leaching and water
150 infiltration and as result num-num plants have started growing on termite mounds. In return,

151 the thorns and “cagey” shrubs of num-num trees provide termite mounds with protection from
152 predation.

153 The first objective was to determine the frequency of association between the termite
154 mounds and the num-num plants. The frequency of num-num plants with termite mounds
155 occurrence will determine whether the association is random or indicative of a mutualistic
156 trend. I predict that a random occurrence should show no significant difference in association.
157 The second objective was to compare the level of predation between singular occurring termite
158 mounds and mounds covered by num-num plants. If there is a protection benefit for the termites
159 in the association, then there should be less predation in such cases, compared to those that
160 occur alone. Thirdly, I compared the size (tree height and canopy diameter) of num-num plants
161 found on termite mounds with those that stand alone during the wet and dry seasons. The
162 seasonal variations influence environmental factors such as temperature, water availability and
163 radiation energy, which consequently affect plant growth. Therefore, it is predicted that plant
164 size, occurrence and even growth rate will decrease as one moves further away from a drainage
165 line, since there will be less water and nutrient availability, as stipulated by the “soil catina”
166 hypothesis (Young 1972). As such, the drainage line was taken into consideration to determine
167 how termite mounds will affect the growth and health of the plants that are further away from
168 water. Termite mounds have a relatively higher nutrient and moisture availability compared to
169 the surrounding landscape (Smith and Yeaton 1998) and therefore num-num plants that
170 associate with mounds should have better growth, size and appearance (i.e. leaf colour) all year
171 round, compared to those that “stand alone”. The fourth objective was to access the
172 thermoregulatory differences in mounds covered by num-num plants and those in direct
173 sunlight. This objective was tested during the wet summer and dry winter seasons. It was
174 predicted that the shading effect of num-num plants coupled with the activity of the mound
175 (active vs inactive) will influence the internal mound temperature fluctuation. Mounds that

176 were inhabited by termites were categorised as “active”, and those uninhabited as “inactive”.
177 Therefore, I hypothesized that active mounds under num-num cover will display minimum
178 internal temperature fluctuations, meaning the association of mounds with num-num plants
179 also has a thermoregulatory advantage.

180

181

182 **Thesis outline**

183 In the second chapter, I initially determined the frequency of num-num and mound association,
184 and further investigated the benefits of association on plant growth (i.e. canopy diameter, plant
185 height and fruiting), and mound state (activity, diameter, height and levels of predation). The
186 third chapter focused on daily and seasonal thermoregulation of mounds under num-nums and
187 those in the open. General Additive Mixed Models were used to assess internal mound
188 temperatures in relation to prevailing ambient and shade temperatures. In the closing chapter,
189 I synthesise the findings of the study and suggest direction for future research areas. The second
190 and the third chapters were written as stand-alone manuscripts (i.e. introduction, methods,
191 results, discussion and references) to ease the process of journal publication. Therefore, some
192 level of repetition is expected especially in the methods and some parts of the introduction
193 between these two chapters.

194

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CHAPTER TWO

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Association benefits between Snouted Harvester termites (*Trinervitermes trinervoides*) and Num-num plants (*Carissa bispinosa*) in a semi-arid savanna setting.

Abstract

Diversity in terrestrial ecosystems is mostly driven by mutualistic relationships. However, very few mutualistic plant-insect associations have been documented in semi-arid savannas of Africa. The reciprocal benefits that termites receive from their association with other species in the ecosystem remain poorly studied. I studied the seasonal level of association between *Carissa bispinosa* (thorny shrub), and *Trinervitermes trinervoides*, termites in Nylsvley nature reserve, South Africa. The objective was to determine the type of association between the two species and possibly to quantify the benefits accruing to one or both species. I hypothesised that termite mounds provide nutrients for plants, hence trees establish better on mounds and, in return, *C. bispinosa* plants protect the mounds from predation. I measured plants (height, canopy diameter, leaf appearance and fruiting) and mounds (height, diameter, damage and activity) and also evaluated soil nutrient properties from mounds with active colonies and the adjacent matrix. There was a significant benefit accrued to both species from the plant-insect association. *C. bispinosa* plants on mounds were larger (~ 33% taller), greener and fruited more in the dry season compared to matrix plants. Mounds under shrubs were significantly less damaged compared to exposed mounds. Sodium, magnesium, potassium, sulphur and copper were enriched in mounds relative to the matrix. Which further validates the high productivity of plants on mounds. The study unravelled a plant-insect association and an anti-predation defence strategy that termites use in semi-arid environments.

366 *Keywords:* Mounds, Mutualism, Plant-insect interactions, Predation, Soil nutrients, South
367 Africa.

368

369 **Introduction**

370 Ecosystem engineers enhance the availability of resources to other organisms by directly or
371 indirectly changing the physical state of an ecosystem (Jones *et al.* 1997). Termites (Infracorder
372 Isoptera) are key ecosystem engineers in several semi-arid savanna ecosystems because of their
373 role in nutrient cycling (Holt and Lepage 2000, Jouquet *et al.* 2011). The mound-building
374 activities of termites translocate soil and nutrients from various underground depths (Lee and
375 Wood 1971), bringing fine soil particles with a different proportion of clay mineral
376 composition to the surface. Studies by Fleming and Loveridge (2002) and López-Hernández
377 (2001) show that termite mounds have a higher pH, moisture, organic matter and mineral (such
378 as carbon, nitrogen, calcium, magnesium, potassium and phosphorus) content than the
379 surrounding matrix. Therefore, given the wide distribution of termites in savanna landscapes,
380 these modifications improve the functioning of the ecosystem at various spatiotemporal scales
381 (i.e. Smith and Yeaton 1998, Jouquet *et al.* 2006).

382 Termite mounds form high moisture patches in dry savannas , creating a more
383 hospitable habitat for other soil microorganisms (Jouquet *et al.* 2006) and also attract plant
384 growth near or on top of inactive mounds (Sileshi *et al.* 2010, Davies *et al.* 2014). Tree canopies
385 (width and height) of plants growing on mounds are relatively larger in comparison to the
386 surrounding matrix (van der Plas *et al.* 2013, Davies *et al.* 2015). Furthermore, the plant
387 communities in association with a mound is reported to be more diverse (Moe *et al.* 2009,
388 Davies *et al.* 2015), with a higher proportion of evergreen species (van der Plas *et al.* 2013)
389 and are generally preferred by ungulates and birds for food and habitat (Mobæk *et al.* 2005,

390 Joseph *et al.* 2014). These plants on mounds sustain a diverse animal abundance in semi-arid
391 savannas.

392 Beyond their ecosystem engineering role, termites are also an important protein-rich food
393 source for terrestrial vertebrates such as birds (Abe *et al.* 2000, van Huis 2017) and mammals
394 (Cooper and Skinner 1979, Richardson and Levitan 1994). Animals such as armadillo
395 (*Orycteropus afer*), aardwolf (*Proteles cristata*) and pangolin (*Smutsia temminckii*) also
396 consume large quantities of termites on daily basis and their extraction methods usually result
397 in significant mound damage and even a total destruction of a termite colony (Sheppe 1970).
398 The mound protects the nest from both predation and environmental fluctuations (Korb 2010)
399 and, as such, damage to the mound can have detrimental effects. Given the predation risk,
400 termites should have adapted defence strategies to survive an attack. *Macrotermes sp.* have
401 fairly large ‘mandibulate soldier’ termite castes with serrated mandibles that bite and deter a
402 predator (Stuart 1969). The *Trinervitermes trinervoides* (Sjöstedt) (Termitidae:
403 Nasutitermitinae) termites secrete a chemical that contains a mixture of diterpenes and
404 monoterpenes which to some extent deter predators (Richardson and Levitan 1994). However,
405 it remains largely unknown how these small species, like *T. trinervoides*, avoid predation of
406 their mounds beyond chemical secretion.

407 The benefits of termites as ecosystem engineers to the ecosystem are well documented
408 and studied (e.g. De Bruyn and Conacher 1990, Moe *et al.* 2009, Sileshi 2010). However, the
409 reciprocal benefits that the termites receive from their association with other plant species in
410 the ecosystem remain poorly studied. In this study, I tested the hypothesis that the association
411 between mounds of smaller termite species (also poorly studied) and spiny plants has a mutual
412 benefit to both species involved. My study assessed the benefits of association between the
413 common num-num plants (*Carissa bispinosa*) and the snouted harvester termite
414 (*Trinervitermes trinervoides*) mound in a semi-arid savanna setting. The specific objectives of

415 the study were to understand whether the relationship between *C. bispinosa* and the *T.*
416 *trinervoides* mounds: (1) benefits both species (mutualistic in nature), or (2) if it was only
417 beneficial to one entity, and (3) determine to what extent the relationship benefits the species
418 involved. I further evaluated substrate particle size (clay, silt and sand), macronutrients (Na,
419 Ca, Mg, K, S and P), micronutrients (Cu, Fe, Mn, Zn, B) and pH from ten active mounds
420 compared to the adjacent matrix.

421

422 **Materials and methods**

423 *Study site*

424 This study was conducted in a semi-arid savanna landscape at Nylsvley nature reserve (24° 39'
425 17.28" S, 28° 41' 27.6" E) in Limpopo province. Nylsvley nature reserve is a 3120 ha protected
426 area, lying on the seasonally inundated floodplain of the Nyl river (Scholes and Walker 2004).
427 The area lies at the intersection of three different geological formations, leading to five distinct
428 soil groups (Scholes and Walker 2004). Seven discrete plant communities occupy the area
429 (Scholes and Walker 2004, Mistry and Beradi 2014). *Acacia sp.*, *Burkea africana*, *C. bispinosa*
430 and *Commiphora sp.* trees dominate the surrounding tree vegetation. *Eragosteae* and *Panicaceae*
431 *sp.* are more common in the grass layer. The reserve has a variety of native herbivores and
432 burrowing animals. There are also several termite mounds of *Macrotermes sp.* and *T.*
433 *trinervoides* scattered throughout the reserve. The area is characterised by a hot summer rainfall
434 period (mean 28.4 °C and 584 mm) from October to March, and cool dry winters (mean 22.3
435 °C and 43 mm) from April to September months (Werner 2009).

436

437 *Sampling*

438 Fieldwork was conducted during the wet (April) and dry (June-July) season of 2017. A total of
439 twenty linear transects were placed perpendicular to the river channel. Each transect was

440 approximately 250 m in length and 10 m wide, starting from the river channel extending out to
441 the flood plains and terrestrial habitats. Transects were placed at least 30 m apart and each
442 transect was GPS-marked for repeated monitoring.

443

444 *Num-num plants*

445 The common num-num plant is a fast-growing medium-sized (2 – 5 m in height) thorny shrub
446 that is drought resistant and found in most parts of the southern African region (Coates-
447 Palgrave 2002). All num-num plants within each transect were recorded, noting their
448 occurrence in the intermound matrix (at least 5 m from a mound) and on top of or close to a
449 termite mounds. The height and canopy diameter of the num-num plants were also measured
450 to the nearest cm using a tape measure attached to a 5 m straight pole and recorded. The
451 appearance of the plants (i.e. the colour and nature of the canopy leaves) was noted by scoring
452 on a five-point scale: 1 = shrub and all leaves looked wilted; 2 = branches and most
453 (approximately two thirds) leaves had turned brown and wilted; 3 = approximately two thirds
454 of the leaves looked green and a third were brown and wilted; 4 = more than two-thirds of the
455 leaves and branches were green and few scattered brown leaves persisted; 5 = All leaves and
456 branches were green with no visible sign of brown wilted leaves. Presence of fruits was also
457 noted as present or absent. Only plants with a canopy diameter of at least 200 cm were
458 considered, in order to prevent the inclusion of young none-established plants in the study.
459 Plants that were clustered and could not be accessed and measured individually were also
460 excluded from the study.

461

462 *Termite mounds*

463 Snouted harvester termite (*T. trinervoides*), recognised by a snout on the head, are found in
464 most arid and semi-arid subtropical region of South Africa (Adam *et al.* 2018). This species

465 predominantly inhabits grasslands and builds compact dome shaped mounds (Adam *et al.*
466 2018). The height and diameter of the termite mounds were measured using a measuring tape
467 and only termite mounds higher than 25 cm above the ground were considered in this study.
468 Termite mounds lower than 25 cm were considered too small. The appearance of the mounds
469 was recorded and scored according to the absence or presence/extent of predator damage: 0 =
470 no damage; 1 = some damage but the mound is still intact; and 2 = extreme damage and the
471 mound is exposed. Damage observed was consistent with that of digging by a mammal and
472 portions of the mound were broken off. We checked mounds for evidence of termite activity
473 i.e. looking for termites, entrance holes to internal channels, foraging activity and recent
474 damage repair. Damage repair was noticeable by a roughly textured soil with a darker colour.
475 Mounds that were inhabited by termites were categorised as “active”, and those uninhabited as
476 “inactive”.

477

478 *Soil composition and nutrients*

479 Mound soil samples were collected during the winter season at cardinal directions on the base
480 of each mound (n = 10) using a 10 cm soil core sampling tool. A paired soil sample was
481 collected from the matrix, (10 m away from the mound or any other mound) at cardinal
482 directions from the focus mound. The first one centimetre of the top soil was scrapped off to
483 remove any vegetation matter before the soils were cored out. All four soil samples collected
484 from a single mound were mixed and combined into one sample representative of that mound
485 and the same was done for the four matrix soil samples. Approximately 1.5 kg of soil was
486 collected at the base of the mound and an equal amount was also collected from the matrix.

487 Soil analyses were carried out following the methods detailed in Van Reeuwijk (2002).
488 Soil samples were air dried, sieved to < 2 mm and then bagged in brown paper bags and sent
489 to Bemlab, a soil testing laboratory in Bloemfontein, Free State, South Africa for analyses of

490 primary water extractable macronutrients. Samples were first digested in a nitric acid (HNO₃)
491 and hydrogen peroxide (H₂O₂) mixture. The resulting solutions were analysed for Na (sodium),
492 K (potassium), Ca (calcium) and Mg (magnesium), Zn (zinc), Mn (manganese), Cu (copper),
493 and Fe (iron) using inductively coupled plasma atomic emission spectrometry (Agilent 7500
494 ICP-MS, ChemStation California, US). Phosphorus was extracted using the Bray-1 method
495 (Bray & Kurtz, 1945). Hot water extraction was used for B (Boron), which was then analysed
496 using spectrometry. For ammonium (NH₄) and nitrate (NO₃), 1 M Potassium chloride (KCl)
497 extract was used followed by an analysis using the continuous-flow colorimetry. The tricalcium
498 phosphate Ca₃(PO₄)₂ extract was used for S (Sulphur). A three-fraction particle size analysis
499 was done to determine soil composition (clay, silt and sand).

500

501 *Data analyses*

502 A Kruskal Wallis (non-parametric analyses of variance - ANOVA) test was used to determine
503 any differences in frequency of single and associated occurrence of termite mounds and num-
504 num shrubs. The presence of predation between singular occurring termite mounds and mounds
505 covered by num-num shrubs was compared using the chi-square independence test. Fruiting of
506 plants between stand-alone and on mound plants was also compared using the chi-square
507 independence test. A two-way analysis of variance (ANOVA) was used to compare the size of
508 num-num shrubs found on mounds with those not associated with termite mounds during the
509 wet and dry seasons. Canopy cover appearance scores of stand-alone plants vs plants on
510 mounds were compared using the Mann-Whitney U test. A series of paired *t*-tests with a False
511 Discovery Rate (FDR) correction for multiple comparisons (Benjamini and Hochberg 1995)
512 were also used to compare soil particle size, macronutrients, micronutrients and pH values
513 between ten active mounds and the adjacent matrix. Data were analysed using the statistical
514 package IBM SPSS 25 (IBM Corp. 2017).

515

516 **Results**

517 A total of 116 num-num plants were recorded from all transects, of which 63 were stand-alone
518 plants and 53 were on mounds. There was a total of 92 termite mounds, of which 39 were
519 stand-alone mounds. Mean height (\pm standard deviation) of plants on mounds (mean = 203.26
520 \pm 63.03 cm) was significantly taller ($t = -7.13$, $p > 0.001$) than that of plants in the matrix (mean
521 = 138.44 \pm 32.26 cm; Fig. 1A).

522

523 *Num-num plants*

524 In the wet season there was no difference in mean num-num canopy diameter ($t = -2.37$, $p =$
525 0.25) between stand-alone plants (mean = 316.43 \pm 93.19 cm) and plants on mounds (Fig. 1B).
526 There was also no difference ($U = 3859$, $p = 0.98$) in canopy cover appearance scores between
527 stand-alone plants (median score = 4) and plants on mounds (median score = 5). In contrast,
528 during the winter season, num-nums on mounds (median score = 4) had significantly higher
529 ($U = 2049.5$, $p = 0.02$) canopy cover appearance scores compared to stand-alone plants (median
530 score = 2). There was a significant association of plant fruiting and location ($X^2_{(1)} = 5.80$, $p =$
531 0.03), with most plants on mounds fruiting compared to stand-alone plants. The presence of
532 fruits on num-num was also significantly dependant on season ($X^2_{(1)} = 6.31$, $p = 0.007$). Most
533 plants fruited in the dry winter ($n = 110$, of which 63 were on mounds i.e. 100 %) compared
534 to the wet summer season ($n = 16$, of which 11 were on mounds).

535

536 *Termite mounds*

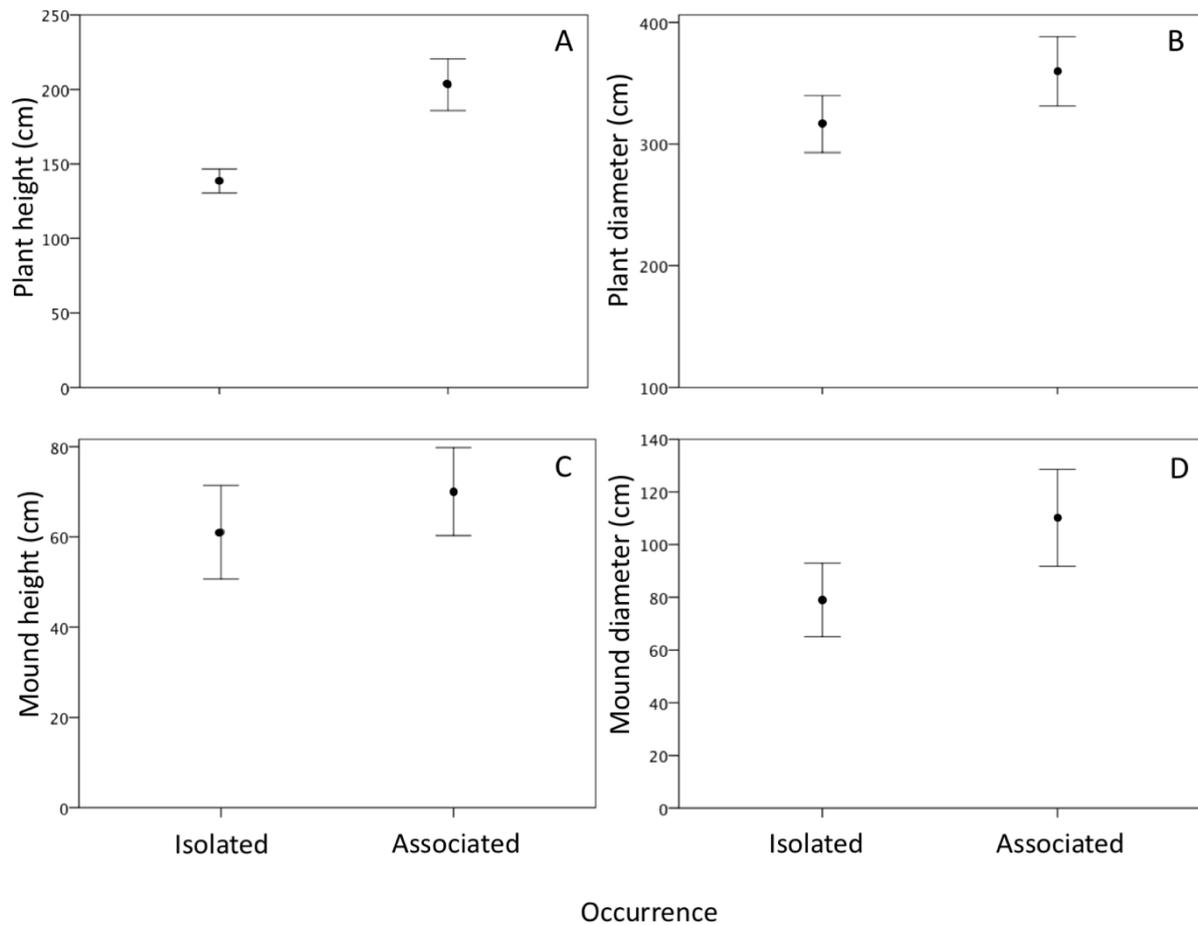
537 Overall the height of exposed mounds (mean = 61.03 \pm 32.04 cm), regardless of season, was
538 similar ($t = 1.256$, $p = 0.97$) to that of mounds under num-num plants (mean = 70.04 \pm 35.40
539 cm, Fig. 1C). However, the diameter of mounds under plants (mean = 110.23 \pm 78.97) was

540 significantly wider ($t = 2.551$, $p = 0.024$) than that of mounds in the open (mean = $78.97 \pm$
541 42.98 , Fig. 1D).

542 During the wet season, three of the mounds with num-num plants were inactive while
543 12 were active. The activity of 38 mounds under the num-num trees could not be determined
544 accurately as it became difficult to penetrate the num-num cover. I, therefore, did not analyse
545 mounds activity data further.

546 In the wet summer season mound damage was significantly heterogeneous according
547 to location ($X^2_{(2)} = 9.80$, $p = 0.04$). The median score of exposed mounds was 2, while that of
548 mounds under num-num plants was 0. However, during the dry winter season, some of the
549 previously damaged mounds had now been repaired and hence there was no significant
550 difference in damage scores ($X^2_{(2)} = 1.70$, $p = 0.09$) between exposed (median = 1) and mounds
551 under trees (median = 0).

552



553

554 **Figure 1.** Size (\pm SD) comparison of isolated and associated common num-num (*Carissa*
 555 *bispinosa*) plants (n = 116) and termite (*Trinervitermes trinervoides*) mounds (n = 92) across
 556 transects sampled in the late wet season (April 2017) at Nylsvley nature reserve, South Africa.
 557 A: mean heights of isolated and plants associated with termite mounds, B: mean plant canopy
 558 diameter of isolated and plants associated with termite mounds, C: mean mound height of
 559 isolated and plant associated mounds, D: mean mound diameter of isolated and plant associated
 560 mounds.

561

562 *Soil composition and nutrients*

563 Soil particle size and pH was similar between the mounds and the surrounding matrix (clay p
 564 = 0.66, silt p = 0.249, sand p = 0.10, pH = 0.07; Table 1). Mg, K, Na, S, and Cu were enriched

565 in mounds relative to the matrix soils (Table 2 and 3). This enrichment was particularly marked
566 for Na ($p < 0.001$). Detailed concentration values for the soil analyses are in the Appendix.

567

568 **Table 1.** Mean soil particle composition of termite mounds ($n = 10$) and the surrounding matrix
569 ($n = 10$) in Nylsvley nature reserve, Limpopo.

570

Soil particle	Mean (\pm SD) mound composition (%)	Mean (\pm SD) Matrix composition (%)	<i>t value</i>	<i>P value</i>
Clay	13.095 (\pm 4.23)	12.49 (\pm 2.06)	0.462	0.655
Sand	77.090 (\pm 4.39)	78.81 (\pm 3.14)	-1.811	0.104
Silt	11.245 (\pm 5.43)	9.08 (\pm 2.98)	1.232	0,249
pH	4.524 (\pm 0.76)	4.37 (\pm 0.59)	2.06	0.07

571

572

573

574 **Table 2.** Relative concentration of macro- and micronutrient samples from live termite mounds
 575 (n = 10) in Nylsvley nature reserve, South Africa.

576

	Mean (\pm SD) mound concentration (mg/kg)	Mean (\pm SD) matrix concentration (mg/kg)	<i>t</i> value	<i>p</i> value
<i>Primary macronutrients</i>				
NO ₃ (nitrate)	21.5 (\pm 24.47)	10.70 (\pm 8.67)	1.16	0.27
NH ₄ (ammonium)	28.15 (\pm 7.69)	21.91 (\pm 1.82)	2.35	0.04
P	7.76 (\pm 3.52)	7.58 (\pm 2.88)	0.21	0.84
K	216.58 (\pm 85.29)	151.08 (\pm 76.07)	3.60	0.01
<i>Secondary macronutrients</i>				
S	11.73 (\pm 6.91)	5.56 (\pm 3.23)	3.48	0.01
Mg	174.90 (\pm 102.07)	105.73 (\pm 62.94)	3.19	0.01
Ca	563.33 (\pm 349.38)	366.53 (\pm 281.59)	2.14	0.06
<i>Micronutrients</i>				
B	0.31 (\pm 1.32)	0.22 (\pm 0.15)	1.32	0.11
Na	14.48 (\pm 3.48)	8.70 (\pm 2.42)	5.41	< 0.001
Cu	1.62 (\pm 0.42)	1.22 (\pm 1.16)	3.90	0.004
Zn	2.93 (\pm 1.15)	2.28 (\pm 2.57)	1.37	0.20
Mn	81.20 (\pm 42.93)	61.32 (\pm 53.33)	2.63	0.03
Fe	198.29 (\pm 227.12)	156.86 (\pm 104.41)	0.71	0.49

577

578

579

580 **Table 3.** False Discovery Rate (FDR) correction of the seven out of 17 soil property
 581 comparison tests with a significant p value. Only five of the seven previously significant
 582 outcomes were significant after the FDR correction analyses.

583

Soil property	p value	p value rank (i)	FDR corrected p value ($i*0.05/17$)	Statistical significant after FDR correction ($\alpha = 0.05$)
Na	0.0004	1	0.0029	Significant
Cu	0.0040	2	0.0059	Significant
K	0.0057	3	0.0088	Significant
S	0.0069	4	0.0118	Significant
Mg	0.0111	5	0.0147	Significant
Mn	0.0275	6	0.0176	Significant
NH ₄	0.0430	7	0.0206	Significant

584

585 **Discussion**

586 Num-num plants on termite mounds were taller and with relatively wider canopies compared
587 to plants growing in the matrix. This indicates that the enriched termite mound soils were
588 beneficial to the growth of these plants. It is well established that termites improve soil fertility
589 on and around mounds (van der Plas *et al.* 2013, Seymour *et al.* 2014) through their foraging
590 (Badertscher *et al.* 1983) and building activities (Laker *et al.* 1982) which concentrates organic
591 compounds and essential nutrients collected from the surrounding matrix and underground
592 excavations respectively. These activities concentrate the immediate surrounding with
593 nutrients previously locked up in mound building substrate when they are weathered down by
594 rain or excavated by predators. In the case of our study, the mounds presented a highly fertile
595 environment that enhanced the growth of plants on mounds. Similar soil enrichments by this
596 species have been documented by Laker *et al.* (1982). Furthermore, some of the larger mounds
597 were not only associated with *C. bispinosa*, but also with other trees such as *Peltophorium*
598 *africanum*, *Acacia* spp, *Grewia bicolor* and *Ziziphus mucronata*.

599 The added advantage of plants growing in nutrient rich soils (i.e. next to mounds) is the
600 increased fruiting success of the plants (Brody *et al.* 2010, Joseph *et al.* 2014). Although canopy
601 cover of all plants, regardless of association, was greener and leafy in the wet season, the num-
602 num plants growing on mounds retained their green leaves well into the dry season. This
603 probably suggests that termite colonies provide a local source of water that sustains the
604 associated trees during the dry season. However, I did not explicitly measure the seasonal soil
605 moisture content. In addition to the network of soil macro pores that promote the infiltration of
606 water into soils (De Bruyn and Conacher 1990), termites are also known to transport moisture
607 from the underground water table, which they then mix with soil to make mud used to build or
608 repair the mounds (Turner *et al.* 2006, Davies *et al.* 2014). This moisture may also be available

609 to the nearby trees and hence these trees comparatively maintain greener canopies even during
610 the dry season.

611 Most of the termite mounds (71 %) seen in the transects were located under num-num
612 plants, however, there were far more plants that were found without mounds underneath them.
613 Additionally, these mounds under num-num cover had minimal to no damage on them. This
614 indicates that the wellbeing and perhaps even the persistence of mounds in this particular
615 system with Aadvark present, is highly dependent on the availability of num-num cover which
616 deters these mound predators. The spines are in opposite pairs and the stems are branched at
617 an angle from the nodes so that different spines tend to close together at their ends thus making
618 it very difficult for predators to penetrate the bush (Cooper and Owen-Smith 1986, Coates-
619 Palgrave 2002). Given that a higher proportion of num-nums were found independent from
620 termite mounds compared to those in plant-mound associations, one can assume that the
621 recruitment and survivorship of num-nums is generally independent of mounds. However,
622 where this association occurs, it does benefit the plants making them taller and more productive
623 in the dry season. What is not certain is which one comes first between the mound and the
624 plant, so as to elucidate which of the two “seeks” the presence of the other. Hesse (1955) noted
625 that vegetation was rarely observed germinating on top of active mounds, suggesting that
626 mounds are usually built around the root and stems of trees. If this premise is true, it implies
627 that the termites’ mounds are built under the plants. This study further supports this assumption
628 given that there were more proportional trees in isolation than those in association with
629 mounds, whereas more mounds that were active were found under trees. The most
630 parsimonious explanation is that termite mounds were “hiding” from predation under the spiny,
631 dense cover of num-num and, in turn, the plants derived a benefit from the mound enriched
632 soils which also promoted their growth and productivity. Perhaps this is not a classic example
633 of a mutualistic association given that both species can survive in the savanna landscapes

634 without the other, but it illustrates a possible advantage in a semi-arid environment with high
635 levels of termite predation.

636 The diameter of termite mounds was much wider when there was a plant association,
637 whereas there was no difference in mound height, which can be expected since the bush is too
638 dense to allow an easy upwards mound expansion. This may actually present a mound
639 thermoregulation disadvantage since larger mounds of *Macrotermes* are known to have better
640 temperature stability (Ndlovu and Pérez-Rodríguez 2018). However, the microclimate shade
641 presented by the num-num plant may counteract that drawback (see Ndlovu and Pérez-
642 Rodríguez 2018). The wider mound diameter indicates that these protected termites were, able
643 to extend the space the colony can occupy and possibly reproductive effort compared to their
644 exposed counterparts. It is also possible that in addition to protection from predation, the plants
645 protect the mound from abiotic factors such as wind and rainfall which continually erode soil
646 from the surface of the mounds. However, when the mounds are under num-num cover the
647 maintenance labour and loss of resources (soil and water) should be reduced (Turner *et al.*
648 2006) and the termites can focus most of their energy on other activities. Shade cover could
649 also mean less exposure to direct sunlight and dry wind which reduces water evaporation and
650 keeps the mound soils relatively moist (Korb 2011) compared to the surrounding matrix (Hesse
651 1955). Hence I would assume that plants on mounds may be exploiting this water for their
652 productivity even during the dry season.

653 There were insignificant differences in the soil particle size of the termite mounds and
654 the surrounding soil. It can, therefore, be assumed that these termites are not selecting out soil
655 particles from the soil that is available to them when constructing and maintaining mounds
656 (Hesse 1955, Jouquet *et al.* 2005). Interestingly, macronutrients and pH of mounds were not
657 consistently elevated in all mounds, whereas previous studies on other termite species reported
658 a uniformly higher pH, moisture, organic matter and macronutrients content in mounds

659 compared to the surrounding matrix (López-Hernández 2001, Fleming and Loveridge 2002).
660 The only significant differences between *T. trinervoides* mounds and the matrix were in
661 concentrations of Mg, K, Na, S and Cu (Table 3). It suggests that the enrichment activities of
662 the *T. trinervoides* termites were minor and may also be limited by the inherent characteristics
663 of the local soils. Nevertheless, the activities of the snouted-harvester termites are significant
664 to num-num plant growth and reproduction (fruiting). The enrichment of mounds with minerals
665 such as Mg, K, S and Cu will enhance plants with essential functions such as photosynthesis,
666 enzyme activation, metabolism and overall growth and productivity (Black and Okwakol 1997,
667 Wang *et al.* 2013).

668 The limitation of my study was that some of the termite mounds under num-nuns were
669 difficult to inspect for activity. A challenge which possibly also deterred predation on mounds.
670 Even when access was possible, it was not easy to confidently determine activity without
671 damaging the mound. This predicament affected the sample size of the study. Although the
672 study only focused on these two species (*Trinervitermes trinervoides* and *Carissa bispinosa*),
673 the overall dynamics of this relationship extend beyond this plant-insect interaction which also
674 affects the foraging ecology of termite predators such as the Aardvark and Pangolin that
675 damage these mounds. An opportunity of measuring water content of the soil samples was
676 missed due to the importance thereof being underestimated as supporting evidence for higher
677 levels of moisture on termite mounds. Much of the diversity in these semi-arid savanna systems
678 is driven by mutualistic relationships amongst organisms, and unravelling such plant-insect
679 interactions that may determine the persistence of an ecosystem engineer (in this case the
680 termites) is significant. Termites modify the soil's chemistry and morphology which in turn
681 influences diversity and productivity of ecosystems. Few studies have investigated how these
682 seemingly defenceless ecosystem engineers (*Trinervitermes sp.* have comparatively smaller
683 mandibles that *Macrotermes sp.*) have adapted to avoid excessive predation. This study has

684 also made significant knowledge advances in plant-insect mutualism (Bronstein *et al.* 2006)
685 and the defence strategy that this termites species employs in a semi-arid environment with
686 high levels of termite predation.

687

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- 793

CHAPTER THREE

794
795

796 **Influence of Num-num (*Carissa bispinosa*) shade on thermoregulation inside Harvester**
797 **termite (*Trinervitermes trinervoides*) mounds.**

798

799 **Abstract**

800 Thermoregulation inside termite mounds is influenced by the prevailing ambient conditions. It
801 was not clear whether the canopies of num-num (*Carissa bispinosa*) offer any significant
802 microclimate that enhances a stable temperature inside *Trinervitermes trinervoides* mounds.
803 General Additive Mixed Models were used to assess 24-hour internal mound temperature
804 fluctuations in relation to ambient conditions, season, shade presence and mound activity. I
805 tested the hypothesis that the microclimate provided by the *C. bispinosa* shade will improve
806 internal mound thermoregulation. There is a strong effect of ambient temperature on internal
807 mound temperature profiles. The effect changed seasonally but was more pronounced on
808 inactive mounds. Temperature profiles of exposed inactive mounds mirrored those of ambient
809 temperatures. Both active and inactive mounds under shade had fairly constant internal
810 temperature profiles in both seasons. Overall, internal temperature changes were fairly constant
811 (± 6 °C) in active mounds during the 24-hour period in both seasons, while temperature changes
812 in inactive mounds varied more (~ 15 °C). The three best models (i.e. lowest AIC values)
813 obtained explained approximately 96% of variance on the response variable (PseudoR² values).
814 Activity and season (including their interactions), were important in determining the internal
815 mound temperature profiles, while shade presence and mound size were not. Therefore, the
816 shade of Num-num shrubs did not affect nor improve thermoregulation capabilities of mounds.

817

818 *Keywords:* Ambient temperature, Microclimate, Mound, South Africa, Thermoregulation.

819

820 **Introduction**

821 Termites (Isoptera) are among some of the most vulnerable insects (Abe *et al.* 2000). Generally,
822 they lack cuticle pigmentation and are, therefore, susceptible to drying out when exposed to
823 direct sunlight (Abe *et al.* 2000, Davies 2012). Thus, moisture and temperature are major
824 physical factors limiting the dispersal of termites (Collins 1969). Therefore, many termite
825 species in tropical regions construct mounds to counteract this problem by maintaining a
826 homeostatic thermal environment that is humid and well-ventilated inside their mounds.
827 According to Wood (1988), temperatures inside mounds are influenced by ambient conditions
828 and can be modified by activities of the inhabitant termite colony. Nest site selection, mound
829 architecture and orientation are the primary mechanisms used by termites to regulate the
830 microclimate inside the mound (i.e. Darlington 1984, Korb and Linsenmair 1998). The
831 orientation of the nest influences the amount of solar radiation received and the time of day
832 when the highest radiation is received (Jacklyn 1992, Jones and Oldroyd 2006). In addition,
833 structural features (i.e. chimneys) are important for retaining heat, while others are effective in
834 dissipating it (Theraulaz 1998, Korb 2003).

835 Termite mounds are essentially a response to ecological requirements (Jouquet *et al.*
836 2006). Termites have different ways in which they achieve thermal homeostasis inside
837 mounds. Internal mound temperatures of fungus-cultivating *Macrotermes bellicosus* in the
838 savanna is mainly regulated by the ambient temperature via solar radiation, however, the heat
839 budget of the nest is also dependant on the metabolism of the termites and fungi to maintain
840 the optimal nest temperature of approximately 30 °C throughout the year (Korb and Linsenmair
841 2000). In addition to a constant temperature, relative humidity near saturation and low
842 concentrations of CO₂ are required for the growth of the fungi (Wood and Thomas 1989, Korb
843 2003). *M. bellicosus*, therefore, transform their dome-shaped mounds into cathedral-shaped
844 mounds (as the colony grows) with reduced wall thickness and increased surface area to allow

845 for gaseous exchange via air channels (Korb and Linsenmair 1998, Korb 2003). To reduce
846 internal mound temperatures, termites introduce water into the nest which results in evaporative
847 cooling (Korb and Linsenmair 2000). The mounds of *M. subhyalinus* have a single "chimney"
848 which is used to release heat in the hot dry areas (Darlington 1984). It was further postulated
849 that the high moisture-absorptive fungus combs and the carton material also help to control
850 humidity in the nest (Hesse 1955). However, only a few studies report on the mound
851 thermoregulation capabilities of other none *Macrotermes* spp. termites such as *T. trinervoides*
852 (Field and Duncan 2013, Adam *et al.*).

853 Termites are known to concentrate nutrients in their mounds over time and, as a result,
854 plant growth is common over and around mounds (Muvengwi *et al.* 2016). The activity of these
855 insects also increases water retention in the soil, which affects vegetation structure and local
856 primary productivity (Nash and Whitford 1995). Hence mounds are known to harbour
857 evergreen plant species with thick foliage (van der Plas *et al.* 2013, Joseph *et al.* 2014) which
858 provide microclimates significantly cooler than the surrounding matrix (Korb and Linsenmair
859 1998, Joseph *et al.* 2016). Most mound-building species in hot, dry environments are said to
860 locate their mounds under shade (Sands 1965, Lee and Wood 1971) although others are
861 completely exposed.

862 In the previous chapter, I was able to show that mounds of the lesser studied
863 *Trinervitermes trinervoides* termites enrich the soil with macro- and micro-nutrients that
864 promote num-num plant (*Carissa bispinosa*) growth. Here, I further investigate whether the
865 plant growth, over and around *T. trinervoides* mounds, provides a microclimate that enhances
866 a stable temperature inside mounds. *T. trinervoides* build dome-shape mound structures that
867 keep internal mound temperatures stable throughout the year (Field and Duncan 2013). The
868 aim of the present study was to document 24-hour internal mound temperature profiles in
869 summer and in winter, inside active as well as dead termite mounds, and further determine how

870 the shade of num-num shrubs affects internal mound temperature fluctuations. I predicted that
871 the shade of num-num shrubs coupled with termite activity (i.e. active vs inactive mounds)
872 would influence the seasonal internal mound temperature fluctuations.

873

874 **Materials and methods**

875 *Study site*

876 This study was conducted at Nylsvley Nature Reserve (24° 39' 17.28" S, 28° 41' 27.6" E) in
877 Limpopo province, South Africa. The reserve lies at the intersection of three different
878 geological formations with five distinct soil groups (Jones *et al.* 2013) on top of the seasonally
879 inundated floodplain of the Nyl river (Rowberry 2011). The region receives about 600 mm of
880 rainfall and is characterised by a warm wet summer season from October to April, and a cool
881 dry winter season between May and September (Werner 2009). Several termite mounds were
882 present in the reserve: most correspond to the relatively small dome shaped mounds of Snouted
883 harvester termites (*T. trinervoides*), with fewer large *Macrotermes spp.* mounds.

884

885 *Fieldwork*

886 The study was carried out in 2017 at the end of the summer season (March) and during the
887 height of the winter season (July), to capture seasonal variations in internal mound
888 temperatures as affected by prevailing ambient conditions. Termite mounds of similar sizes
889 (approximately 1 m in height and diameter) of *T. trinervoides* were carefully selected for this
890 study. A total of 14 mounds were used for this study, of which eight were active (inhabited)
891 and the other six were inactive; within each group, half of the mounds were exposed while the
892 other half were shaded. Mounds were deemed as shaded if the entire mound was covered by
893 num-num vegetation. Exposed mounds were those totally exposed to sunlight throughout the
894 day with no observed shading from any surrounding vegetation.

895 Mounds were drilled on the side, at 2 cm from the ground level and the drilled hole went in
896 approximately half the diameter of the mound. The aim was to reach the core of the mound.
897 Evidence of termite activity was checked for by extensively looking for termites inside and
898 around the mound before the loggers were inserted. Internal mound and ambient temperature
899 were measured to the nearest 0.1 °C using data loggers (EL-USB-5, Lascar electronics).
900 Loggers were calibrated in a natural convection laboratory oven (MRC, DNO 20, 1100 W, 20
901 l) at 30 °C prior to data collection. Each logger was attached to a 60 cm flexible steel wire (to
902 allow easy retrieval of the logger afterwards) and placed inside a 50 cm × 5 cm PVC pipe. The
903 outer end of the pipe was sealed tight with a thick polyurethane insulation foam. Loggers inside
904 pipes were then inserted inside the mounds in the evening (around 16h00 – 18h00) and the
905 drilled holes were quickly sealed with a mud mixture made from the drilled soil substrate and
906 water. About 10 cm of the logger attachment steel wire remained sticking out of the mound
907 and another logger was attached on this outside end wire so as to record ambient temperature.
908 All data loggers were left in place for at least 72 hours and were programmed to record
909 temperature at 1-hour intervals. Loggers were retrieved at the end of each sampling season and
910 the mound was subsequently sealed again.

911

912 *Data analyses*

913 Mound internal temperature (continuous variable) was analysed in relation to mound activity
914 (categorical variable, active vs. inactive), shade cover (categorical variable, open vs shaded),
915 mound volume (continuous variable, mound size) and external temperature (continuous
916 variable); using General Additive Mixed Models (GAMMs) following Ndlovu and Pérez-
917 Rodríguez (2018). Given that the number of sampled mounds was relatively limited for each
918 treatment, I did not exhaustively test all the possible interactions and only limited my analyses

919 to the three interactions which made biological sense i.e. between the ambient temperature and
920 the three factors. The tested interactions were:

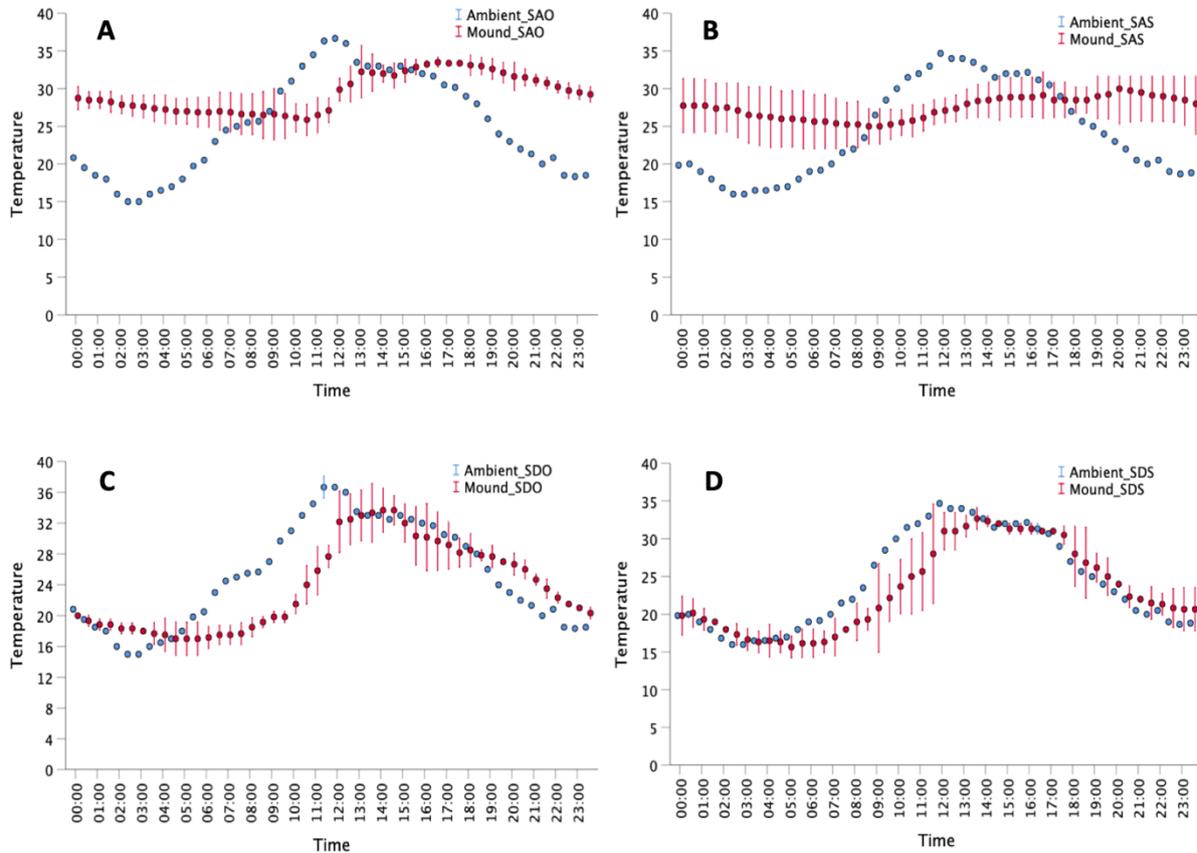
- 921 • Interaction 1: Ambient_temperature*Hour*Activity. Does the effect of ambient
922 temperature change during the day? Does the mound temperature profile vary between
923 active and inactive termite mounds?
- 924 • Interaction 2: Ambient_temperature*Hour*Shade. Does the effect of ambient
925 temperature change during the day? Does the mound temperature profile vary between
926 shaded or exposed mounds?
- 927 • Interaction 3: Ambient_temperature*Hour*Season. Does the effect of ambient
928 temperature change during the day? Does the mound temperature profile vary between
929 seasons?

930 All statistical analyses were carried out in R 3.4.2 (R Development Core Team 2017), using
931 the command *uGamm* of the R package *mgcv* to fit GAMM models with Gaussian distribution
932 and an identity link function. GAMMs allow fitting of models to nonlinear variables without a
933 priori specifications of their functional form (Zuur *et al.* 2014).

934

935 **Results**

936 Temperature profiles of all 14 mounds were successfully recorded at 30 mins intervals during
937 both sampling seasons. In summer, ambient temperatures fluctuated greatly with maximum
938 temperatures of 37 °C recorded around midday (11h00 –13h00), while minimum temperatures
939 of 15 °C were recorded in the early morning hours between 02h00 – 04h00. Temperatures of
940 exposed active mound were relatively constant at mean = 29.5 ± 2.72 °C (\pm standard deviation),
941 while shaded mounds had a slightly low temperature of 27.5 ± 2.24 °C. Exposed inactive
942 mounds had a temperature of mean = 23.7 ± 5.67 °C, while shaded mounds were slightly higher
943 at mean = 23.3 ± 5.72 °C (Fig 1).



945

946 **Figure 2.** Mean internal mound (*Trinervitermes trinervoides*) and ambient temperature (°C)
 947 profiles at Nylsvley nature reserve during the summer season. Temperature was recorded at 30
 948 min intervals over a 24-hour period. A: exposed active mounds, B: active mounds under num-
 949 num (*Carissa bispinosa*) shade; C: exposed inactive mounds, and D: inactive mounds under
 950 num-num shade. [SAO = Summer Active Open; SAS = Summer Active Shade; SDO = Summer
 951 Dead Open; SDS = Summer Dead Shade].

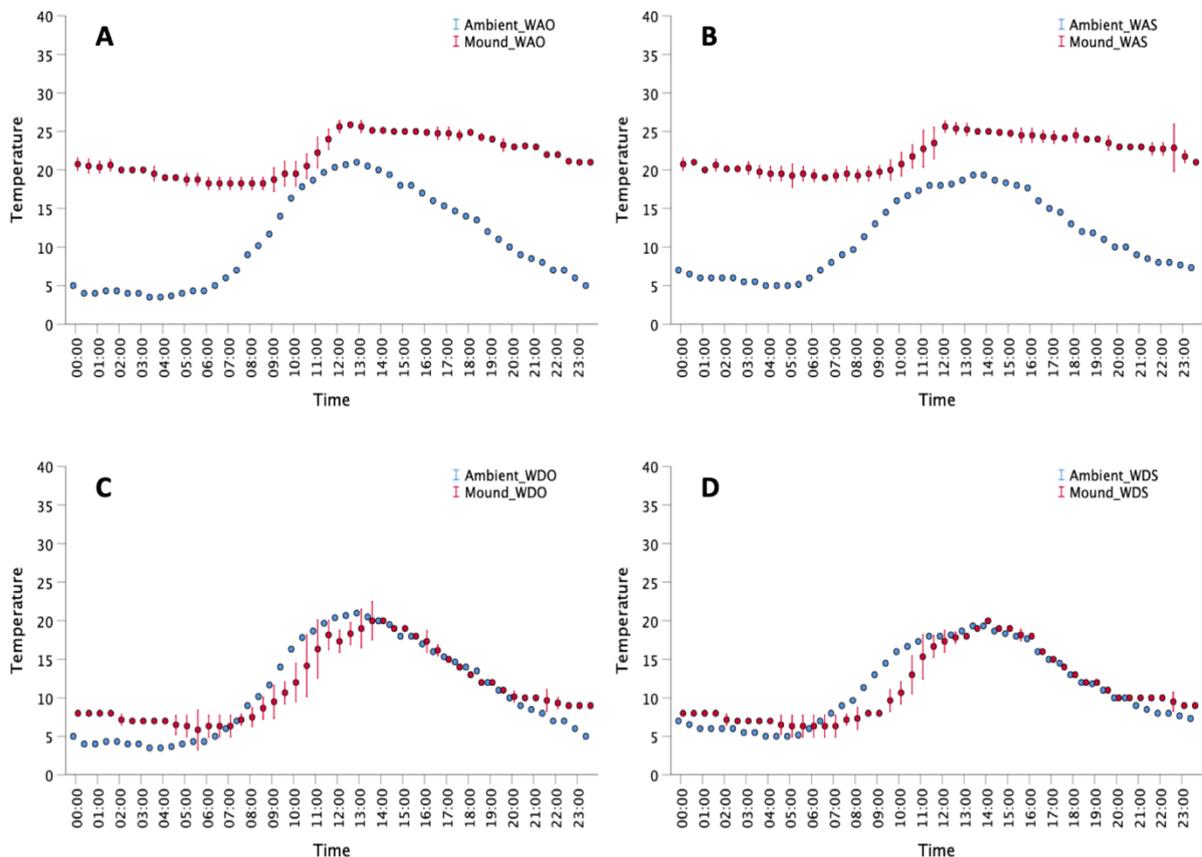
952

953 Lower ambient temperatures were recorded during the winter season with maxima
 954 temperatures of 21 °C recorded around 13h00 –14h00, while minima temperatures of around
 955 3.5 °C were experienced in the early morning hours (02h00 – 04h00). Exposed active mound
 956 temperatures were relatively constant (mean = 21.8 ± 2.62 °C), while shaded mounds (mean =
 957 22.0 ± 2.23 °C) were slightly more stable. Exposed inactive mounds (mean = 11.38 ± 4.56 °C)

958 and exposed shaded mounds (mean = 11.2 ± 4.42 °C), relatively fluctuated with ambient
959 temperature and they never peaked above 20 °C (Fig. 2).

960

961



962

963 **Figure 3.** Mean internal mound (*Trinervitermes trinervoides*) and ambient temperature (°C)
964 profiles at Nylsvley nature reserve during the winter season. Temperature was recorded at 30
965 min intervals over a 24-hour period. A: exposed active mounds, B: active mounds under num-
966 num (*Carissa bispinosa*) shade; C: exposed inactive mounds, and D: inactive mounds under
967 num-num shade. [WAO = Winter Active Open; WAS = Winter Active Shade; WDO = Winter
968 Dead Open; WDS = Winter Dead Shade].

969

970 There was a strong effect of ambient temperature on internal mound temperature profiles. The
971 effect changed seasonally but was more pronounced on inactive mounds (Fig. 1). Temperature
972 profiles of exposed inactive mounds mirrored those of ambient temperatures in both seasons.

973 Both active and inactive mounds under shade had fairly constant internal temperature profiles
974 during both seasons. Overall, internal temperature changes were fairly constant (± 6 °C) in
975 active mounds during the 24-hour period in both seasons, while temperature changes in inactive
976 mounds varied more (~ 15 °C).

977 The full model that included all available variables, including the interactions among
978 them, had the following form:

```
979 Tfull <- uGamm(Mound_temperature ~ Active * Size + Active * Shade + Season*Active +  
980 te(Hour, Ambient_temperature, by = Factive), random = list(Mound=~1,Hour=~1).
```

981

982 All nested models within the full model were also tested, i.e. all possible combinations of
983 variables, including/excluding interactions. Three best models (i.e. lowest AIC values) were
984 obtained and all three models explained approximately 96 % of variance on the response
985 variable (PseudoR² values, Table 1).

986

987

988 **Table 4.** Set of best-ranked models, according to Akaike Information Criterion (AIC) values,
 989 examining variations in internal temperature of termite mounds sampled in the Nylsvley nature
 990 reserve. Each line of the table represents a model that includes those variables with estimate
 991 values. The estimates of the effect of each variable were averaged across the three models with
 992 AIC values differing less than four units from the best model. The relative importance of each
 993 variable was calculated using the Akaike weights (amongst all possible 51 models). te: (Hour,
 994 Ambient temperature, by = F active). NA indicates that the particular model does not include
 995 that variable. The presence of interactions is shown with an '+'.
 996

(Intercept)	Active	Season	Shade	Te	Active: Season	Active: Shade	df	AIC	Delta AIC	Weight	PseudoR2
21.59	+	+	+	+	+	+	19	4637.75	0	0.668	0.964
21.82	+	+	NA	+	+	NA	17	4640.04	2.28	0.213	0.961
22.01	+	+	+	+	+	NA	18	4641.21	3.45	0.119	0.961

997
 998
 999 The estimate average values of the coefficients across the three models revealed that activity
 1000 and season (including their interactions), were important in determining the internal mound
 1001 temperature profiles. Shade and mound size did not have any significant effect in determining
 1002 the internal temperatures profiles of mounds (Table 2).
 1003
 1004

1005 **Table 5.** summary of the model estimated average variable coefficients. Column headings Adj
 1006 SE = Adjusted Standard Error, Pr(>|z|) = p value, Sig. = level of significance.

1007

	Estimate	std Error	Adj SE	Z value	Pr(> z)	Sig.
(Intercept)	21.692	0.365	0.366	59.304	<0.001	***
ActiveYes	5.694	0.722	0.723	7.879	<0.001	***
SeasonWinter	-8.329	0.223	0.223	37.397	<0.001	***
ShadeYes	0.249	0.495	0.495	0.504	0.614	
ActiveYes:SeasonWinter	4.347	0.880	0.881	4.932	<0.001	***
ActiveYes:ShadeYes	-0.944	0.833	0.833	1.132	0.257	

1008

1009

1010 **Discussion**

1011 The structure of the *T. trinervoides* mounds provided little buffer against ambient temperature
 1012 fluctuations. It appears the termites are actively maintaining the internal temperature of a
 1013 mound and keep it within a narrow temperature range. This study does reveal that seasonal
 1014 changes in temperature, as well as the activities of the termites, are a strong determinant
 1015 towards internal mound temperature profiles. It appears that, in general, *T. trinervoides* termites
 1016 struggle to maintain higher optimal mound temperatures during winter, hence this could be the
 1017 reason why the species tend to be inactive during cold winter months (Adam et. al. 2018). The
 1018 energetic costs of opening the mound, going out to forage and later returning to the mound,
 1019 sealing it and using metabolic activity to heat up the mound interior could be highly costly
 1020 (Sands 1965).

1021 Similar to the findings of Field and Duncan (2013), who found that *T. trinervoides* at
 1022 the same site kept their internal mound temperature within narrow limits of 20 °C in winter and

1023 around 30 °C in summer, I also found that there was a significant difference in internal mound
1024 temperatures between seasons, even though stable within a season. The temperature stability
1025 profile remained similar but the termites were maintaining a significantly lower constant
1026 internal temperature in winter. Perhaps such flexibility is simply because this species of
1027 termites does not cultivate fungi (Adam et. al. 2018) which is sensitive to extreme temperature
1028 fluctuations (Abe et. al. 2000). Indeed, a stable temperature is required by all termites, and in
1029 this case, *T. trinervoides* maintained mound conditions within the ranges of 24 – 34 °C in
1030 summer and 18 – 26 °C in winter.

1031 In the summer season when the savanna temperatures reached above 35 °C, active
1032 termite mounds that are completely exposed maintained temperatures that are closer to, but not
1033 exceeding 30 °C. Temperatures of smaller mounds such as those of *T. trinervoides* positively
1034 correlate with ambient temperature, reaching 30 °C only if the ambient temperature is high
1035 (Korb and Linsenmair 1998). When active mounds were shaded by *C. bispinosa*, internal
1036 temperatures were slightly less than those that are not shaded, but still close to 30°C. This is
1037 because introducing shading results in a drop of the ambient temperature followed by a decline
1038 in mound temperatures (Korb and Linsenmair 1998).

1039 In winter, temperatures of mounds correlated with the lower ambient temperatures,
1040 however, exposed and shaded mounds with live colonies all maintained temperatures much
1041 higher (close to 25 °C) than the ambient temperature (11.3 °C). This suggests that the termites
1042 modified the temperature to ensure a hospitable internal environment since during these times
1043 *T. trinervoides* termites never leave the mound (Field and Duncan 2013, Adam et. al. 2018).
1044 Inactive mounds had temperatures that closely resembled the ambient temperature, regardless
1045 of whether they were in the open or under shade. It was interesting to find that the ambient
1046 temperature was slightly higher under shade than it was in the open. This could be an indication
1047 that in the colder season, the “umbrella canopy” of the plant creates a warmer microhabitat that

1048 is protected against the cold wind in the open. The effect of ambient temperature on internal
1049 mound temperatures was more pronounced in inactive mounds. Although there were no
1050 termites to regulate internal temperatures and produce metabolic heat, inactive mound
1051 temperatures did not exponentially increase as would be expected with the influence of ambient
1052 temperatures (Joseph *et al.* 2016). This is likely due to the soil insulation properties that the
1053 mound structure naturally provides, which acts as a buffer against ambient temperatures (Wood
1054 1988).

1055 A recent study on Savanna *Macrotermes sp.* found that the contribution of plant shade
1056 towards the internal temperature stability was negligible and hence it was suggested that the
1057 size of termite mound plays a significant role in buffering the mound interior against external
1058 conditions (Ndlovu and Pérez-Rodríguez, 2018). Given this premise, it made sense to
1059 hypothesise that the smaller sized and temperature vulnerable mounds of *T. trinervoides* should
1060 somehow be relying on microclimates provided by plant canopies in hot climates to buffer
1061 some of the strain from ambient temperature fluctuations (Sands 1965, Lee and Wood 1971).
1062 However, it appears that even the Num-num (*C. bispinosa*) shade has little effect on the
1063 temperature profiles of these smaller mounds. The plant shade does appear to lower (though
1064 insignificant) the immediate ambient temperature around the mound, which in turn slightly
1065 decreases the mean internal mound temperature. The effect of the shade does not assist towards
1066 stabilising the internal mound temperature profiles since both exposed and shaded mounds per
1067 season (and according to activity) had a similar internal temperature fluctuation.

1068 Given the relatively low thermoregulation capabilities of *T. trinervoides* termite
1069 mounds compared to *Macrotermes sp.* in the same landscape (Field and Duncan 2013, Joseph
1070 *et al.* 2016, Ndlovu and Pérez-Rodríguez, 2018), one begins to wonder how these termite
1071 species will survive in face of stream temperature fluctuations. The two possible adaptations
1072 are that the mounds of *T. trinervoides* may increase in volume to counteract the effects of

1073 ambient temperature as predicted with *Macrotermes sp.* (Ndlovu and Pérez-Rodríguez, 2018),
1074 or the seemingly insignificant role of microclimates provided by the num-num shade will help
1075 to offload the few temperature degrees (excess heat) which may help the species
1076 thermoregulate better. This prediction can be tested by future studies during a heat wave
1077 episode. Chapter 2 also revealed that mounds under num-num shade had a much wider diameter
1078 and resultant volume of which this may also help to buffer against extreme temperature
1079 fluctuations as shown by the study of Ndlovu and Pérez-Rodríguez (2018).

1080

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CHAPTER FOUR

Synthesis

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Mound building termites are ecosystem engineers that interact with plant-litter-soil system through their nesting, feeding, and foraging activities which promotes habitat heterogeneity and functional complexity in landscapes (Holt and Lepage 2000, Jouquet *et al.* 2011). Activities of termites are known to enrich soils with essential nutrients and also increase the content of clay which in turn enhances the water holding capacity of soils. The accumulated nutrients on mounds, makes these structures more fertile than the surrounding matrix (Wood 1988, De Bruyn and Conacher 1990, Holt and Lepage 2000, Jouquet *et al.* 2011, Davies *et al.* 2014, Joseph *et al.* 2014, Muvengwi 2016).

Although the effects of termites on ecosystem functioning have previously been documented (De Bruyn and Conacher 1990, Holt and Lepage 2000, Jouquet *et al.* 2011, Davies *et al.* 2014, Joseph *et al.* 2014), what is unique about my study, is the novelty of the relationship that exists between a vulnerable termite species and a thorny tree species at a semi-arid savanna system with relatively pronounced levels of termite predation. Therefore, my study explored the existence of a mutualistic association between Num-num (*Carissa bispinosa*) and the mound building Snouted harvester termite (*Trinervitermes trinervoides*) in a semi-arid savanna setting. In particular, I wanted to understand whether the relationship between *C. bispinosa* and the *T. trinervoides* mounds benefits (1) both species (i.e. mutualistic in nature), or (2) only one entity (parasitic), and (3) further determine the extent of the accrued benefits to the species involved. I investigated how plant performance differed seasonally (wet vs dry) when growing on mounds compared to those in the surrounding matrix. I also measured and quantified mound predation damage between protected and exposed mounds. In order to determine how the termite mounds were affecting plant growth, I further evaluated soil properties and nutrients

1180 on mounds and the surrounding matrix (Chapter 2). I found that there was a facultative
1181 mutualistic relationship between the termites and the plants. Termite mounds were less
1182 susceptible to predation in summer when found under num-num. In turn num-num plants on
1183 mounds benefited from the enriched soils and hence were significantly more productive (in
1184 terms of reproduction, drought resistance and above ground biomass) than those in the matrix.
1185 Num-num cover did significantly deter mound predation and assist in termite colony survival.

1186 The second aspect of my investigation was to establish how effective the shade of num-
1187 num contributed towards the thermoregulatory capacity of mounds underneath. The
1188 assumption was that num-num canopies create a microclimate that was buffered from the
1189 prevailing environmental conditions. I found that ambient temperature was a stronger driver of
1190 mound thermoregulation regardless of num-num shade availability. Termites activity
1191 contributed more towards the maintenance of a homeostatic internal mound environment, and
1192 this became easier during the warm summer season. This was in contrast with patterns reported
1193 for *Macrotermes sp.* (Ndlovu and Pérez-Rodríguez 2018) that showed a stable internal mound
1194 temperature when found under trees. Perhaps this contrast also lies in the differences in mound
1195 size between the two species of termites.

1196

1197 **Limitations and proposed future research**

1198 Due to some difficulties in accessing some of the termite mounds, I was not able gather much
1199 accurate data on mound activity and let alone analyse that dataset. As result, I could not
1200 confidently determine the activity of mounds under num-num plants. It still remains uncertain
1201 whether these mounds were occupied or just older dead mounds that were now nutrient rich
1202 heaps of soil. Future studies may look into increasing the mound activity sample sizes under
1203 num-num by devising a remote way to assess mound activity, perhaps using thermal imaging
1204 tools. Such findings would determine the survival of mounds under num-num.

1205 All my data collection was limited to a once off study of a single site in two consecutive
1206 seasons. Another limitation of the study that might have given more accurate results of thermal
1207 buffering by num-num shaden is that sampling was not done at the peak of each season.
1208 Perhaps further replicates of seasons that were sampled and an expansion of the study into other
1209 sites could valid (or even challenge) the finds of this exploratory research. Due to the small
1210 sample size and limited repetitions (inherent in most ecological studies within an MSc
1211 timeframe), I acknowledge that my findings may be open to several interpretations. However,
1212 despite the limitations of this study, to my best knowledge, this is the first study that revealed
1213 a previously undocumented survival strategy displayed by the *Trinervitermes trinervoides*
1214 termites use to escape extreme temperature and predation pressure at a semi-arid savanna.

1215

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1247 **Appendix**

1248 **Table 1.** Soil particle analyses results for samples collected from the mound and the matrix

1249

Sample	Site	Soil particle composition (%)		
		Clay	Silt	Sand
1	mound	7.00	7.25	87.21
	matrix	10.60	5.20	83.50
2	mound	14.35	15.00	71.41
	matrix	14.45	12.95	72.45
3	mound	18.65	4.80	78.62
	matrix	13.45	12.50	76.45
4	mound	11.85	13.60	76.72
	matrix	16.45	4.80	81.46
5	mound	15.50	11.75	75.17
	matrix	12.65	8.00	80.55
6	mound	6.65	21.20	74.22
	matrix	9.95	11.25	77.99
7	mound	10.50	13.60	75.86
	matrix	12.90	8.85	77.25
8	mound	15.15	10.40	76.89
	matrix	13.20	7.15	80.29
9	mound	12.60	12.60	74.06
	matrix	10.70	12.10	77.43
10	mound	18.70	2.25	80.75
	matrix	10.50	8.00	80.68

1250

1251 **Table 2.** Soil nitrate (NO₃) and ammonium (NH₄) concentrations for collected samples.
 1252 Samples denoted with an M and a number were collected from mounds, whereas those with an
 1253 additional “open” level were collected from the matrix.

1254

Sample	Lab number	NO ₃		NH ₄	
		mg/l	mg/kg	mg/l	mg/kg
M1	851	0.27	2.7	2.45	24.5
M2	852	2.77	27.7	3.72	37.2
M3	853	0.29	2.9	3.13	31.3
M4	854	0.26	2.6	2.27	22.7
M5	855	0.44	4.4	2.27	22.7
M6	856	8.46	84.6	4.45	44.5
M7	857	2.31	23.1	2.55	25.5
M8	858	2.52	25.2	2.98	29.8
M9	859	2.19	21.9	2.34	23.4
M10	860	1.99	19.9	1.99	19.9
M 1 open	863	1.95	19.5	1.95	19.5
M2 open	864	2.09	20.9	2.09	20.9
M3 open	865	2.12	21.2	2.12	21.2
M4 open	866	2.13	21.3	2.13	21.3
M5 open	867	0.47	4.7	2.62	26.2
M6 open	868	0.40	4.0	2.11	21.1
M7 open	869	0.29	2.9	2.24	22.4
M8 open	870	0.28	2.8	2.23	22.3
M9 open	871	0.48	4.8	2.32	23.2
M10 open	872	0.49	4.9	2.10	21.0

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1256

1257 **Table 3.** Concentrations (mg/kg) of Soil Calcium (Ca), Magnesium (Mg), Sodium (Na),
 1258 Potassium (K), Sulphur (S), Phosphate (P), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn)
 1259 and Boron (B) measured from collected soil samples.

1260

Sample	Site	Ca	Mg	Na	K	S	P	Cu	Fe	Mn	Zn	B	pH
1	Mound	256.26	62.82	17.61	151.36	5.15	7.60	0.83	147.77	29.17	1.51	0.23	4.49
	Matrix	236.27	47.62	15.06	103.11	2.99	5.80	0.71	75.94	25.43	1.09	0.12	4.37
2	Mound	312.15	74.04	20.96	118.57	15.96	12.20	1.78	834.65	33.42	2.86	1.10	4.34
	Matrix	127.00	36.82	8.30	74.30	9.12	7.20	1.87	325.90	11.24	1.19	0.44	3.51
3	Mound	186.34	96.21	15.03	299.16	3.50	5.40	1.54	112.72	37.52	4.75	0.18	3.86
	Matrix	180.78	82.65	8.30	169.35	2.54	4.80	1.51	93.06	27.39	2.28	0.14	4.17
4	Mound	156.28	59.94	12.31	147.35	6.96	4.80	1.71	213.08	41.48	4.09	0.16	3.73
	Matrix	158.98	63.91	7.03	139.62	2.87	7.20	1.30	87.21	36.33	2.98	0.13	3.93
5	Mound	356.21	130.52	10.07	150.14	11.14	6.20	1.11	153.77	103.15	1.56	0.27	4.50
	Matrix	272.73	82.19	7.75	129.94	7.12	11.20	0.96	258.75	114.58	2.88	0.39	3.49
6	Mound	828.04	210.18	15.81	137.79	27.84	15.60	1.99	143.75	152.49	2.67	0.25	5.21
	Matrix	557.56	98.66	7.89	118.92	7.55	14.20	1.23	97.05	122.55	2.85	0.15	4.41
7	Mound	811.74	284.92	14.93	276.30	9.84	6.20	1.54	94.65	100.02	3.05	0.19	5.54
	Matrix	348.34	129.37	8.10	134.36	3.89	6.00	0.97	81.85	47.32	1.89	0.11	4.80
8	Mound	1166.14	334.10	16.42	293.14	13.94	8.60	1.58	68.85	93.22	2.57	0.24	5.23
	Matrix	461.20	150.93	8.37	147.63	4.58	7.00	1.01	209.03	68.59	3.26	0.21	4.98
9	Mound	806.55	259.16	11.39	356.53	13.61	5.80	2.31	108.17	98.50	1.88	0.24	5.12
	Matrix	1069.53	254.09	9.87	354.77	11.98	6.00	1.55	228.06	100.43	3.07	0.33	5.20
10	Mound	753.59	237.06	10.29	235.50	9.33	5.20	1.80	105.47	123.07	4.34	0.20	4.37
	Matrix	252.92	111.01	6.31	138.81	2.92	6.40	1.08	111.77	59.34	1.28	0.15	4.79

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