



Exploring the fine line between ecology, conservation and utilisation of a commonly exploited resource: A case study of mopane worms (*Gonimbrasia belina*) in southern Africa

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DECLARATION

I, **Mutali Winnie Netshanzhe**, hereby declare that this thesis submitted to the University of Venda for the degree of Doctor of Philosophy in Life Sciences: Zoology has not been submitted previously for any other degree at this or any other institution and that it is my own work in design and execution and that all materials contained therein have been duly acknowledged.

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SUMMARY

Mopane worms, the larvae of the mopane moth (*Gonimbrasia belina*), have a wide distribution in south and central Africa. They are valued highly as a food source and thus create local income in rural areas. They also contribute to ecosystem services by being part of the food chain and facilitating nutrient cycling on a landscape level. However, mopane worms are threatened by high harvest rates, habitat loss and the changing climate. Significantly, the existing research focuses on the utilisation of mopane worms, such as nutrient content, for human consumption. Very little research is done on mopane worms' ecology and their ecological contribution and importance.

This study aims to determine the extent of mopane worms' distribution across southern Africa, specifically in relation to protected and non-protected areas, to evaluate to what extent harvesting is restricted or can potentially be addressed, as well as determine the potential ecosystem services of mopane worms by measuring the consumption rate and frass production on a landscape scale and comparing nitrogen release through mineralization from frass and mopane leaves.

This was achieved through four main objectives: 1) review current research to evaluate the type of research topics, consolidate current knowledge, and identify gaps and future research directions; 2) quantify the suitable habitat of mopane worms in several southern African countries; estimate the effectiveness of protected areas to capture suitable habitat, and, explore variables affecting the suitable habitat of mopane worms; 3) assess the impact of mopane worms on the mopane veld ecosystem in Mapungubwe National Park through herbivory and 4) compare the N mineralisation rate as indicated by N-NH_4 and N-NO_3 release from frass from mopane worms and leaves from mopane trees, to establish whether the presence of mopane worms' frass leads to faster nutrient release for plant uptake.

The review found that most studies focus on mopane worm utilisation, such as their importance as a food and income source, while very few research efforts focus on biology or ecology. Although research on mopane worms has drastically increased over the years, these research efforts are not equally distributed among African countries, with most studies being done in South Africa, while other countries have very few or even no studies. Presence-only modelling identified the suitable mopane worm habitat and correlated this with protected and unprotected areas. Most of the harvesting occurs in non-

protected areas, where there are relaxed regulations on harvesting. The extent to which mopane worms contribute to defoliations and frass production, was determined by measuring and calculating the consumption rate and frass production for the landscape. Data show that a wide variation exists, which can be due to factors such as weather patterns, type of vegetation, predation, etc. This was followed up with a laboratory incubation trial to determine the difference between nutrient release from mopane worm frass (when a healthy population of mopane worms are present) with the mineralization and nutrient release from mopane leaves (when no mopane worms are present). More nutrients are released from frass compared with leaves, indicating that mopane worms facilitate nutrient cycling in the landscape.

This study highlighted the significance of mopane worms in the natural environment and then provided a suitable habitat for the worms that need management to ensure their sustainability.

Keywords: distribution, frass production, leaf consumption, mineralisation, protected areas, suitable habitat, utilisation.

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1 CHAPTER 1: INTRODUCTION



A yellow and black mopane worm feeding on a leaf of a mopane tree in the Ga-Selwane communal land, Phalaborwa.

1.1 Background

Edible insects have been part of the human diet for ages, with over 1900 species exploited as food (van Huis et al., 2013). Edible insects are full of nutrients, containing high levels of crucial nutrients such as protein, iron, and zinc and have thus been an essential food source in developing countries, enhancing food security. They have also been explored as animal feed for different animals such as poultry and fish (Moyo et al., 2019). In addition of being a food/feed source, they also provide seasonal income as they are traded in both the rural and urban markets where harvesters sell to consumers (Hlongwane et al., 2021). This highlights their importance to livelihoods and how they are vulnerable to over-harvesting.

While their importance in terms of provision have been clearly established (Nemadodzi et al., 2023), other services such as their influence to ecological function and nutrient cycling have been overlooked in studies (de Swardt et al., 2018) yet insects have been reported to contribute more to the ecological functioning through arthropod herbivory, with their biomass almost matching that of mammal herbivory (de Swardt et al., 2018). For example, when herbivorous insects feed on leaves, they release frass to the ground, which is often fast-decomposing, and together with their cadavers can enhance the nutrients pool in the environments, accelerating nutrients cycling (Reynolds & Hunter, 2004). Fürst et al. (2023) reported that the recent decline in arthropod herbivory have raised concerns on their conservation status. Coupled with the notion that they have high abundance, rapid life cycles and can replenish easily and are also seen as pests, this has resulted into little conservation efforts directed to them (Reid, 1999). Mopane worms have encountered the same fate of being highly valued as food and income source (Nemadodzi et al., 2023) resulting in an increase in demand, causing overexploitation. The potential impact of reduced populations due to overharvesting, habitat loss and climate change is poorly understood.

1.2 Problem statement

Mopane worms face multiple threats that endanger their survival. Overharvesting for food and commercial purposes has led to declining populations. Habitat destruction due to deforestation and agricultural conversion results in the removal of mopane trees. Climate change increases these pressures by changing rainfall patterns and temperatures, which influences the worm's survival and

breeding success. These combined threats could decrease mopane worm populations, impacting biodiversity and communities.

The lack of formal national laws over the use of insects means that the governance of their exploitation depends on the regulations in the land where they are being harvested, and they are mostly harvested in communal lands where the lands are under local chiefs. There is a knowledge gap on how much harvestable mopane worms are available and how much of this is already protected from harmful harvesting methods.

Mopane worms provide a critical ecosystem service in semi-arid regions by facilitating nutrient cycling in mopane woodland and forming part of the various food chains during all the instar phases. The knowledge on the conversion of mopane leaves to frass by mopane worms remains less studied, i.e., leaf consumption, frass production by worms, and how the frass aids in nutrient cycling in the ecosystem. Currently, mopane worms are extensively harvested, which can lead to local extinctions, and significant impacts on ecosystems. This study aims to contribute to the knowledge of mopane worm ecology and to understand the services provided and the value of mopane worms

1.3 Aims

The main aim of this study was to determine the extent of mopane worms' distribution across southern Africa, to evaluate to what extent harvesting is restricted or can potentially be addressed, as well as determine the potential ecosystem services of mopane worms by as a way to quantify their ecological importance and hence promote sustainable use of mopane worms.

The aim was divided into sub-aims for each data chapter in this thesis:

- To determine the current state of knowledge and research on mopane worms (Chapter 2)
- To determine the spatial distribution of mopane worms in southern Africa and how effective protected areas are in capturing these suitable habitats (Chapter 3)
- To determine the population dynamics and ecosystem services of mopane worms by measuring growth dynamics and nutrient cycling (Chapters 4 & 5)

1.4 Objectives

- Conduct a systematic review of the type of research topics, consolidate current knowledge, and identify gaps and future research directions on mopane worms (Chapter 2)
- Quantifying the suitable habitat of mopane worms in several southern African countries (Chapter 3)
- Estimating the effectiveness of protected areas to capture suitable habitats (Chapter 3)
- Exploring variables affecting the suitable habitat of mopane worms (Chapter 3)
- To determine the consumption rate of leaves by mopane worms and their rate of frass release at various instar stages of development (Chapter 4)
- Compare N-NH_4 and N-NO_3 release between mopane worms' frass and mopane leaves to indicate the rate of nutrient return between frass and mopane leaves (Chapter 5)

1.5 Thesis layout

This thesis is divided into four data chapters prepared as standalone papers that have either been submitted or are in preparation for submission. These chapters are Chapter 2: Mopane worms: A comprehensive overview of research efforts, Chapter 3: Using presence-only modelling to quantify the suitable habitat of a communally exploited edible insect, the mopane worm (*Gonimbrasia belina*), Chapter 4: Leaf consumption and frass production by the mopane worm (*Gonimbrasia belina*) in the Mapungubwe National Park, and Chapter 5: The role of mopane worms in nutrient cycling: Insights from nitrogen incubation studies. This is followed by a synthesis plus a conclusion report.

1.6 References

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2 CHAPTER 2: Mopane worms: A comprehensive overview of research efforts



Early instar mopane worms going through ecdysis in Ga-Selwane communal land, Phalaborwa.

Mopane worms: A comprehensive overview of research efforts

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2.1 Abstract

Mopane worms are widely distributed in south and central Africa and have traditionally been utilised as a food and protein source for humans and animals. Mopane worms contribute to the local economy and provide essential ecosystem services. Due to their high cultural significance, studies have focused mainly on the significance of mopane worms as a food and income source and less on their ecological role. High harvest levels, habitat loss and climate change could threaten mopane worms' sustainability. A systematic review was conducted to collate published research on mopane worms, to identify the *status quo* in current knowledge, and to identify research gaps and future research needs. There is a need to study and understand the ecology of this species and its possible status as an ecosystem engineer through nutrient cycling and as prey of other species. The review identified 151 peer-reviewed publications that fit the search criteria. More studies were in South Africa and Botswana (25% each), followed by Zimbabwe (22%). 78% of the studies focused on mopane worms' utilisation, while the remaining 22% focused on biology and ecology. The review indicates a limited understanding of the dynamics of outbreaks, making any statements about the sustainability of harvesting difficult. This review shows that there are still no rules of thumb for harvesting sustainably, but regulations or monitoring would be advised without more information.

Keywords: biology, ecology, review, sustainable use, utilisation

2.2 Introduction

"Mopane worms" are late instar stage caterpillars of the mopane moth, *Imbrasia belina* or *Gonimbrasia belina* (Westwood, 1849), from the Lepidoptera order and the Saturniidae family (Ditlhogo et al., 1996; Gondo et al., 2010). Mopane worms are distributed throughout southern Africa, including parts of Angola, northern Namibia, northern and eastern Botswana to Zimbabwe, central and northern Mozambique, Zambia, Malawi, and northern South Africa (Makhado et al., 2012).

Mopane worm outbreaks can occur twice a year, from November to January and from February to May (Kozanayi & Frost, 2002; Makhado et al., 2014). Early-stage caterpillars (instar stages I-III) live in cluster groups of 20-200; however, the caterpillars disperse during instar stage IV. At the end of instar stage V, the caterpillars crawl down to the base of their host tree, burrow into the ground, and pupate to emerge as a moth to lay eggs (Gondo et al., 2010; Makhado et al., 2014). Mopane worms are host specific, with mopane tree, *Colophospermum mopane* (J. Kirk ex Benth.) J Leonard Kirk J. (1860), being their most popular food plant in southern Africa.

Not only are mopane worms a traditional food source rich in protein (Potgieter et al., 2012) and are considered a delicacy by many people in southern Africa, they also provide food to animals during outbreak periods (Gaston et al., 1997; Madibela et al., 2007; Potgieter et al., 2012), are a source of income to local communities (Stack et al., 2003; Makhado et al., 2014; Baiyegunhi et al., 2015), and provide essential ecosystem services by facilitating nutrient cycling and contributing to soil fertility (De Swardt et al., 2018). Therefore, mopane worms play an essential role in food security, the economy and ecology of the region.

In mopane woodlands, the primary nutrient cycling process appears to be modulated by mammal herbivory (De Swardt et al., 2018). Grazing mammals increase foliar nitrogen (N) by excreting N in forms readily available for plants (De Swardt et al., 2018). However, leaf fall and decomposition are essential in the nutrient cycling in mopane woodlands. For example, although *C. mopane* is leguminous (Makhado et al., 2014), it does not "nodulate" (De Swardt et al., 2018), and as such, one of the mopane trees' main nutrient cycle pathways is through leaf flush, leaf fall, and decomposition. However, plant litter is not readily available as nutrients for plant uptake, as decomposition and subsequent release of plant-

available nutrients take time (Belovsky & Slade, 2000; Reynolds & Hunter, 2004). As such, processes that can facilitate the breakdown of mopane leaves into readily available plant nutrients would be essential for the functioning of mopane woodlands. One such process could be the consumption of leaves by the mopane worm and subsequent frass production.

Sustainable harvesting of mopane worms requires thorough knowledge of their biology and ecological role in mopane woodlands. This chapter reviews the current research to evaluate the type of research topics, consolidate current knowledge, and identify gaps and future research directions.

2.3 Materials and Methods

2.3.1 Selection of studies

A systematic literature review was conducted, covering 1961 to 2023, based on a priori-defined set of criteria to allow for replication of the review process. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement and Checklist) guidelines was followed when including or excluding publications during screening stages. The search engines used to find peer-reviewed research papers, included African Journals Online (AJOL), Google Scholar, and Web of Science. The online databases were searched using the keywords; 'mopane worms', 'mopane caterpillar', '*Imbrasia belina*', '*Imbrasia belina* caterpillars', and '*Gonimbrasia belina*.' All publications were evaluated according to the title, keywords and abstract to select only studies focusing on mopane worms. Grey literature, such as reports, presentations, dissertations, and book chapters, was not included. The Google Scholar search resulted in more than 1000 papers, and the evaluation of papers was stopped when there were no usable records in a sequence of more than 20 records. The records returned at this point consisted of grey literature, presentations, abstracts, or the same publications already captured. After removing duplicates from various databases, 151 papers were retained (Figure 2-1).

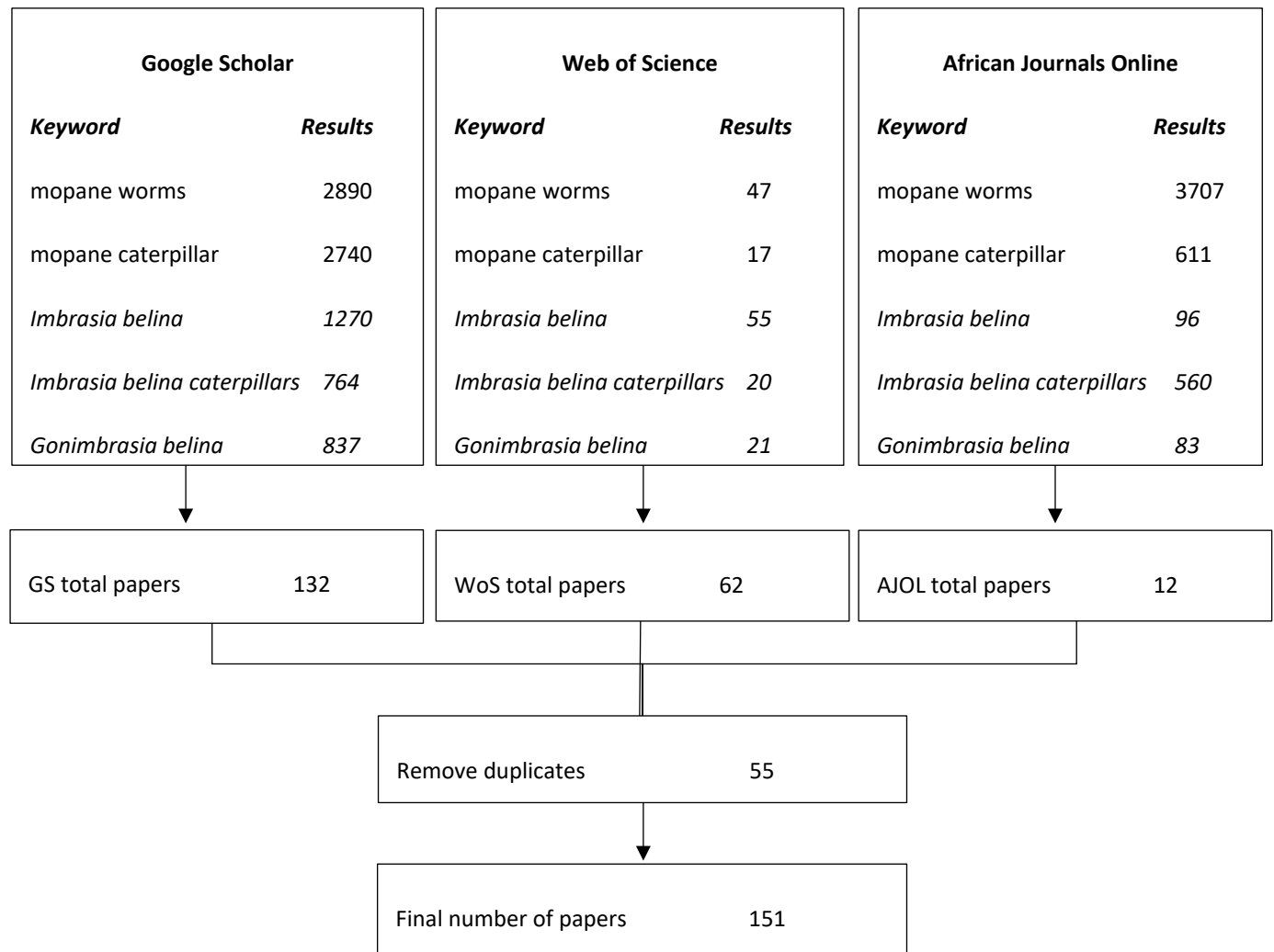


Figure 2-1. PRISMA flow diagram illustrating the literature search and selection process for studies included in the review.

Papers were categorised into seven themes: i) biology and life cycle, ii) ecological role, iii) cultural and economic significance, iv) sustainable management and conservation, v) processing and utilisation, and vi) health and safety. These seven themes were clustered into two broad groups, namely biology and ecology of mopane worms (biology and life cycle, ecological role), and secondly, utilisation of mopane worms (cultural and economic significance, sustainable management and conservation, processing and utilisation, health and safety). By organising the information into these two broad groups, the review effectively addressed the diverse aspects of mopane worm research, distinguishing between research focusing specifically on the natural aspects of mopane worms and research focusing on the utility of mopane worms, which includes sociocultural aspects, economy, consumption and subsequent results from mopane worm utilisation, such as health impacts and allergic reactions.

The following information was extracted from the selected publications: title, authors, publication date, study type (such as review publication or experimental trial), topic, study area (location of the study area), and main result.

2.3.2 Data analysis

The Chi-Square test (Franke et al., 2012) was used to investigate the effect of the country on the research theme and the number of studies. The Mann-Kendall test was used to test for a significant temporal trend in research publications (Pettitt, 1979) and Sen's estimator for the slope of the trend (Sen, 1968). Analysis was done in R (R Core Team, 2023) with the 'Kendall' package for trend analysis (McLeod, 2011) and the “trend” package for estimation of slope (Pohlert, 2016).

2.4 Results

2.4.1 Temporal research trend

A total of 151 papers met the selection criteria (Figure 2-1). Analysis reveals a significant increase in publications related to *G. belina* (Mann-Kendall test: $\tau = 0.642$, $p < 0.0001$; Figure 2-1A), with an average of 2.4 publications per year. However, the trends varied by study theme (Figures 2-1B & C). Publications focusing on the biology and ecology of mopane worms exhibited a positive trend (Mann-Kendall test: $\tau = 0.287$, $p = 0.004$; Figure 2-1B) during the review period, averaging 0.46 papers per year. In contrast, articles centred on utilising mopane worms showed a significant positive trend (Mann-Kendall test: $\tau = 0.714$, $p < 0.0001$; Figure 2-1C), averaging 1.9 papers per year. The slope of the trend for papers focused on utilisation was 0.063 ($z = 7.4593$, $p < 0.0001$), whereas the slope for biology and ecology papers was 0 ($z = 2.8463$, $p = 0.004$). This indicates that the publication rate for topics related to the use of mopane worms - such as health benefits, risks, consumption, harvesting, marketing and cultural and economic aspects, is approximately four times higher compared to those focused on biology and ecology.

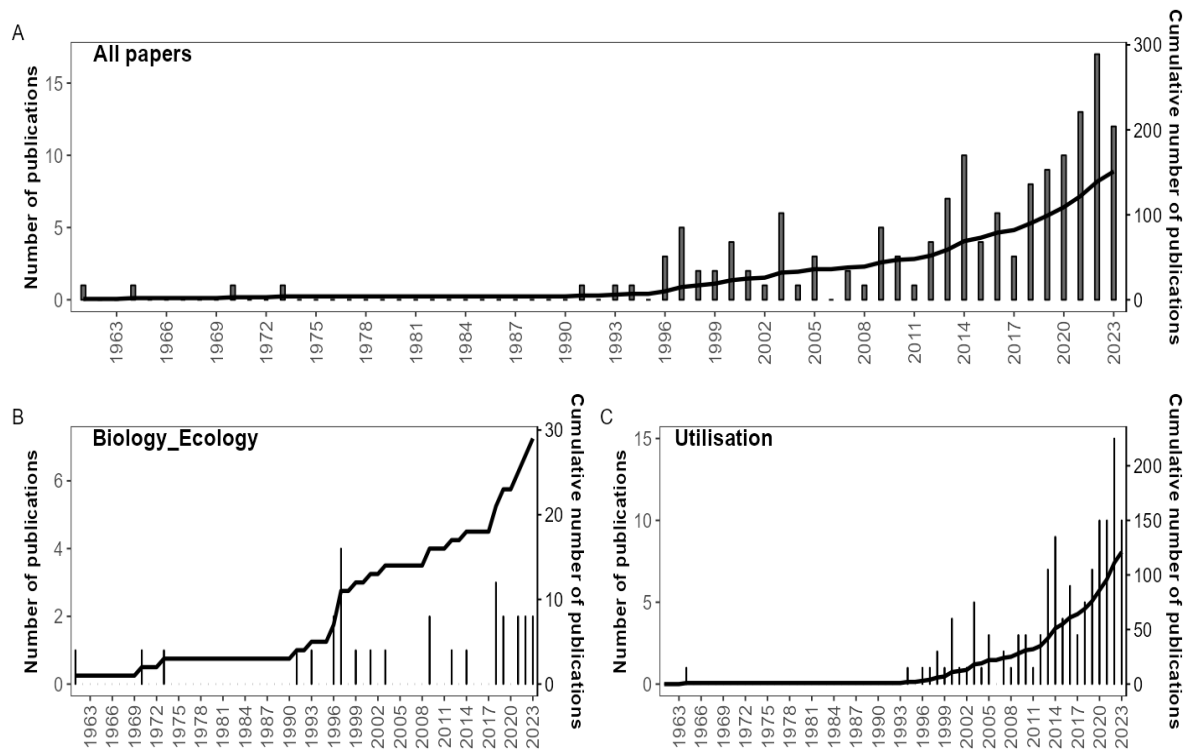


Figure 2-2. The trend in the number of research papers on mopane worms published per year for all papers (A), biology and ecology papers (B), and utilisation papers (C). The Y-axis gives the number of papers published per year, represented by the bars, while the secondary Y-axis represents the cumulative number of documents, represented by the black line.

The publication of studies in mopane worms only gained momentum in 1991. Earlier studies primarily investigated ecological aspects of mopane worms, such as the distribution and seasonal occurrence of worms in the Transvaal (Van den Berg et al., 1973) or the chemical composition of mopane worm guts (Cmelik, 1970; Glew et al., 1999). This increase in studies might be a response to the rise in pressure on natural resources in dry and semi-arid regions. Increased population growth and poverty in these areas increase pressure on natural resources, such as wood and wildlife (Makhado et al., 2012). As pressure on natural resources increases, some populations might become locally extinct, increasing food insecurity for local communities that depend on them (Kamwi et al., 2020). Increased pressure on food sources can highlight the importance of food security, local economies, and the ecological impact of

extinction on the environment, resulting in increased research interest and publications (Makhado et al., 2012; Kamwi et al., 2020).

Utilisation studies were more often on communal and private land where other forms of resource extraction, like wood harvesting, occur. In contrast, ecological studies were conducted primarily on reserves, particularly in South Africa. Such a spatial bias in studies could hamper the ability to inform policies around sustainable use and the ecological role of mopane worms. For example, the density of mopane worms in private land can be affected by factors not necessarily present in non-protected areas, including wood harvesting, variation in livestock grazing/browsing densities, and land clearing activities. By extrapolating mopane worm densities from protected areas, there is an overestimation of abundance in private areas, resulting in overharvesting.

2.4.2 *Spatial representation of studies*

A total of 193 study locations were identified and distributed in seven countries (Figure 2-2). Some publications listed several locations, while others were not linked to any specific locations (e.g. reviews). Study sites were spatially autocorrelated, concentrated in specific areas, and the number of studies varied between countries ($\chi^2_6 = 90.375$, $p < 0.000$; Figure 2-3). Most of the studies were carried out in South Africa and Zimbabwe (25% each), followed by Botswana (22%), Namibia (6%), and Zambia (3%), while single studies came from Malawi, Kenya, Nigeria and Congo. A total of 14% of the publications covered more extensive areas labelled as 'Southern Africa'. Despite the fact that mopane worms are widespread in southern Angola and central and northern Mozambique (Makhado et al., 2012), no published papers were found for these countries.

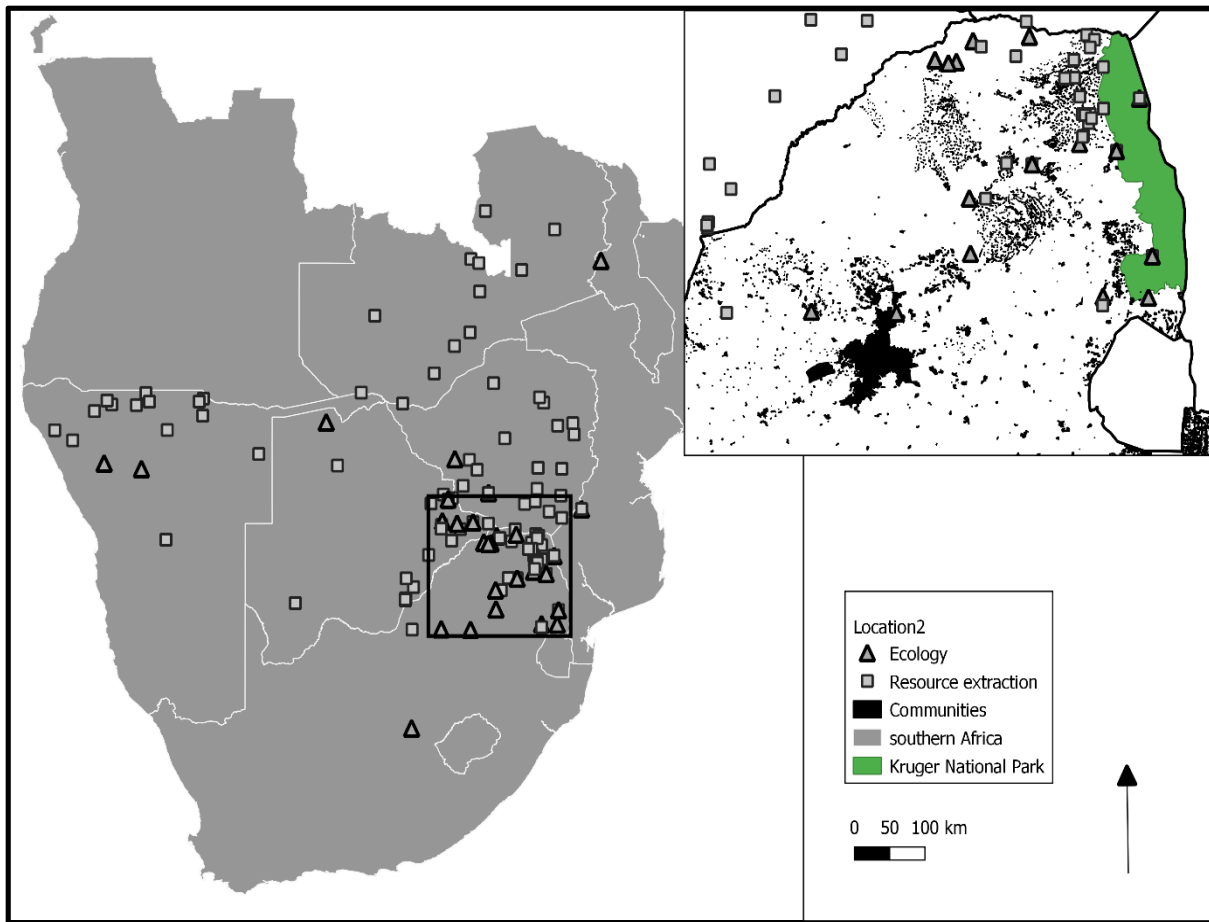


Figure 2-3. The spatial distribution of mopane worm studies done in Southern Africa

2.4.3 Study themes

The number of publications varied significantly among the broad themes ($X^2_1 = 43,104$, $p < 0.000$). Most of the research (78%) concentrated on utilisation, with biology and ecology comprising only 22% of the research effort (Figure 2-3A). To evaluate the effect of research under the broad themes and countries, all countries with less than two publications were pooled into 'other category' (Malawi, Nigeria, Zambia, Congo) to allow a robust chi-square test. There was a significant association between broad theme research and countries ($X^2_5 = 19.659$, $p = 0.001448$), where ecological research was primarily centred in South Africa (11 papers) and utilisation studies in Botswana (15 publications; Figure 2-3A). South Africa (8 publications), Zimbabwe (5 publications) and Botswana (4 publications) together represent 86% of all published research in biology and ecology (Figure 2-3A). Similarly, there was a strong association

between broad and sub-themes ($\chi^2_6 = 134$, $p < 0.00$; Figure 2-3B), where research under ecology and conservation was dominated by ‘ecological role’ (Figure 2-3B), while ‘nutritional value’ (37% of research) research dominated utilisation studies (Figure 2-3B).

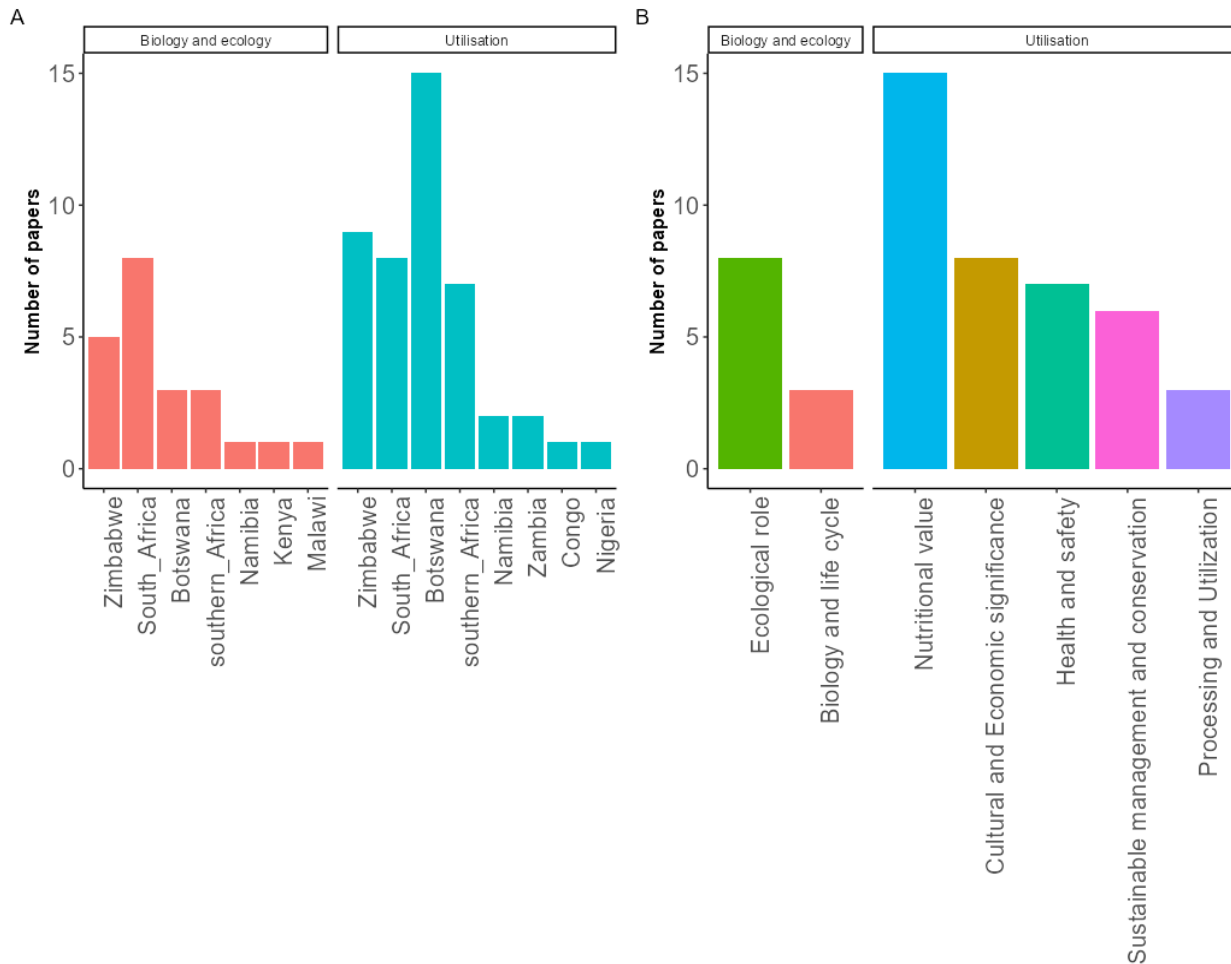


Figure 2-4. The number of papers published within different broad themes per country (A) and sub-themes per broad themes (B).

The biology and ecology of mopane worms were significantly overshadowed in research by their utilisation. Most studies focused on the utilisation of mopane worms, with the most common aspect being their nutritional value. Secondary aspects of utilisation, such as marketing, job creation, and allergic reactions due to the processing or consumption, were also included under this broad theme.

The ecological importance and conservation of insects are often overlooked in research and society as they are considered pests (Reid, 1999), with efforts mainly focused on larger mammals (Potgieter et al., 2012). Furthermore, the mopane worm populations undergo periodic population explosions, reaching millions of individuals (Thomas, 2013), which further suggests low conservation status or management actions.

A summary of the research extracted from the review papers is presented under each theme:

Biology

Greyling et al. (2001) found that two geographically distant (approximately 170 km apart) *G. belina* populations had high levels of polymorphism (42% and 47%) and heterozygosity (0.182 to 0.210). While their overall genetic divergence was insignificant, there was a significant gene flow between these two populations and a high variation. The different generations in a year were genetically similar, suggesting that two generations are not subspecies and that pupae from the first generation are the moths that emerge in the second generation (Greyling et al., 2001). In contrast, four morphologically distinct variants of mopane worms were found in KwaZulu Natal, South Africa (Fakazi et al., 2021).

The mitogenomes of *G. belina* and *Gynanisa maja* are the first representatives of African Saturniidae, thus laying a foundation for assessing the genetic diversity, population structure, and phylogeography of African edible worms (Langley et al., 2020)

Ecology

Most mopane worms' ecological studies studied the interaction between the host tree and habitat impacts, while some focused on habitat distribution and life cycles (such as Van den Berg et al. (1973) and Douglas (1991).

Although most of the mopane worms occurred in semi-arid regions of southern Africa, mopane worms are not entirely restricted to the mopane woodland and feed on several other trees, such as *Salix bebbiana* (willow), *Searsia lancea* (karee), *Protorhus longifolia* (red beech), *Terminalia sericea* and *Ficus*

craterostoma (wild fig) (Douglas, 1991), *Sclerocarya birrea* (marula) (Fakazi et al., 2021), *Anacardium occidentale* (cashew nut) (Kusia et al., 2021), and *Julbernardia paniculata* (Mughogho & Munthali, 1993). However, mopane worms could not ingest and assimilate *T. sericea* leaves and showed slower growth rates when feeding on *S. birrea*, while *C. mopane* provided better nutrition than *S. birrea* (Teferra et al., 2000). This could be co-evolution rather than preference, i.e. the presence of more mopane trees resulted in mopane worms co-evolving and becoming more adapted to mopane leaves.

Some studies attempted to relate mopane tree properties with mopane worm occurrence. Leaf nutrition (Nunu et al., 2019) or tannin levels (Mufandaedza et al., 2018) did not influence their distribution. However, Sibula and Dube (2022) found that tannins are not produced by mopane trees when the trees are completely defoliated, compared to partial damage, therefore not a feeding deterrent for mopane worms. Hrabar et al. (2009b) also found that the nutritional status of leaves does not influence mopane moth oviposition. These studies suggest that leaf chemistry does not influence egg mass density, implying that mopane moths prefer resource quantity rather than quality (Ditlhogo et al., 1996; Hrabar et al., 2009b; Hrabar & Du Toit, 2014). This was further supported by Wiggins (1997a), who found that eggs are more likely to be deposited in clusters on conspecific trees, while small trees or solitary trees rarely receive egg clutches. Other aspects that can determine outbreaks include parasitism, time of the year, and amount of rainfall (Bara et al., 2022).

Wiggins (1997b) examined fluctuating asymmetry in leaf length and mean leaf size and found variation in the presence of eggs. However, Hrabar et al. (2009b) found that leaf size seems to influence the presence of eggs, at least in Venetia Nature Reserve, because the trees that mopane moths preferred had larger leaves. Preferred trees were also significantly taller, had greater shoot weight, and had larger shoot biomass than neighbouring non-utilised trees. The abundance of egg mass also showed an increase with increasing tree height. Trees with larger canopies were preferred (Hrabar et al., 2009b). Thus, the tree size primarily influences oviposition at the individual tree level. However, this is not the only driver in tree selection, and this topic needs further research (Hrabar et al., 2009b).

Mopane worms not only remove leaves but also impact host tree regrowth. When mopane worm defoliation was compared to natural pruning (e.g., elephants (*Loxodonta africana* Blumenbach, 1825), the natural pruning resulted in longer shoots and leaves, whereas defoliation produced shorter shoots and leaves (Hrabar et al., 2009a). Despite the difference between pruning and defoliation, there was no

visible trade-off in investment between tolerance and resistance, as mopane trees are adapted to both (Hrabar et al., 2009a). Heavy pruning resulted in a halving of egg masses observed in the next season because of less leaf biomass (Hrabar & Du Toit, 2014). The worms' defoliation pressure also affects the trees' fecundity (Ditlhogo et al., 1996). Only 14% of defoliated trees produced seeds, in contrast to 84% undefoliated trees (Ditlhogo et al., 1996).

Burning is another aspect that impacts host plant preference. Early burning increased the preference of mopane worms for *Brachystegia spiciformis*, *Terminalia sericea* and *Diplorhynchus condylocarpon* (Müll.Arg.), while late burning increased mopane worms preference for *Brachystegia manga* (Mughogho & Munthali, 1993). The persistent utilisation of mopane veld by mammalian herbivores and the presence of ants negatively impacted the worms' presence and resulted in the localised absence of the worms (Styles & Skinner, 1996).

Aspects of nutrient cycling and soil quality have been studied as well. The nutrient cycling effect of mopane worms was studied by De Swardt et al. (2018), who suggested that defoliation and frass release may influence nutrient cycling, especially potassium (K) and, to some extent, phosphorus (P), in their environment. Leaf litter, together with frass, resulted in higher K and P values in the soil under mopane worm-infected areas. Styles and Skinner (1996) observed the presence of mopane worms exoskeletons in fine textured soil in areas signifying occurrence of mopane worms as opposed to the absence of the exoskeletons in very coarse textured and rocky soil (Styles & Skinner, 1996). Bangira et al. (2023) similarly found that mopane worms were more likely to burrow and pupate in less compacted sandy soil with relatively high organic matter and leaf litter. Mufandaedza et al. (2018) found that soil texture only influences the worms' distribution indirectly through the distribution of mopane trees southeastern Lowveld of Zimbabwe, as soil chemical properties such as N and P did not affect the worms' distribution (Mufandaedza et al., 2018).

Only one study investigated the effects of competition between *G. belina* and *G. maia* in Kasungu National Park, Malawi (Mughogho & Munthali, 1993). Both species prefer similar food but are able to exist without competition by feeding at different heights in the same tree. This divergence may have evolved due to interspecific competition for the same food. The abundance of the two species varied between tree host and height. *G. belina* was dominant in *Brachystegia spiciformis* at heights over 3 m,

while *G. maia* was abundant at the lower height classes. Both feed on mature leaves rather than a new flush (Mughogho & Munthali, 1993).

Regarding the effect of climate and precisely temperatures, Frears et al. (1997) noted that mopane worms have physiological mechanisms for thermoregulation and found the only behavioural mechanism to reduce high temperatures is hanging upside down. Frears et al., 1999 reported that while temperature influences the worms' foraging activity of worms, it is not the sole factor influencing foraging, but factors such as predation also have an impact.

Nutritional value

The ever-growing human population has driven the search for alternate protein sources. Insects as a protein source have attracted increasing attention (Siulapwa et al., 2014; Chagwena et al., 2019), and in semi-arid regions, seasonally abundant mopane worms could be an ideal protein replacement (Illgner & Nel, 2000; Obopile & Seeletso, 2013; Nantanga & Amakali, 2020). With a protein content measured at 50% (Rapatsa and Moyo, 2022) to 55% (Mnisi et al., 2022), these worms are a significant food source for many rural communities, and most studies in this review focused on the value thereof for human and animal nutrition.

Around 250 insects in Africa are consumed by humans (Van Huis, 2003), and mopane worms are among the most popular ones (Quin, 1964; Dube et al., 2013). A survey of schools in Limpopo found that up to 60% of students included mopane worms in their food intake (Mbhenyane et al., 2005). Similarly, Baiyegunhi et al. (2016) found that 61.7% of the sampled population in the Mopani district (Limpopo province) consumed the worms at least three to five times weekly. Only 10% of the study sample in Zimbabwe did not eat insects, mainly because of religion, stigma, or repulsion (Dube et al., 2013).

Cmelik (1970) studied the sterols in the diet and some organs of the worm and found that the main sterol from the mopane tree was B-sitosterol, while the sterol fractions of the worm's organs were made of mixtures. The gut and fat bodies were rich in cholesterol as a major component, along with smaller amounts of β -sitosterol, campesterol, and di-unsaturated sterol (Cmelik, 1970). He also conducted laboratory studies, measuring the fat content of insects (Cmelik, 1969). There was no significant

difference in the nutritional content of worms from different regions (Jennings et al., 2001). Mopane worms have a comparatively high nutritive value; with a protein content of 38-74 % (Motshegwe et al., 1998; Moyo et al., 2019, Moyo et al., 2021), or more specifically, 55-57 % (Jennings et al., 2001; Siulapwa et al., 2014; Dube et al., 2013; Musundire et al., 2016), fat content of 10 % (Siulapwa et al., 2014) to 16 % (Jennings et al., 2001), and carbohydrate content of 7.8 % (Siulapwa et al., 2014). They also contain high quantities of essential minerals, including Ca, Fe, Mg, Mn, and Zn (Glew et al., 1999), as well as a high content of selenium (0.12 g/100 g), magnesium (0.17 g/100 g), zinc (0.011 g/100 g) (Kapepula et al., 2022), Na (42.1 mg/100 g), Fe (26.7 mg/100 g) (Amadi and Kiin-Kabari, 2016) with Fe at 122-350 % of the Daily Recommended Intake (DRI), and Zn at 125 – 450 % (Payne et al., 2015). The protein, zinc, and iron contents are higher than those of cowpeas (Nantanga & Amakali, 2020).

Mopane worms contained ten essential amino acids in varying amounts (Amadi & Kiin-Kabari, 2016). The predominant amino acid was glutamic, and the essential amino acid was arginine (Siulapwa et al., 2014). The primary fatty acids included palmitic, oleic, and linoleic (Amadi and Kiin-Kabari, 2016), of which linoleic acid was the most abundant (Motshegwe et al., 1998; Yeboah and Mitei, 2009; Pharithi et al., 2004); however, a significant variation in the composition of palmitic and linoleic acids in the lipid content from early III to late V instar phase was observed (Pharithi et al., 2004). Oil profiles are comparable to that of seed oil (Motshegwe et al., 1998; Zinzombe and George, 1994), which is considered a healthier source of essential fatty acids as compared to other animal food sources (Motshegwe et al., 1998).

Compared to ox, pig or chicken, the worms' cholesterol content was relatively high. However, the presence of a significant amount of β -sitosterol and campesterol (which are blood plasma cholesterol-lowering phytosterols) can mitigate this relatively high cholesterol (Yeboah & Mitei, 2009).

Besides humans, worms also provide food for different animals as they possess protein, Zn, Fe (Moyo et al., 2021), an adequate protein replacement for fish nutrition (Rapatsa and Moyo, 2022), ruminants (Madibela et al., 2009), broilers, layers, and guinea fowl (Moyo et al., 2019). Mopane worms can replace soybean products in quail diets without compromising the quails' performance, health, or meat quality (Marareni & Mnisi, 2020). Compared to fish meal and soybean, mopane worms contain greater concentrations of essential amino acids, such as threonine, valine, tryptophan, and phenylalanine. Thus, its inclusion into diets may balance the essential amino acids level. Mopane worms are composed of all

essential amino acids required by tilapia (Rapatsa & Moyo, 2017) and are a lipid source that provides essential fatty acids (Moyo et al., 2019; Olaleya et al., 2021), with cholesterol as the significant sterol present. The most abundant fatty acid was palmitic acid at 29.3%. Mopane worms had 4.36% of docosahexaenoic acid (DHA). The total saturated fatty acids were high (39.9%) (Olaleya et al., 2021). Unsaturated fatty acids can improve the concentration of conjugated linoleic acids (Madibela et al., 2007). While it has high levels of omega-6 fatty acid, the low omega-3 fatty acid is low and should be supplemented with food rich in omega-3 (Olaleya et al., 2021). No significant difference was found in the dietary fat levels of mopane worm meal and that of fish meal (Moyo et al., 2019). Urea content was the only biochemical component that increased in response to mopane worm meal (Nyuliwe et al., 2022).

Inclusion of 4.5% (Nobo et al., 2012a), 9% (Chiripasi et al., 2013), 11% (Nyuliwe et al., 2022), 12% (Moyo et al., 2021) and 13% (Mogwase et al., 2018) of mopane worm meal for animal consumption were deemed safe for animal feed and it was found that it would not affect growth patterns, mineral intake, or taste of meat products. Similarly, mopane worm inclusion did not affect Tswana hens' growth rate, egg production (Manyeula et al., 2018) or meat taste (Manyeula et al., 2019). However, too much mopane worm meal (higher than 13%) could negatively affect growth. It is possibly unpalatable and reduces the overall food intake (Nobo et al., 2012a; Nobo et al., 2012b; Moyo et al., 2019). Much higher ratios of mopane worm substitutions were tested for tilapia nutrition: up to 60% inclusion was considered optimal (Rapatsa and Moyo, 2017), while others suggested 50% inclusion yielded the highest growth performance (Mwimanzi & Musuka, 2014). In contrast, Rapatsa & Moyo (2019) reported too high mopane worm inclusion could lead to liver degradation.

Mopane worms were found to have a lower protein efficiency ratio than other edible insects, possibly due to limited amino acids (Chagwena et al., 2019). It also contains 27% chitin, which could impede digestion, thus limiting nutrient absorption (Kwiri et al., 2014).

Regardless of the potential as poultry and fish meal, factors such as chitin, the risk of overexploitation, unpalatability, and indigestion caused by higher inclusion levels in diets may limit the use of mopane worm as protein substitutes in animal feed (Moyo et al., 2019). Rasengwatshe and Madibela (2005) also found that mopane worm has a higher effective degradability than carcass meal. Thus, more mopane worms would be fermented in the rumen than carcass meal.

Cultural and economic significance

Mopane worm harvesting has monetary, economic, and social benefits (Swemmer et al., 2015) and is particularly important in rural livelihoods. They supplement nutritional requirements and generate income and up to 10,000 seasonal employments in southern Africa (Makhado et al., 2014). In Zambia, Mbata & Chidumayo (2003) estimated that an average family of six can generate US\$309 from sun-dried worms per harvesting season, more than they generated from agricultural produce.

Several socio-economic factors influence mopane worm harvesting (Hope et al., 2009; Baiyegunhi et al., 2015). This included age, gender, education, income from mopane worm sales, social capital (group affiliation), household size, lack of institution/law to regulate the use, distance to the nearest harvesting site, and regional locations (Hope et al., 2009; Baiyegunhi et al., 2015). Although poorer households tend to harvest more, mopane worms are collected by households from all income groups (Stack et al., 2003). Thus, it is predicted that the increase in profitability and productivity could exclude poorer communities, as better-off communities have access to technologies that can lead to overexploitation (Stack et al., 2003). Stack et al. (2003) also reported the trend that mopane worms had been traditionally harvested as subsistence food; however, there has been a shift to trade, e.g., in some sites in Zimbabwe, 59% of mopane worms' stocks were sold for cash, 19% exchanged for goods, while only 9% were consumed at home. While 75% of the respondents in the Kruger National Park consumed all their harvested worms at home, others sold their harvest and earned an average amount of R517.14 per person (Swemmer et al., 2020). Mbata and Chidumayo (2003) found that even when harvest volumes are relatively small (an average of 19.3 litres per household), it can contribute positively to households in terms of consumption and income generation in Zambia.

There is a need to improve rural livelihoods by balancing utilisation and conservation (Baiyegunhi et al., 2016). Most (96%) of the harvesters are female (Stack et al., 2003) and are also more dependent on mopane worms as an income source (Togarepi et al., 2020). However, prevailing institutions and practices in rural communities must be more inclusive towards men. However, Mabossy-Mobouna et al. (2022) reported that women were the primary mopane worm marketers, which helped with income generation for them, highlighting the need for national and local governments and local authorities to create inclusive institutions and involve women in decision-making and increase their access to resources (Hlongwane et al., 2021; Sithole et al., 2021).

Consumers (81.7%) and harvesters (76.6%) are the primary contributors to the mopane worm value chain. Factors that affect this value chain include gender, household size, marital status, religion, and household assets (Manditsera et al., 2022). As much as 63% of harvested worms in the Mopani district, South Africa, are sold within a production year (Baiyegunhi & Oppong, 2016). The product's utility is a function of its form, location, concentration, and availability time. Changes in these attributes along the marketing chains add value to the product as it moves from producers (collectors) to consumers (Kozanayi & Frost, 2002). Factors influencing commercialisation decisions and intensity were household head, household size, income, the average distance to the nearest market, transporting, communication assets, and lack of institutions/laws regulating the use and management of woodland resources (Baiyegunhi & Oppong, 2016). Export prices are highly influenced by local availability, especially close to the border of South Africa, where the price for mopane worms is higher than local prices in Botswana or Zimbabwe.

Sustainable management and conservation

Some studies suggest that mopane worms are being used in an unsustainable way (Gondo, 2010; Thomas, 2013; Kusia et al., 2023). Several factors affect the sustainable use of mopane worms. These include a growing demand both locally and in urban populations, urbanisation, increased human activities (Togarepi et al., 2020), rise in prices in urban markets (which increase financial incentives), maximising harvest volume (which can lead to overexploitation, Roberts, 1998; Thomas, 2013; Baiyegunhi & Oppong, 2016), burning of mopane veld (Togarepi et al., 2020), damage to mopane trees during harvesting process (Roberts, 1998) and disregarding indigenous knowledge (Ngwenya et al., 2021). Furthermore, human settlement growth results in reduced vegetation cover and mopane woodlands and, consequently, a decline in harvests (Ndlovu et al., 2019). Natural factors causing mopane worms' decline included increased temperatures and unreliable rainfall (Ngwenya et al., 2021).

The lack of regulatory laws to govern mopane worms' extraction gives rise to excessive harvesting or damage to mopane trees, and thus, the government needs to establish regulations and rules to control harvesting (Baiyegunhi & Oppong, 2016; Moyo et al., 2019), not just locally, but also among African countries to coordinate sustainable use (Musundire et al., 2021). A study in the Vhembe and Mopani districts showed that private and public-governed land exercised more control and monitored mopane

worm harvesting than communal systems, as one must negotiate and pay a set price for a specified period (Sekonya et al., 2020). There should also be strict regulatory laws to govern the harvesting of mopane trees for firewood or logs by giant commercial companies.

Measures to improve sustainability include improving property rights and institutional frameworks that regulate mopane worm utilisation by supporting communities to establish Indigenous natural resource management systems (Thomas, 2013), allocating woodlots to members of the community (Dube and Dube, 2010), and developing policies and regulations, either at the community level or self-organised governance (Ndeinoma and Wiersum, 2017), such as restricted harvest periods, a tax to account for undervaluation of the value of mopane worms (Akpalu et al., 2007) or properly enforced permit system (Mufandaedza et al., 2015).

Processing and utilisation

Post-harvest processing and storage of mopane worms impact the quality or safety of their consumption (Dangwa et al., 2014; Kachapulula et al., 2018), and the need to improve and standardise current culturally acceptable processing techniques to support sustainable and safe processing has been identified (Manditsera et al., 2022). A review revealed that processing methods such as boiling, frying, and roasting significantly affect the safety of edible insects (Murefu et al., 2019; Potgieter & Ramalivhana, 2020). Degutting before preparation decreases bacteria (Mujuru et al., 2014), improves the quality of the end product, and increases the protein concentration and digestibility (Madibela et al., 2007; Madibela et al., 2009; Ruzengwe et al., 2022). Cooking and frying reduce metal concentrations (Murefu et al., 2023), while it is recommended to boil the worms for 20 minutes and dry at 40°C before further processing into various products (Ruzengwe et al., 2023). Although cooking is the recommended method, it can result in varying nutrient concentrations because it was reported to affect nutrient retention in the worms (Hlongwane et al., 2022). When mopane worms that were field-dried and laboratory-dried were tested for microorganisms, both types were contaminated with fungi and bacteria, including *E. coli*, *K. pneumoniae*, *A. flavus*, *Penicillium* sp, and *Fusarium* sp (Gashe et al., 1997; Simpanya et al., 2000). Climatic factors can also influence post-harvest processes; for example, if mopane worms are harvested during heavy rain, the humidity can interfere with the harvesting and drying process, causing spoiled products (Mataboge et al., 2016).

Studies on dried stored mopane worms indicated they were subject to pest infestation, including bacteria, moulds, insects (Mpuchane et al., 2000), and fungi (Nawases et al., 2018). The preferred packaging that can help maintain the quality of stored products was identified as high-density polyethylene, which outperformed traditional storage methods (Monyetsware and Marenya, 2023).

Health and Safety

In some communities, the popularity of mopane worms has declined (Obopile & Seeletso, 2013) due to fear of recent cases of allergic reactions (Kung et al., 2013) or religious reasons such as the Zion Christian Church, which forbids its members from consuming insects (Baiyegunhi et al., 2015), for example approximately 26% of sampled households were form part of religious groups that prohibit the consumption of mopane worms (Baiyegunhi et al., 2015).

Some potential risks exist in consuming and handling mopane worms. Collectors of mopane worms are more exposed and thus have a higher risk of respiratory-related problems because mopane worms were found to be an inhalant allergen (Ndlovu et al., 2021; Ndlovu et al., 2022). A few instances of allergic reactions to mopane worm consumption were described by Okezie et al. (2010), Kung et al. (2013), Potter (2013), and Ribeiro et al. (2017). Symptoms included headache, dyspnoea, cough, wheezing, and tongue and lip swelling within 10 minutes after consumption (Kung et al., 2011). Although allergens are associated with the presence of polymorphism tropomyosin gene (Dlamini et al., 2023), contributing factors for allergic reactions can also be the presence of microbes, yeast and mould. Regarding microbiological quality in open markets, Ramashia et al. (2020) found low food safety and hygiene standards. All dried insects were sold from open containers, with insects handled with bare hands. Vendors also lacked appropriate shelters and clean, suitable utensils (Ramashia et al., 2020). Yeast and mould were some of the microbial infections found, and although these were still within an acceptable range, it indicates possible sources of contamination (Ramashia et al., 2020; Potgieter & Ramalivhana, 2020). According to Mpuchane et al. (1996), fungi, such as *Aspergillus*, can occur in up to 47% of samples and reach toxic levels in some postharvest conditions.

Another health-related concern regarding mopane worm consumption is heavy metal contamination. Pollution from industries and mines could contaminate surrounding trees by heavy metal deposition on

leaves. Ekosse et al. (2005) found heavy metal concentrations (Ca, Cu, Al, Zn, Co, Cd & Mn) in mopane worms close to a smelter were higher than those from 56 km away. It is recommended that food safety regulations should be in place as these have potential health risks when consuming mopane worms that feed on these leaves (Greenfield et al., 2014).

2.5 Conclusion

This literature review highlighted the spatial and temporal trends in mopane worm research. Although mopane worms extend to countries north of the Kunene and Zambezi rivers, there was a spatial disparity in terms of studies in these areas. They highlighted the gap in knowledge on mopane worms' studies and distribution that still exists, as there is limited knowledge about the biology, ecology, and utilisation of mopane worms in countries north of the Kunene and Zambezi rivers. In terms of the study theme, most research focused on the utilisation and socioeconomic impacts of mopane worms as contrasted to the biology and ecology of these worms. Ecological research and topics related to the ecological role of mopane worms in the ecosystem received less attention. Only 22 % of the papers focused on biology and ecology, while 78 % focused on utilising mopane worms. Several subthemes were identified under the broad category of 'utilisation', including 'nutrition', 'sustainability and conservation', 'culture and economics', 'health and safety', and 'processing and utilisation'. Of these subgroups, most papers focused on nutrition, emphasising the use of mopane worms as food and fewer concerns around sustainability. Very few studies have, therefore, considered the long-term sustainability of local populations.

The published research shows that the worms provide a readily available and cheaper animal protein source that contains higher quantities of protein, fat, carbohydrates, and valuable minerals in comparative levels. This provisioning extends to humans, livestock, poultry and fish. Several researchers highlighted the importance of retaining the tradition of entomophagy, not only for its nutritional benefits but also for its cultural and social significance. Entomophagy, the practice of eating insects, is deeply rooted in the culture of many communities where mopane worms are consumed, and its continuation is vital for maintaining cultural identity and social cohesion.

The financial influence of mopane worms is significant to the livelihoods of those involved in harvesting and trading. Mopane worms' commercial utilisation depends on the correct post-harvest practices, and educating harvesters could improve the cleanliness and safety of mopane worms' consumption. Additionally, semi-domestication and domestication, which involve rearing mopane worms in controlled environments, should be explored as viable alternatives. These practices could potentially increase the availability of mopane worms while alleviating the pressure on wild populations and improving the quality and safety of the product.

While this review highlighted the limited research on ecology, several relevant topics emerged. Mopane worms are important mopane tree defoliators and could play a significant role in nutrient cycling, tree-grass dynamics and ecosystem stability. However, the extent of this role remains unexplored. Studies that quantify the significance of this process to mopane veld are critical so an appropriate value can be placed on services provided by mopane worms.

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Authors contribution

MWN:	Literature search, data extraction, writing, editing.
CMS	Conceptualisation, literature search, data extraction, writing, editing,
AJG	Writing, advising, editing.
LHS	Conceptualisation, data analysis, writing, editing.

Data availability statement

Data will be made available on publication at dryad.com

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Conflict of interest statement

The authors declare no conflicts of interest.

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3 CHAPTER 3: Using presence-only modelling to quantify the suitable habitat of a communally exploited edible insect, the mopane worm (*Gonimbrasia belina*).



Yellow and orange mopane worm feeding on a leaf of the mopane tree in Ga-Selwane communal land, Phalaborwa.

Using presence-only modelling to quantify the suitable habitat of a communally exploited edible insect, the mopane worm (*Gonimbrasia belina*).

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3.1 Abstract

The mopane worm (caterpillar stage of *Gonimbrasia belina*), a communally exploited edible insect, is a crucial component in the savanna ecosystems. Harvesting remains informal, largely unregulated, and mostly on communal and private land. Recent increases in demand have raised concerns about sustainability. This study takes the initial step toward developing sustainable harvest strategies by quantifying suitable habitat, estimating each country's contribution to total suitable habitat, estimating the effectiveness of protected areas to capture suitable habitat, and exploring variables affecting the suitable habitat of mopane worms. This study used presence-only modelling to create full and restricted ensemble models by combining generalised additive models (GAM), MAXENT, and random forests (RF). The restricted model was restricted to mopane woodland (*Colophospermum mopane*) to evaluate potential populations which can sustain harvest. The full and restricted ensemble models classified 15.1% and 40% of the model landscape as suitable for mopane worms, respectively. Under the restricted model, Zimbabwe contributed 26.8% to the suitable habitat, followed by Botswana (25.3%), South Africa (15.4%), Namibia (14.7%), and Mozambique (11.1%). In terms of protected areas, 36.2% of

the suitable habitat fell within protected areas, while the remaining 63.2% fell in non-protected areas. It is concluded that local communities, governments, and regional conservation authorities should address the harvest management of this communally exploited edible insect.

Keywords: biodiversity conservation, communal resources, protected areas, suitable habitat

3.2 Introduction

Insects represent the most abundant group of organisms on Earth, surpassing the biomass of all other animals by up to 30% (Dube et al., 2013). Their high abundance, rapid life cycles and high nutritional and protein content have made insects readily accessible and appealing food sources used by humans, as well as a protein substitute for animal feed (Banjo et al., 2006; Dube et al., 2013; Chagwena et al., 2019). In rural southern Africa, insect protein is often substituted for animal protein, which is crucial to combating malnutrition (Dube et al., 2013). Consequently, they are harvested and traded, providing seasonal income and supporting rural livelihoods. This growing demand for insects as food and income sources drive their commercialisation (Baiyegunhi & Oppong, 2016). Specifically, the trade of mopane worms, influenced by outbreaks and prevailing weather conditions, can generate between \$ 39 and \$ 100 million annually (Potgieter et al., 2012).

In Africa, insect protein plays a crucial role in human nutrition, with regional preferences varying significantly. For example, palm weevil grubs (*Rhynchophorus phoenicis fabr.*) and termites (*Macrotermes bellicosus*) are favoured in western Nigeria, particularly in the Delta and Edo states (Banjo et al., 2006). In Uganda, termites (*M. bellicosus*), grasshoppers (*Zonocerus variegatus*), and beetles (*Analeptes trifasciata*) are preferred (Banjo et al., 2006). Mopane worms (*Gonimbrasia belina*) are highly popular in southern African countries such as Botswana (Obopile & Seeletso, 2013), Namibia (Thomas, 2013), South Africa (Baiyegunhi & Oppong, 2016), and Zimbabwe (Chagwena et al., 2019). The dual role of insects as food and income sources can lead to conflicts between utilisation and conservation, particularly in the absence of formal harvesting laws or policies (Sekonya et al., 2020). Sustainability is further threatened by factors such as habitat loss and the changing climate. Since one of the important hosts for mopane worms is the mopane trees (*Colophospermum mopane*), any disruption to the

mopane veld, including wood harvesting and urbanisation, can adversely affect their populations (Togarepi et al., 2020) and decrease the ability to maintain a sustainable mopane veld environment.

Although formal regulations still need to be improved in insect harvesting (Sekonya et al., 2020), many cultural or local restrictions are in place to guide harvest (Mapendembe & Mujere, 2018; Musundire et al., 2021). For example, in the Uukwaluudhi area, Namibia, there are by-laws to govern mopane worm harvesting, including announcing the commencement of the harvesting period, permits for harvesters, and prohibiting illegal felling of host trees (Thomas, 2013). On communal lands in South Africa, chiefs announce the beginning of harvesting to limit early harvesting, while in protected areas such as the Kruger National Park (Sekonya et al., 2020), rangers monitor harvest rates to ensure sustainability. As such, edible insects are primarily harvested in communal areas where rural communal stewardship persists (Musundire et al., 2021). However, these stewardship programs face significant pressure and are eroded in many communities (Mbata et al., 2003; Musundire et al., 2021), necessitating the need to develop sustainable harvest strategies and conservation plans.

Species distribution models (SDM) are used to assess the distributions of species and identify suitable habitats, informing management strategies. These models rely on species-occurrence data, including either presence-absence or presence-only records (Tsoar et al., 2007). Despite the variety of edible insect species, research on their distribution is often limited to basic assessments of the extent of occurrence (Lobo, 2016). Recent advances in presence-only models have significantly enhanced their ability to address the limitations inherent in presence-only data (Tsoar et al., 2007; Montoya-Jiménez et al., 2022). SDMs now regularly include multiple algorithms (e.g., random forest (RF), maximum entropy models (MAXENT), generalised linear models (GLM), and generalised additive model (GAM; Bai et al., 2022), which, when combined, produce ensemble models for more improved model performance (Meller et al., 2014). Presence-only models have been used for conservation planning of many species (Tsoar et al., 2007), including predicting the potential distribution of insects (Fand et al., 2014; Fand et al., 2020; Fan et al., 2020; Pangga et al., 2021) and informing the International Union for Conservation of Nature (IUCN) Red List (Syfert et al., 2014). Ngarega et al. (2021) used Maxent to forecast the effects of climate change on the potential distribution of the mopane tree (Ngarega et al., 2021), while Shen et al. (2023) used bioclimate envelopes (BIOCLIM), GLM, and RF to forecast the possible decline in the distribution of mopane worms due to climate change. Therefore, SDMs can be an important tool for

developing potential distribution models for edible insects, which can serve as a basis for formulating sustainable harvest plans.

This study used presence-only modelling on a communally exploited edible insect, the mopane worm, to take the initial step toward developing sustainable harvest strategies by firstly quantifying the suitable habitat of mopane worms in several southern African countries, secondly estimating the effectiveness of conservation areas to capture suitable habitat, and, thirdly, exploring variables affecting the suitable habitat of mopane worms. The mopane worm is the larvae/caterpillar of the mopane emperor moth (*Gonimbrasia belina*) and will be referred to by the colloquial name ‘mopane worm’ throughout the text.

3.3 Materials and Methods

3.3.1 Occurrence data

Mopane worms’ occurrence (presence-points) data was downloaded from the Global Biodiversity Information Facility (GBIF.org, 2022). Each presence point included a country of origin, a date, a spatial coordinate, and a basis of recognition (human observation, preserved specimen, or material sample, Supplementary Figure S1). The key area of interest were areas south of the Kunene and Zambezi rivers, which include Namibia, Botswana, South Africa, Zimbabwe and Mozambique south of the Zambezi.

3.3.2 Predictor data

Climate and soil properties were included as predictor variables based on their ecological importance to mopane worms’ distribution (Table 3-1). The climate data, with a resolution of 2.4 m, was obtained from Worldclim (<https://worldclim.org/data/index.html>), and included: annual mean temperature (bio1 in °C), maximum temperature of the warmest month (bio5 in °C), minimum temperature of the coldest month (bio6 in °C), annual precipitation (bio12 in mm), precipitation of the wettest month (bio13 in mm), and precipitation of the driest month (bio14 in mm) (Supplementary Table S1). Soil data, specifically clay content in the upper 30 cm, was sourced from ISRIC (<https://www.isric.org/instruction->

[wms](#)). This depth is relevant because mopane worms burrow primarily within the upper 30 cm of soil during their pupation stage (Ghazoul, 2006).

Multicollinearity among predictor variables was evaluated using variance inflation factors (VIF; sdm R package (Naimi et al., 2014). Initially, two variables with VIF values greater than 15 (bio1 and bio12) (Chatterjee & Hadi, 2006) were excluded, then the VIF was recalculated (Hijmans et al. 2005). Variables with VIF values below 5 (Supplementary Table S1) were then retained. The remaining bioclimatic variables reflect their significance in influencing the distribution of mopane worms and their host tree (Makhado et al., 2014; Table 3-1).

Table 3-1: Environmental variables used to model the suitable habitat for mopane worms

Variable	Variable description	Ecological reason	Reference
Clay	Clay % on the top 30 cm of the soil (g/kg)	Soil physical content (e.g. clay content) affects the burrowing process of worms. Mopane worms seem to prefer to burrow into less compacted sandy soil.	Bangira et al. (2023)
Bio 5	Maximum temperature of the warmest month (°C)	Mopane worms emerge during the rainy seasons in southern Africa which are also the hottest periods; thus, cold temperatures could decrease worm outbreaks.	Gondo et al. (2010)
Bio 6	Minimum temperature of the coldest month (°C)		
Bio 13	Precipitation of wettest month (mm)	Mopane worms occur during the rainy, wet season thus dry season might lead to a decrease in mopane worms, while the wet season could influence tree growth and consequently food for the worm and thus increase in mopane worms.	Gondo et al. (2010)
Bio 14	Precipitation of driest month (mm)		

3.3.3 Models

Five modelling algorithms were fitted and evaluated for their performance based on AUC (receiver operator curve statistic, Elith et al., 2006), with each algorithm replicated 5 times (25 replicates per

model). The AUC indicates the degree to which the output of each algorithm aligns with the original occurrence data, with higher scores representing better model performance (Elith et al., 2006). evaluated two regression models (GAM & GLM), two machine learning models (MAXENT & Random Forest), and a climate envelope model (BIOCLIM) (Montoya-Jiménez et al., 2022) were evaluated, and based on their AUC performance (models with AUC > 0.8; Supplementary Tables S2, three models (GAM, MAXENT, and Random Forest) were selected. The selected algorithms were then combined to generate an ensemble model (Araújo and New, 2007) that significantly improves model performance (Tsoar et al., 2007). Habitat suitability probability in each cell across all three algorithms was averaged and weighted by each algorithm's calculated AUC value (Elith et al., 2006) obtained from five-fold cross-validation. The influence of each environmental variable on the mopane habitat model was assessed using the Pearson correlation test and the AUC test. Both tests employ a permutation approach to evaluate the importance of variables. This is achieved by comparing the model performance with the permuted variable to a model with the original variable (Naimi & Araujo, 2016). The getVarImp function in the sdm package (Naimi and Araujo, 2016) was used to implement the tests. All modelling was done in R (R Core Team, 2022), using the *sdm* package (Naimi & Araujo, 2016).

3.3.4 Quantifying the extent of suitable habitat

To quantify the extent of suitable habitat, the output from the models were visualised graphically using a logistic score for each pixel representing spatially explicit probabilities of species presence (Phillips & Dudík, 2008). A threshold that maximised the True Skill Statistic (TSS; Meller et al., 2014) was applied to classify the model predictions into suitable and unsuitable habitats. TSS is often used for threshold estimation since it shows good performance and seems to be independent of species prevalence (Meller et al., 2014). Finally, since the focus of the study was the southern African region, the model output was restricted to the mopane tree, the primary host tree for this region (Ngarega et al., 2021). A published Maxent mopane tree model (Ngarega et al., 2021) was used to clip the full mopane worm model to produce the restricted model representing mopane worms that can be harvested (suitable for outbreaks). The suitable mopane worm habitat was then estimated for each country and expressed as the proportion of the suitable habitat based on the full and restricted ensemble model.

3.3.5 Effectiveness of protected areas in capturing mopane worm habitat

The effectiveness of protected areas in capturing suitable habitats for mopane worms, was achieved by calculating the percentage of suitable habitats that fell within protected areas. Protected areas were obtained from the Protected Planet website (UNEP-WCMC, 2023), which characterizes protected areas by the IUCN category (Supplementary Table S3). The IUCN categories include Ia (strict nature reserve), Ib (wilderness area), II (national park), III (natural monument), IV (habitat or species management area), and V (protected landscape). If a protected area does not have an assigned IUCN category, it was assigned to the lowest protection category (Category V; protected landscape). The effectiveness of protected areas was expressed on two levels. The first level looked at the contribution of the protected area network in each country toward the total suitable mopane worm habitat across the modelling space, while the second level looked at the effectiveness of the protected area network in each country in capturing suitable habitats within countries.

3.4 Results

3.4.1 Model performance

The three modelling algorithms used (GAM, MAXENT, RF) performed well, and all achieved an AUC above 0.80 (Supplementary Table S4). Subsequently, the ensemble model achieved an AUC of 0.95 with a prevalence of 0.21 (Supplementary Table S5).

3.4.2 Potential distribution and contribution of suitable habitat to each country

The full ensemble model classified 15.1% (1 349 997 km²) of the model landscape as suitable habitat for mopane worms (Figure 3-1B), while restricting the ensemble model (Restricted model, Figure 3-1A) to *C. mopane*, resulted in 40% (602 934 km²) of the model landscape as suitable habitat for mopane worms (Figure 3-1A). As such, only around 40% of the restricted model landscape has the potential for mopane worms to reach outbreak populations needed for harvest in areas where *C. mopane* is the primary host tree. Although the suitable habitat predicted from the full ensemble model closely followed the distribution of *C. mopane* (Figure 3-1C), large sections of the suitable habitat predicted fell outside *C.*

mopane distribution (Figures 3-1B and C), extending to countries such as Tanzania, Kenya, and provinces such as the Kwazulu-Natal and Eastern Cape in South Africa.

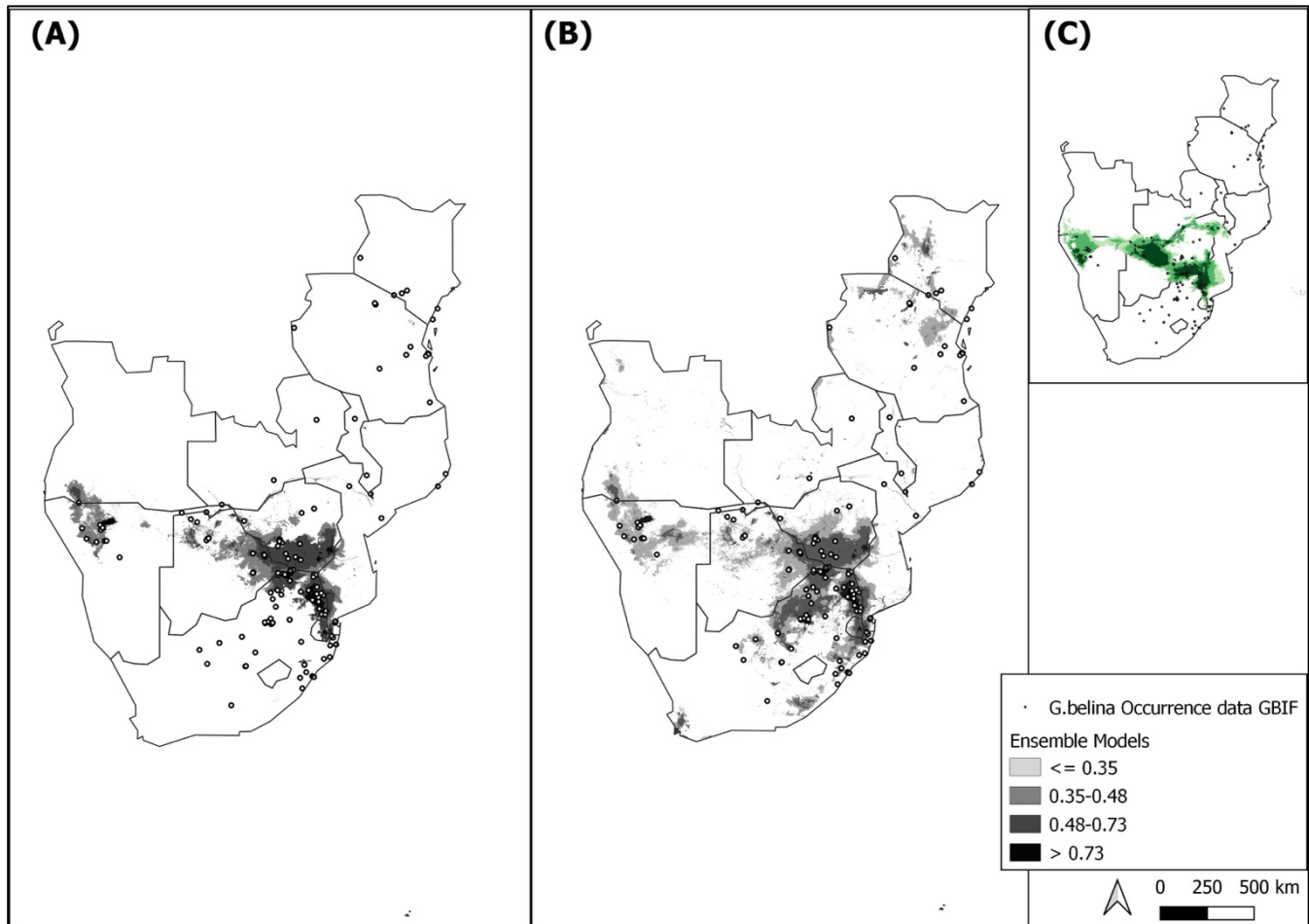


Figure 3-1: Predicted suitable mopane worms' habitat from the restricted ensemble model (A) and the full ensemble model (B). C represents the distribution of the mopane woodland.

3.4.3 Contribution of each country to the overall suitable habitat.

Under the full ensemble model, South Africa had the largest area of suitable habitat (389 217 km²; 28.8% of total suitable habitat, Table 3-2), followed by Botswana (217 767 km²; 16.1%), Zimbabwe (210 464 km²; 15.6%; Table 3-2, Figure 3-1B) and Namibia (189 198 km²; 14%; Table 3-2, Figure 3-1B). The rest of the southern African countries contributed less than 100,000 km² (less than 10% of the total suitable habitat, Table 3-2).

Not surprisingly, under the restricted model, suitable mopane worm habitat was confined to the mopane woodland belt (Figure 3-1A). Zimbabwe (161 874 km²; 26.8%; Table 3-2, Figure 3-1A) and Botswana (152 556 km²; 25.3%; Table 3-2, Figure 3-1A) contributed the bulk of the suitable habitat for mopane worms where harvesting can potentially occur. South Africa (92 760 km²; 15.4%; Table 3-2), Namibia (88 405 km²; 14.7%; Table 3-2), and Mozambique (66 924 km²; 11.1%; Table 3-2) all contributed more than 10% of the total suitable habitat under the restricted model, while the rest of the southern African countries contributed little (Table 3-2) under the current modelling scenarios.

In terms of evaluating each country's suitable habitat for mopane worms' harvest (restricted model), Zimbabwe had the highest potential, where 41.8% of the country had suitable habitat for mopane worms' harvest (Table 3-2, Figure 3-1A), followed by Botswana (26.9%; Table 3-2, Figure 3-1A). The rest of the southern African countries had 10% or less of their landmass suitable for mopane worm populations where harvest can occur (Figure 3-1A). Even though Eswatini had more than 50% of its land classified as suitable habitat under the restricted model (Table 3-2), the relative amount of suitable area (8 760 km²) contributed little to the overall suitable mopane worm harvest area (1.5%; Table 3-2).

Table 3-2: Total area per mopane worm host country, suitable area of mopane worm habitat per country, and proportion of the suitable area of habitat per country and in all countries.

Country	Total Area (km ²)*	Restricted model			Full ensemble model		
		Total suitable area (km ²)**	Percentage of country (%)#	Percentage overall (%)##	Total suitable area (km ²)	Percentage of country (%)	Percentage overall (%)
Zimbabwe	392 573	161 874	41.8	26.8	210 464	54.4	15.6
Botswana	581 163	152 556	26.9	25.3	217 767	38.4	16.1
South Africa	1 224 385	92 760	7.6	15.4	389 217	32.0	28.8
Namibia	827 465	88 405	10.7	14.7	189 198	23.0	14.0
Mozambique	791 082	66 924	8.5	11.1	90 870	11.6	6.7
Angola	1 255 218	28 178	2.3	4.7	50 516	4.1	3.7
Eswatini	17 336	8 760	50.9	1.5	15 364	89.3	1.1
Zambia	755 640	2 700	0.4	0.4	13 978	1.9	1.0
Tanzania	947 253	730	0.1	0.1	98 602	11.2	7.3
Malawi	118 860	47	0.1	0.0	3 982	4.2	0.3
Kenya	586 770				68 958	12.1	5.1
Lesotho	30 495				923	3.0	0.1
Total	125 528 010 022	602 934	149.3	100	1 349 997	285.2	99.8

*Surface area of each country (km²) (UNEP-WCMC, 2023)

**Area per country that is suitable mopane worm habitat (Maxent model output)

#The percentage of the country that is suitable mopane worm habitat (suitable area/total area) x 100

##Of all the suitable mopane worm habitats in the study area, this is the contribution of each country (Total suitable area/Σ (Total suitable area of all countries) x 100

3.4.4 Effectiveness of protected areas in capturing harvestable populations

To evaluate the effectiveness of conservation areas, findings from the restricted ensemble model are reported, given that most mopane worm harvesting occurs within these regions (Ngarega et al., 2021). Of the 574,757 km² of suitable mopane habitat, only 37% are within protected areas. In Botswana, 51,747 km² (33%) of the suitable habitat of 152,556 km² is protected, while 100,809 km² remains unprotected. Similarly, in Zimbabwe, only 43,041 km² (26.6%) of the 161,874 km² suitable habitat is protected, and 118,833 km² is unprotected. South Africa has 29,954 km² (32.3%) of its 92,760 km² suitable habitat under protection. Collectively, conservation areas in Namibia (11.5%), Botswana (9%), and Zimbabwe (7.5%) represent 32.5% of the potential habitat under the restricted model. Additionally, conservation areas in South Africa (29,954 km²) and Mozambique (19,971 km²) contributed significantly to the total suitable habitat for mopane worms in the restricted model (Table 3-3).

Evaluating the effectiveness of country-specific protected area networks in capturing suitable mopane worm habitat under the restricted model shows that protected areas generally conserve approximately 25% of suitable habitat per country (Table 3-3). Importantly, protected areas in Botswana (33.9% of suitable country habitat), Zimbabwe (26.6%), South Africa (32.3%), Mozambique (29.8%), and Namibia (74.7%; Table 3-3) all perform adequately in capturing suitable mopane worm habitat under the restricted model. Regarding IUCN-protected status categories, only protected areas in Botswana contained suitable mopane worm habitats in IUCN Category Ib (Table 3-3). Most suitable mopane worm habitats in conservation areas fall under IUCN Categories V and VI (Table 3-3). From the analysis, no suitable mopane worm habitat contained in IUCN category 1a was found (Table 3-3).

Table 3-3: Contribution of each country's conservation area to the total suitable habitat for mopane worms under the restricted model

Country	Total suitable mopane habitat per country (km ²) ^a	Total conservation area per country (km ²) ^b	Non-protected areas		Protected or conservation areas		Total suitable habitat in conservation area, per country and IUCN categories (%) ^h						
			Total suitable habitat in non-protected area/ country (km ²) ^c	Total suitable habitat, as % of overall suitable habitat for Southern Africa ^d	Total suitable habitat in protected/ country (km ²) ^e	Total suitable habitat, as % of overall suitable habitat for Southern Africa ^f	Suitable habitat in conservation areas, as % of overall suitable habitat for each country ^g	Ib	II	III	IV	V	VI
Zimbabwe	161 874	111 120	118 833	20.7	43 041	7.5	26.6	0	10.6	0	0.1	14.1	1.8
Botswana	152 556	169 370	100 809	17.5	51 747	9.0	33.9	8.7	0.7	0	1.2	23.4	0
South Africa	92 760	113 601	62 806	10.9	29 954	5.2	32.3	0	0.0	0	0	32.3	0
Namibia	88 405	313 534	22 394	3.9	66 011	11.5	74.7	0	24.4	0	0	50.3	0
Mozambique	66 924	233 249	46 953	8.2	19 971	3.5	29.8	0	23.7	0	0.2	0.0	5.9
Swaziland	8 760	738	8 673	1.5	87	0.0	1.0	0	0.7	0	0	0.3	0
Zambia	2 700	311 773	2 270	0.4	430	0.1	15.9	0	4.5	0	0	2.2	9.2
Tanzania	730	378 341	534	0.1	196	0.0	26.8	0	0	0	0.6	26.2	0
Malawi	48	27 190	30	0.0	17	0.0	36.6	0	0	0	3.3	33.3	0
Total	574 757	1 676 034	363 304	63.2	211 453	36.8							

^a Logistic pixel probability of occurrence of 0.3 was used as a threshold to define suitable mopane worms' habitat from the Ensemble model.

^b Total area under protected status per country (UNEP-WCMC, 2023)

^c Total suitable habitat for mopane worms, in non-protected regions for each country

^d Suitable habitat in non-protected regions, expressed as a % of all suitable habitat in southern Africa

^e Total suitable habitat for mopane worms, in protected or conservation regions for each country

^f Suitable habitat in protected or conservation regions, expressed as a % of all suitable habitat in southern Africa

^g Suitable habitat in protected or conservation regions, expressed as a % of all suitable habitat for each country

^h Subdividing suitable habitat in protected or conservation areas per country (g) into IUCN categories for the type of protected areas, expressed in percentage

3.4.5 Variable importance

From both the Pearson correlation test (Figure 3-2A) and the AUC test (Figure 3-2B), bio13 (precipitation of the wettest month) had a high influence on all modelling algorithms, followed by bio6 (minimum temperature of the coldest month), the clay content of the soil and bio14 (precipitation during the driest month) (Figure 3-2). Although the influence of variables on distribution models varied with modelling algorithms (Figure 3-3), the general pattern of variable importance remains consistent between algorithms (Figure 3-2).

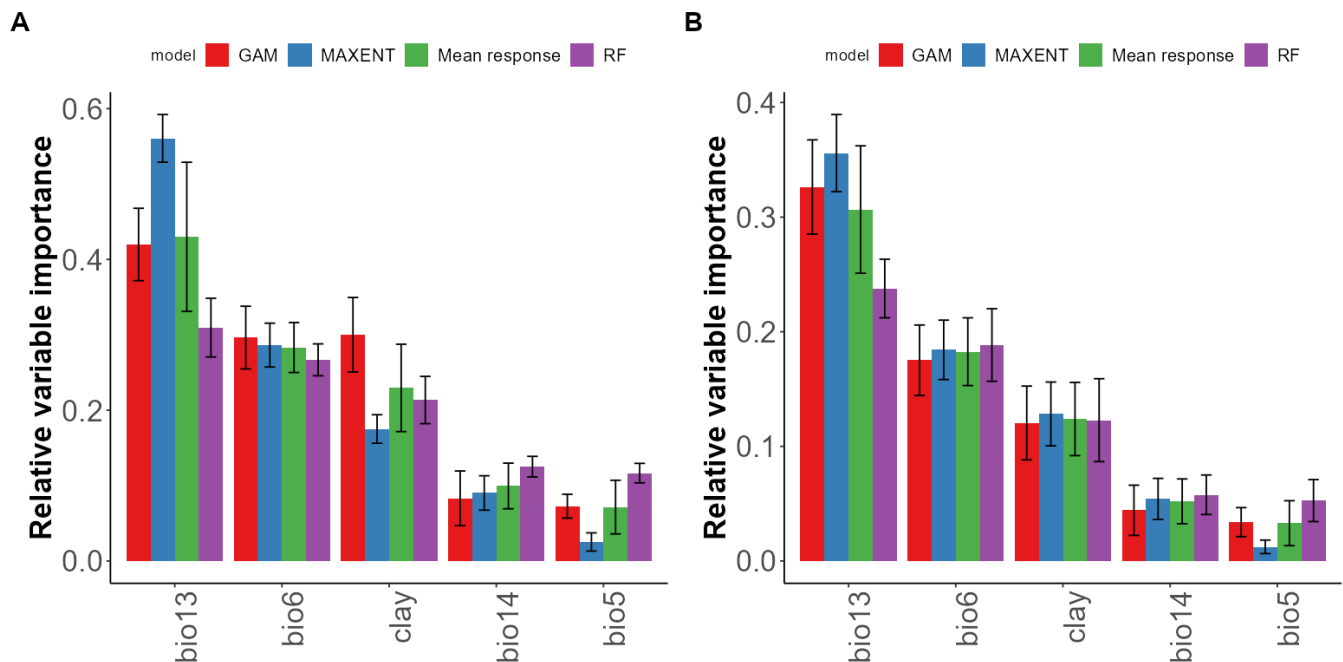


Figure 3-2: Pearson correlation test (A) and AUC test (B) for evaluating the relative contribution of predictor variables in predicting the probable distribution of the mopane worm. Predictor descriptions : bio 5 (Maximum temperature of the warmest month (°C)), bio6 (minimum temperature of the coldest month (°C)), bio13 (precipitation of the wettest month (mm)), bio14 (precipitation during the driest month (mm)), clay content of the soil (g/kg). The error bars represent the standard deviation (variability in the estimate of relative variable importance for each predictor across the different models).

There was a variation in the response of mopane worms to the various environmental predictor variables (Figure 3-3). The presence of mopane worms was strongly associated with bio13 (precipitation during the wettest month), bio14 (precipitation during the driest month), bio6 (minimum temperature

of the coldest month), and clay content (Figure 3-3). Specifically, mopane worms' presence was significant in areas where precipitation during the wettest month (bio13) ranged from 10 to 150 mm (Figure 3-3). Furthermore, mopane worms showed a preference for warmer climates, with predicted occurrences in regions where maximum temperatures during the warmest months (bio5) ranged from 20°C to 35°C, and minimum temperatures during the coldest months (bio6) ranged from 0°C to 5°C (Figure 3-3). The worms preferred soil with clay content between 0-150 (g/kg) for all modelling algorithms (Figure 3-3).

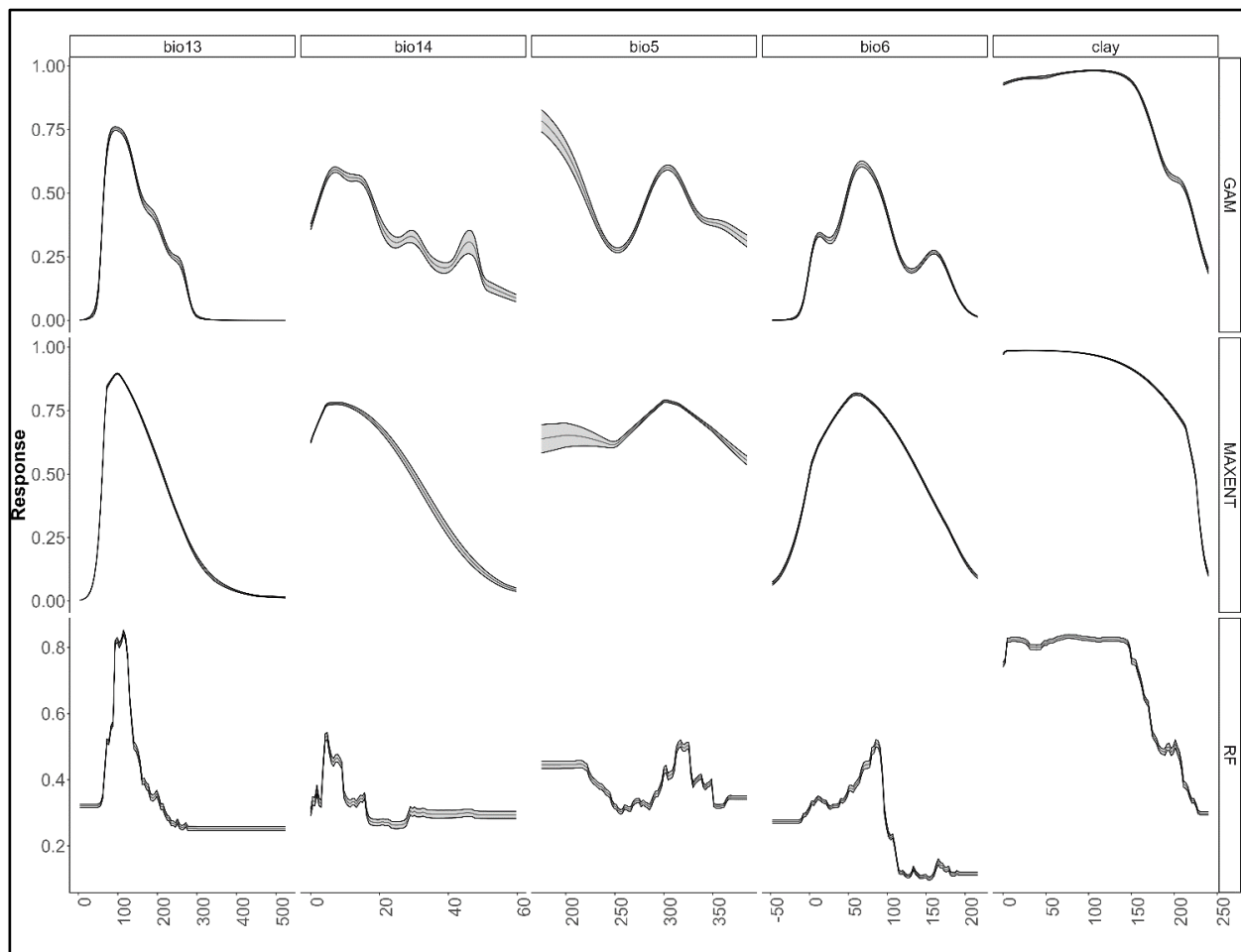


Figure 3-3: The response curves of the five environmental predictors used in the mopane worm ecological niche model. Predictor abbreviations are given in Table 3-1; x-axis units for bio13 (mm), bio14 (mm), bio5 (°C), bio6 (°C), and clay (g/kg). The scaling of temperature-related variables is by a factor of 10.

3.5 Discussion

3.5.1 Potential distribution and suitable habitat per country

The full ensemble model predicted that 15.1% (1,349,839 km²) of the model landscape is suitable habitat for mopane worms, consistent with Shen et al. (2023) (non-network model), who estimated 1,480,000 km² in southern Africa. Both models likely underestimated the total habitat due to sampling bias, as most data comes from southern Africa, with limited data for areas north of the Kunene and Zambezi rivers. For example, several studies have reported significant mopane worm outbreaks in Zambia, Congo (Mabossy-Mobouna et al., 2022; Kapepula et al., 2022), Kenya (Kusia et al., 2021), and Nigeria (Olaleye, 2020). Yet, few mopane worm records are available on GBIF for these areas. Furthermore, mopane worms also utilise other host trees, including *Brachystegia spiciformis*, *Julbernadia paniculata* (Mughogho & Munthali, 1993), *Terminalia sericea*, and *Sclerocarya birrea* (Ditlhogo et al., 1996; Teferra et al., 2000) and *Diospyros mespiliformis*, (Mopane Worms Enterprises [PBC], 2020). This bias in presence-only modelling can lead to an underestimation of potential habitat (Yackulic et al., 2013; Gomes et al., 2017). Thus, the potential habitat for mopane worms is likely broader than the present model suggests and highlights that the modelling approach in this study is probably more applicable to a suitable mopane worm habitat in the mopane belt (Ngarega et al., 2021).

Clipping the full ensemble model to the distribution of *C. mopane* indicated that 40% (602,934 km²) of the restricted model is suitable for mopane worms (Makhado et al., 2014; De Swardt et al., 2018), which is comparable to the predictions of Shen et al. (2023) based on Bayesian network models (649,000 km² vs. 602,934 km²). This suggests that the restricted modelling approach accurately captures potentially suitable mopane worm habitats, even without considering biotic and abiotic interactions. Restricting the full model to *C. mopane* allows a more focused evaluation of harvested populations' conservation status and management.

Several hypotheses explain the close relationship between mopane worms and mopane trees. Mopane worms developed faster and survived up to pupation more successfully when feeding on *C. mopane* compared to *Sclerocarya birrea* (Teferra et al., 2000). Leaves of *C. mopane* have higher crude protein and energy content compared to *S. birrea* and *Terminalia sericea* (Teferra et al., 2000). However, Hrabar et al. (2009) suggested that the quantity of leaves, rather than their quality, determine host tree selection, and since mopane trees often form dense monodominant savannah stands (Duffy et al., 2018), they can provide substantial amounts of food for the consumption of

mopane worms (Ditlhogo et al., 1996; Makhado et al., 2014). Furthermore, the tannin content of mopane leaves does not change with complete defoliation (Sibula and Dube, 2022), allowing mopane worms to exploit a resource that might be limited to other herbivores (Hrabar et al., 2009). While there is a close relationship between mopane worms and trees, it is highlighted that mopane worms also have close relationships with other host tree species in other areas in Africa.

In areas south of Kunene and Zambezi rivers, four countries, Zimbabwe, Botswana, South Africa, and Namibia, accounted for over 82% of suitable habitat. Botswana and Zimbabwe alone contributed up to 52%, which makes these countries key producers and exporters (Rasengwatshe & Madibela, 2005; Madibela et al., 2007; Dube & Dube, 2010), while consumption and trade primarily occur in Botswana, Zimbabwe, and South Africa (Nemadodzi et al., 2023). Cross-border mopane worm trade has increased significantly in southern Africa, but much of it is informal and under-researched (Makhado et al., 2014; Nemadodzi et al., 2023, Ghazoul, 2006). Few studies detail the quantities of mopane worms imported and exported, as it is mainly informal (Ghazoul, 2006). Furthermore, trade and harvest studies primarily focus on southern Africa, particularly in countries heavily involved in cross-border trade (Makhado et al., 2014; Nemadodzi et al., 2023).

3.5.2 *Effectiveness of protected areas*

The findings of this study indicate that 63.2% (363 304 km²) of the suitable mopane worm habitat in the restricted model (harvestable populations) are in non-protected areas. This highlights the need for better management and conservation in these regions, especially as land tenure systems in southern Africa are complex, ranging from communal land under local chiefs' governance (Mbata & Chidumayo, 2003; Thomas, 2013; Ndlovu et al., 2019), to public and private land (Sekonya et al., 2020). While public and private lands have some regulations, communal land often lacks formal management. This is because these lands are under the governance of local chiefs, and there are issues around disobedience to the rules set by the chief and conflicts about whom the land belongs to between the community and the chief (Thomas, 2013; Mufandaedza et al., 2015; Sekonya et al., 2020). For example, harvesters often cut down branches/ whole trees and harvest immature worms, reducing the worm population, which burrows and emerges as a moth to lay eggs for the next generation (Thomas, 2013).

Furthermore, there is less direct monitoring of communal land, as harvesters have been reported to start harvesting before the chief officially announces the harvesting season (Sekonya et al., 2020). Non-locals are supposed to get permission from the Chief; however, they come in large numbers, harvesting even the juvenile stages of the mopane worms and breaking mopane branches because of less monitoring (Sekonya et al., 2020).

In contrast, private and public systems, where approximately 36.8% of mopane worm habitats occur, exercise greater control and monitoring of mopane worm harvesting (Sekonya et al., 2020). Harvesting is subject to permission and payment and is restricted to a limited time period (Sekonya et al., 2020). For example, harvesters are required to seek permission from parks authority/ farm owner before harvesting in the Gonarezhou National Park (public property) and Gonakudzingwa Small Scale Farms (private property) in Zimbabwe (Mufandaedza et al., 2015). Harvesting is monitored, and breaching the rules results in fines (Mufandaedza et al., 2015). This balance between use and conservation ensures sustainable practices while protecting the species (Swemmer et al., 2020).

Apart from the laws established by local chiefs, national parks, and private landowners to govern worm harvesting, there are no national laws on the exploitation of insects, and therefore, few/no consequences for anyone who overexploits this resource (Halloran et al., 2015; Niassy et al., 2018). Regulatory laws in South Africa do not specifically govern insects but animals (Niassy et al., 2018), making it challenging to protect insects under the current laws formally. It is left to the land management regime to protect the worms from excess use to ensure sustainability (Mufandaedza et al., 2015). However, countries like Thailand, Switzerland, Kenya, and Canada have advocated for laws to include insects in food regulatory law (Halloran et al., 2015).

Regarding protected areas and IUCN categories, only Botswana had suitable habitats in IUCN 1b, while most countries had suitable habitats in IUCN II, V, VI, and IV. Although Botswana, Zimbabwe, and South Africa have low areas of suitable habitat in protected areas, they are reportedly highly involved in the mopane worm trade (Nemadodzi et al., 2023). This indicates that mopane worms are being extensively exploited in these habitats where they are least protected.

3.5.3 Variable importance

The suitability of mopane worm habitat is primarily influenced by precipitation (bio13), minimum temperature (bio6), and soil clay content. Rainfall and temperature are the key factors influencing insect distribution (Togarepi et al., 2020; Meltus et al., 2024). Mopane moths emerge at the beginning of the summer rains; however, the presence of mopane worms declines with rainfall as extreme rainfall negatively impacts larval stages (Thomas, 2013).

Temperature was the second key factor. Although mopane worms are resilient at a range of temperatures, extreme temperatures result in population decline (Mataboge et al., 2016). Heat waves can cause the eggs to burst and larvae to die (Togarepi et al., 2020), while during the coldest months, mopane worms enter diapause (Mataboge et al., 2016).

The clay content of the soil was the third most influential variable, as mopane worms spend a significant amount of time under the soil. They prefer to burrow in sandier, less compact soil (Bangira et al., 2023). Additionally, soil type affects mopane tree distribution, which in turn influences the distribution of worms (Hrabar et al., 2014; Mufandaedza et al., 2018).

3.6 Management and conservation implications

Mopane worm outbreaks can reach millions of individuals (Meltus et al., 2024), which makes it hard to believe that harvesting can have negative impacts. However, even though the harvest of highly abundant species often warrants low conservation concern (Thomas, 2013), several past extinctions of highly abundant species highlight a cause of concern. The importance of invertebrates has often been overlooked in research and society and is therefore overlooked in terms of conservation (Reid, 1999).

Gonimbrasia belina has several characteristics that make it vulnerable to unsustainable harvesting. First, they exhibit bivoltinism, emerging twice a year (Gondo et al., 2010), which supports the idea of their sustained abundance as a source of protein and income. Combined with periodic population explosions over wide areas, contributes to the perception that this species warrants a low conservation status (Chowdhury et al., 2023) and thus are overlooked for conservation efforts and also over harvested (Reid, 1999). Second, outbreak and harvest populations often depend on a preferred single host tree, posing a sustainability risk if the host tree is negatively impacted. Thirdly,

limited suitable habitat is in protected area networks, with the most significant proportion in non-protected land, often in communal landscapes where few laws or policies regulate harvest (Mufandaedza et al., 2015).

As part of effective management, local communities, governments, and regional conservation authorities should address the management of this communally exploited species. Several examples demonstrate how local communities have implemented regulations to manage edible insects' harvesting effectively. In midwestern Zambia, for instance, harvesters of *Gynanisa maja* and *Gonimbrasia zambesina* caterpillars observe temporal restrictions to ensure sustainability (Mbata et al., 2003). The chiefdom manages these restrictions under a senior traditional ruler, with harvesting commencing only after the senior chief announces the harvest period (Mbata & Chidumayo, 2003). Furthermore, the Bisa tradition supplements these restrictions by prohibiting the caterpillars' host plants felling except under special permission from traditional leaders (Mbata & Chidumayo, 2003).

Similarly, southern African customs encourage selective stinkbug harvesting (*Encosternum delegorguei*) only at non-breeding sites (Musundire et al., 2021). The harvest of ground crickets (*Henicus whellani*) in southern Africa is also restricted until after maximum mating and egg-laying (Murefu et al., 2019). In the absence of formal regulations, some local communities in southern Zimbabwe, northern South Africa, and eastern Botswana have formed vigilante groups to coordinate the harvesting of mopane worms (Musundire et al., 2021). Such community actions have sometimes led to the development of formal regulations, as seen in the Gwanda municipality in Matabeleland South, Zimbabwe, which enacted bylaws in 2020 to regulate mopane worm harvesting due to community pressure (Musundire et al., 2021).

3.7 References

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Data availability statement

Data and code will be made available upon publication at dryad.com.

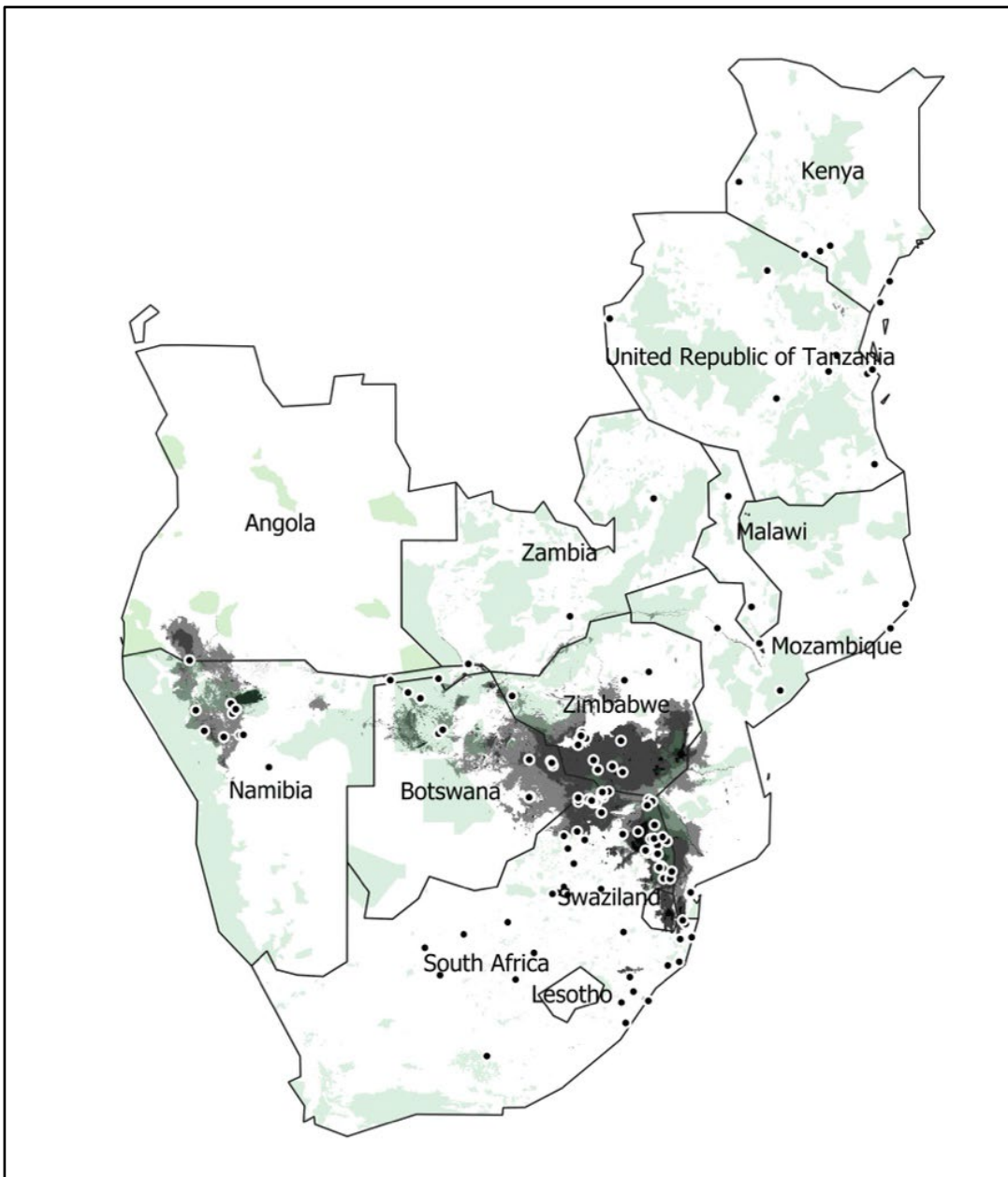
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Conflict of interest statement

The authors declare no conflicts of interest.

3.8 Supplementary information



Supplementary Figure S1: Study area of model

Supplementary Table S1: The bioclimatic variables assessed for importance in the modelling

Code	Description	VIF	VIF**
Bio1	Annual mean temperature	23.195018*	
Bio 5	Maximum temperature of the warmest month	9.806389	1.523976
Bio 6	Minimum temperature of the coldest month	14.152834	2.163152
Bio 12	Annual precipitation	31.467951*	
Bio 13	Precipitation of wettest month	14.387833	2.165840
Bio 14	Precipitation of driest month	10.311164	1.653578
Clay content	Clay content is the upper 30cm	1.084900	1.075754

*Variables with high VIF were removed. VIF after the highly correlated variables were removed

Supplementary Table S2: The five different modelling algorithms evaluated

Methods	AUC	COR	TSS	Deviance
GAM	0.82*	0.51	0.54	0.79
GLM	0.67	0.28	0.37	0.95
MAXENT	0.83*	0.49	0.54	0.98
RF	0.87*	0.64	0.61	0.66
BIOCLIM	0.69	0.32	0.37	1.63

*Models in bold selected to generate the ensemble model

Supplementary Table S3: IUCN categories of management (Source: IUCN ESARO, 2020)

Category	Description	Examples
Ia – Strict Nature Reserve	Strictly protected areas which are set aside to protect biodiversity and also possibly geological/geomorphical features, where human visitation, use and impacts are strictly controlled and limited to ensure protection of the conservation values. There are few examples of this category in the region, though some national parks will have restricted areas within them, rather than being fully categorised as Category Ia.	Tsingy de Bemaraha, Tsaratanana and Betampona (Madagascar) Aldabra Atoll, Cousin, La Digue and Aride (Seychelles)
Ib – Wilderness Area	Protected areas which are usually large unmodified or slightly modified areas, retaining their natural character and influence without permanent or significant human habitation, which are protected and managed to preserve their natural condition.	Moremi, Khutse and Central Kalahari Game Reserves (Botswana) Koko Hill, Mamboya and Ikwamba Forest Reserves (Tanzania)
II – National Park	Large natural or near natural areas set aside to protect large-scale ecological processes, along with the complement of species and ecosystems characteristic of the area, which also provide a foundation for environmentally and culturally compatible, spiritual, scientific, educational, recreational, and visitor opportunities. This is the most common management category in the region.	Parc Marin de Mohéli (Comoros) Amboseli and Masai Mara (National Reserve) (Kenya) Niassa (National Reserve) (Mozambique) Volcans (Rwanda) Kruger (South Africa) Serengeti (Tanzania) Bwindi Impenetrable (Uganda) Kafue (Zambia)
III – Natural Monument or Feature	Protected areas set aside to protect a specific natural monument, which can be a landform, sea mount, submarine cavern, or a geological feature such as a cave or even a living feature such as an ancient grove.	Victoria Falls National Park (Zimbabwe) Popa Game Park and Gross Barmen Hot Springs (Namibia) Toro-Semliki, Karuma, Bugungu and a number of other wildlife reserves (Uganda)
IV – Habitat/Species Management Area	The aim is to protect particular species or habitats and management reflects this priority.	Partial Reserve Namibe (Angola) Maun Game Sanctuary (Botswana) Gash-Setit Wildlife Reserve (Eritrea) Alledoghi and Bale Wildlife Reserves (Ethiopia) Sehlabathebe National Park (Lesotho) Majete and Nkhotakota Wildlife Reserves (Malawi) Poudre d'Or and Trou d'Eau Douce Fishing Reserves (Mauritius) Sabaloka Game Reserve (Sudan)
V – Protected Landscape/Seascape	Protected areas where the interaction of people and nature over time has produced an area of distinct character with significant, ecological, biological, cultural and scenic value, and where safeguarding the integrity of this interaction is vital to protecting and sustaining the area and its associated nature conservation and other values.	Iles Musha and Maskhali (Djibouti) Libhetse Nature Reserve (Eswatini) numerous areas in Madagascar Imatong Forest Reserve (South Sudan)
VI – Protected area with sustainable use of natural resources	These are protected areas which conserve ecosystems and habitats together with associated cultural values and traditional natural resource management systems. They are generally large, with most of the area in a natural condition, where a proportion is under sustainable natural resource management and where low-level non-industrial use of natural resources compatible with nature conservation is seen as one of the main aims of the area.	Dabus Valley, Jikao, Tedo, Omo West and numerous other Controlled Hunting Areas (Ethiopia) Beacon, Booby Island, Etoile and Mamelles Nature Reserves (Seychelles) Matetsi, Sapi and Hurungwe Safari Areas (Zimbabwe)

Source: Dudley (2008); UNEP-WCMC & IUCN (2019a).

Supplementary Table S4: The five modelling algorithms run for mopane worms to evaluate model performance

Methods	AUC	COR	TSS	Deviance
Maxent	0.83	0.49	0.53	0.97
GAM	0.82	0.52	0.54	0.78
RF	0.87	0.64	0.62	0.65

Supplementary Table S5: The ensemble model performance run for the distribution of mopane worms

Ensemble model				
Prevalence	AUC	COR	P-value	Deviance
0.21	0.95	7.350000e-01	2.047204e-214	0.59

4 CHAPTER 4: Leaf consumption and frass production by mopane worms (*Gonimbrasia belina*) in Mapungubwe National Park



Early instar mopane worms feeding on leaves of mopane tree in Ga-Selwane communal land, Phalaborwa.



Leaf consumption and frass production by mopane worms (*Gonimbrasia belina*) in Mapungubwe National Park

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4.1 Abstract

While edible insects have been majorly appreciated and studied for being sources of food and income, they have also been reported to contribute to the ecological functioning of the environment. Most of them ingest leaves throughout their life cycle. They consume leaves and transform them into easily mineralizable frass, enhancing nutrient cycling as leaf litter tends to mineralize slower. This study assessed the impact of mopane worms on the mopane veld ecosystem in the Mapungubwe National Park. The first three instar stages (I-III) of mopane worms were observed for three days to measure and determine the rates at which mopane leaves were consumed, and frass was produced to estimate biomass intake and output by mopane worms and quantify their impact at a landscape scale. There was a significant difference in the dry leaf consumed ($p=0.009$) and the frass produced ($p=0.001$) between the first and third instar. Scaling consumption and frass production rates up to the landscape level revealed that mopane worms could consume between 152 and 9,970 kg of dry leaf mass per hectare and release between 63 and 4,137 kg of frass in one outbreak period.

Keywords: biomass, frass production, instar, landscape level, leaf consumption

4.2 Introduction

Mopane worms, the larval stage of the mopane moth (*Gonimbrasia belina*), play a vital ecological and economic role in semi-arid regions of southern Africa, especially in the mopane woodlands (*Colophospermum mopane*; Makhado et al., 2012). Emerging twice in a year, the moths lay eggs in clusters of 2-10 per tree with 30-355 eggs per cluster (Ditlhogo et al., 1996; Makhado et al., 2014), which develop through five instar stages (I-V) over 4-6 weeks (Gondo et al., 2010; Makhado et al., 2014), with each stage lasting 5-7 days (Gondo et al., 2010). While primarily feeding on the leaves of *C mopane* (Nemadodzi et al., 2023), mopane worms are also known to consume leaves from other trees, including *Terminalia sericea*, *Sclerocarya birrea* (Ditlhogo et al., 1996), *Brachystegia spiciformis* and *Julbernardia paniculata* (Mughogho & Munthali, 1993). However, outbreaks are mostly associated with mopane woodlands, where harvesting activities mostly occur (Makhado et al., 2014; Swemmer et al., 2020).

Apart from being an essential protein source to humans and livestock (Moyo et al., 2019), as well as creating vital seasonal income for rural communities (Baiyegunhi and Oppong, 2016), mopane worms hold a key but often overlooked ecological role (De Swardt et al., 2018). They contribute to the food web by providing sustenance to various animals, including birds (Gaston et al., 1997), aardvarks, bat-eared foxes, bats, baboons, warthogs, mongooses and vervet monkeys (Potgieter et al., 2012). Moreover, large-scale defoliation of trees contributes to nutrient cycling at the landscape level (De Swart et al., 2018). During outbreak periods, mopane worms can defoliate entire tracts of trees (Ditlhogo, 1996), producing large quantities of frass (excrement), which decomposes rapidly, releasing nutrients into the soil and facilitating natural nutrient cycling (Styles, 1996; Belovsky & Slade, 2000; Reynolds & Hunter, 2004).

Despite their ecological significance, research on mopane worms has largely focused on their cultural impact and nutritional importance for human and animal consumption (Netshanzhe et al., 2022), with very limited attention to their role in nutrient cycling. To better understand this, it is crucial to quantify the extent of leaf consumption and frass production. Few studies have explored these variables (De Swardt et al., 2018), which are influenced by factors such as population dynamics, survival, temperature, rainfall, predation, parasitism and tree density (Bara et al., 2022). This study combined field data on leaf consumption and frass production with published literature to estimate the impact of mopane worms on nutrient cycling per hectare.

4.3 Materials & Methods

4.3.1 Study area

Three sites ($22^{\circ}14'18''$ S, $29^{\circ}22'31''$ E; $22^{\circ}14'23''$ S, $29^{\circ}22'40''$ E; $22^{\circ}13'01''$ S, $29^{\circ}21'51''$ E) within Mapungubwe National Park, Limpopo Province, South Africa (Figure 4-1) were sampled (Figure 4-1). This area is dominated by the mopane veld, with a semi-arid climate (Du Toit et al., 2003), which receives low, highly variable summer rainfall of between 350 - 400mm per year, between October and March (Götze et al., 2008). It is characterized by soils with sandy to loamy texture (Götze et al., 2008).



Figure 4-1: Study sites at the Mapungubwe National Park

4.3.2 Rates of herbivory and frass production

Thirty clusters of mopane worms were located on mopane trees within the three study sites. The initial mopane worm number for each cluster was recorded. The instar stages were determined directly through observation. To obtain the herbivory rate, the total number of leaves present in the enclosed branch was recorded at the beginning of the experiment and again at the end of three days to determine the total number of leaves consumed by each mopane worm cluster over the three days (Table A1). This served as the initial and final leaves, and the difference indicates the number

of leaves consumed. Organza bags were placed over the branch and tied to the stem to contain the mopane worms and frass. Each cluster was visited every 12 hours for three days to collect frass. When the supply of mopane leaves became too low for a cluster, all mopane worms were transferred to a nearby branch, and the new leaf and worm numbers were recorded.

To calculate the rate of herbivory, 300 leaves from 17 mopane trees were collected, their wet mass weighed, sun-dried for 48 hours, and weighed again to estimate their dry mass. These values were averaged to represent the average dry leaf mass for a mopane leaf. With this constant, the dry leaf mass consumed per worm for each cluster was calculated with the following formula:

$$\text{Dry leaf consumption} = \frac{[\text{Total leaves consumed} \times \text{average dry leaf mass}]}{(\text{number of worms})}$$

To find the rate of frass production of the sample population, the dry mass of the collected frass was weighed. Then the frass mass produced per worm for each cluster was calculated with the following formula:

$$\text{Frass produced} = \frac{[\text{Total frass produced per cluster}]}{(\text{number of worms})}$$

Rates of herbivory and frass production per worm were grouped by instar stage. An exponential curve was plotted and used to predict similar rates for fourth and fifth-instar worms.

$$\text{Frass produced/hectare by instar stage} = (\text{estimated maximum worm count per hectare}) \times (\text{survival probability per instar stage}) \times (\text{frass production rate per instar stage}) \times (\text{instar duration}).$$

4.3.3 *Worm density on a landscape scale*

To scale up the calculated herbivory and frass production rates to a landscape level, the measured leaf consumption rates and frass production from instar stages 2 and 3 were augmented with published data to calculate estimated upper and lower limit of leaf consumption and frass production per hectare.

To calculate consumption and frass production per hectare, the total consumption and frass production per worm during its life cycle were multiplied by the estimated number of worms per hectare. Consumption and production for each worm were determined by multiplying the daily rate at each stage by the number of days the stage lasted. The following variables were considered to estimate the number of worms per hectare.

- Number of trees per hectare
- Number of trees infected during outbreak periods
- Number of egg clutches per tree
- Number of eggs per clutch
- Proportion of eggs likely to survive

To evaluate extremes in the mopane worm population due to environmental variables, the upper and lower values were calculated from data reported in the literature (Ditlhogo, 1996). These estimates provide a landscape-level understanding of the influence mopane worms have on nutrient cycling.

4.4 Results

4.4.1 *Leaf consumption and frass production*

Dry leaf consumption and frass production increased with an increase in the instar stage (Table 4-1, Figures 4-2 & 4-3).

The average dry leaf consumption per worm per day for instar stages 1, 2 and 3 were -20.34 (± 41.80) mg, 49.80 mg and 194.93 (± 156.31) mg, respectively, while the average frass production per worm per day for the same instars were 5.27 (± 2.03) mg, 23.92 mg and 91.10 (± 45.20) mg respectively (Table 4-1). The negative consumption rate in stage 1 likely results from worms being so

small that their minimal intake leads to negligible changes in mass, making measurements susceptible to experimental error. Due to large variations in field conditions, controlling the experiment is difficult. The large standard deviation indicates that there was a lot of variation. Due to significant variations in field conditions, it was challenging to control the experiment effectively. The large standard deviation in the results suggests considerable variability, indicating that environmental factors and other variables may have influenced the data. This variation highlights the complexity of accurately measuring mopane worm consumption and frass production in natural settings. Additionally, the sample size of individuals in stage 2 was only one, reducing the confidence in accurate data.

Table 4-1: Dry leaf mass consumption rates and frass mass production rates for each instar stage

Instar	Sample size	Average dry leave consumption (mg)/instar	Average total frass (mg)/instar
1	5	-20.34 (± 41.80)	5.27 (± 2.03)
2	1	49.80	23.92
3	24	194.93 (± 156.31)	91.10 (± 45.20)

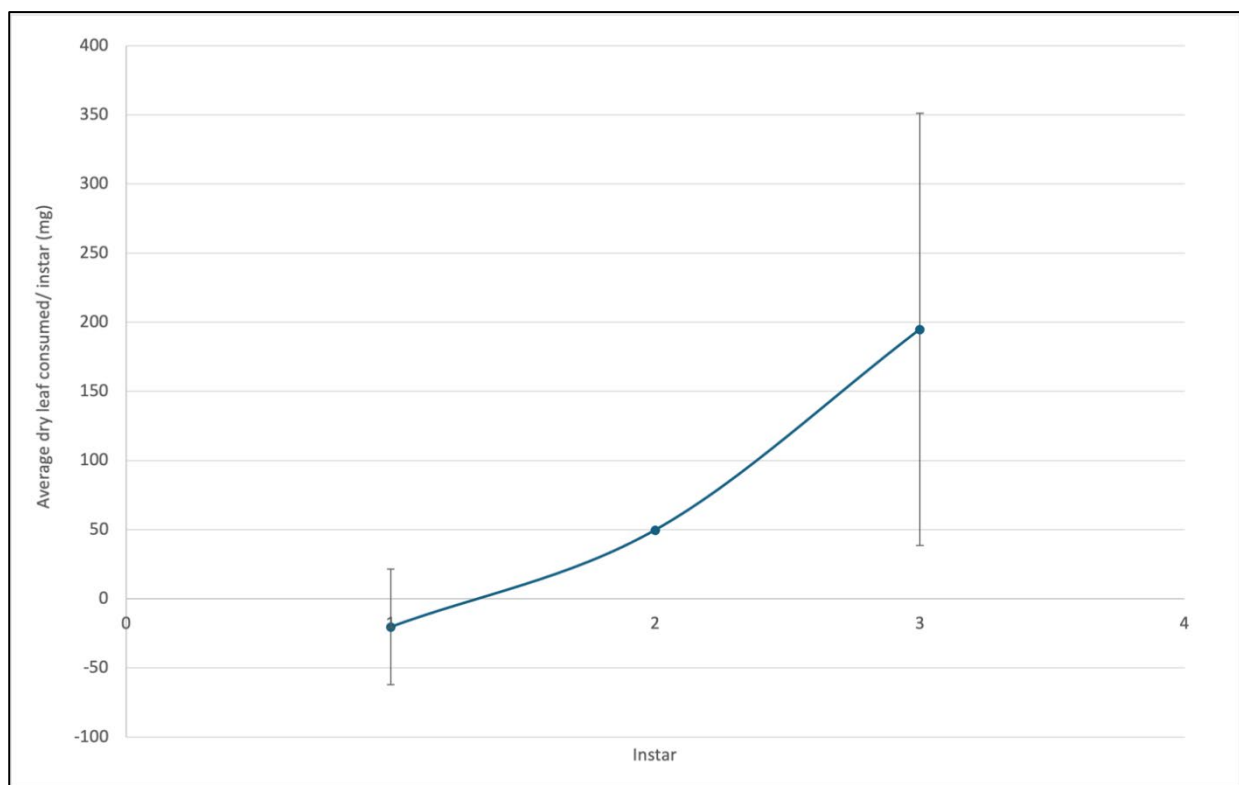


Figure 4-2: Average dry leaf (mg) consumed per instar stage by the mopane worms.

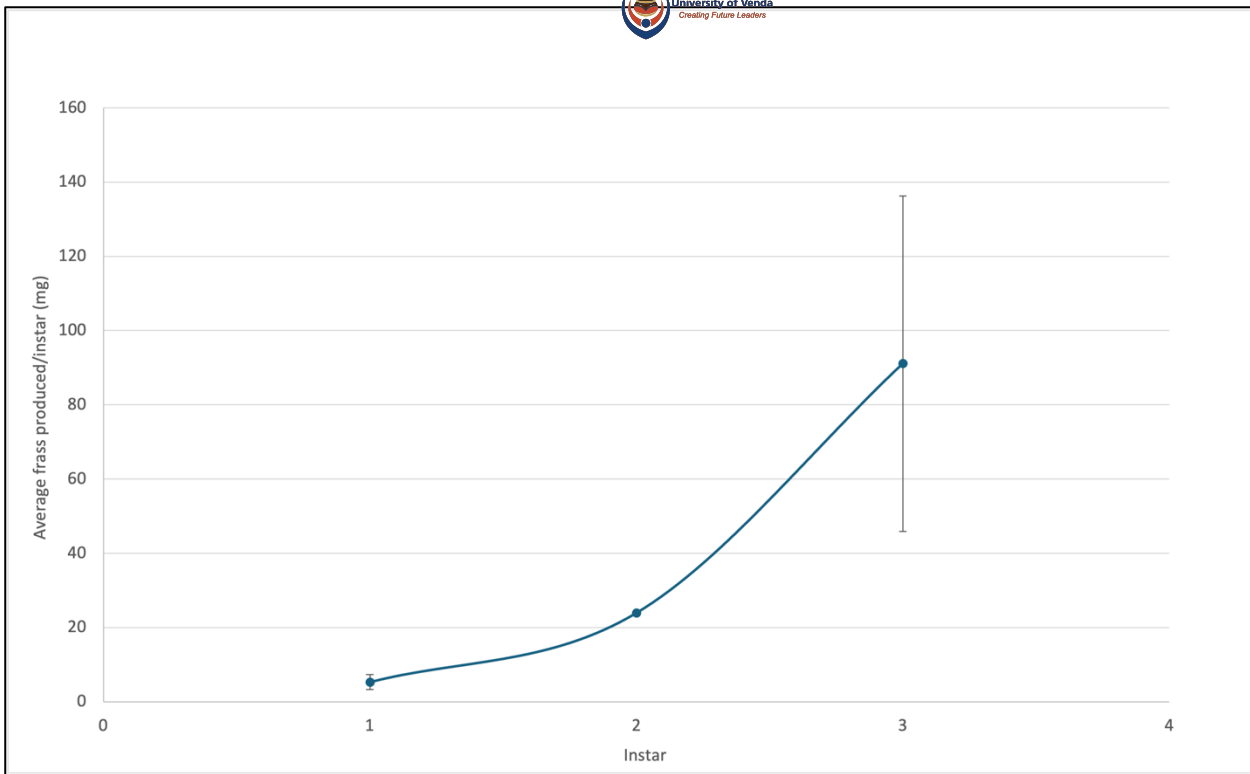


Figure 4-3: Average frass (mg) released per instar stage by mopane worms.

4.4.2 Scaling up to landscape level

To scale up to landscape level, the field data obtained on consumption for stages 2 and 3, was augment with published data. Since the consumption rate is so low in stage 1, only stages 2-5 were used. Variables and sources are shown in Table 4-2.

Table 4-2: Variables and sources to estimate consumption, frass production and number of worms per hectare

Variable	Lower end	Higher end	Source
Consumption, Stage 2 (mg/worm/day)	49.80		Own results
Consumption, Stage 3 (mg/worm/day)	194.93		Own results
Consumption, Stage 4 (mg/worm/day)	1517.79		Calculated based on stage 2, 3 (own results) and stage 5 (De Swart et al., 2018)
Consumption, Stage 5 (mg/worm/day)	3405.44		De Swart et al. (2018)
Frass production, Stage 2 (mg/worm/day)	23.92		Own results
Frass production, Stage 3 (mg/worm/day)	91.10		Own results

Frass production, Stage 4 (mg/worm/day)	629.27		Calculated based on relationship between consumption and frass production
Frass production, Stage 5 (mg/worm/day)	1400		De Swart et al. (2018)
Duration per stage (days)	5	7	Siddiqui et al. (2024)
Egg cluster per tree	1.9	8.9	Ditlhogo et al. (1996)
Eggs per cluster	30 20	355 200	Ditlhogo (1996); Gondo et al. (2010); Makhado et al. (2014)
Success rate of eggs hatching (%)	60		Wiggins (1997)
Average tree per hectare	391		Mosimanyana & Kiflewahid (1991)
Trees affected during outbreak periods (%)	44		De Swart et al. (2018)
Estimated worms/ha	29,000		De Swart et al. (2018)

To calculate the consumption rate and frass production per worm over its life cycle, the daily consumption and production rates were multiplied by the number of days per stage. Five days were used for the lower limit, while seven days were used for the upper limit (Table 4-3) (Siddiqui et al., 2024).

Table 4-3. Consumption per worm across their entire life cycle ranges between 25.84-36.18 g of dry leaves and produces 10.72-15.01 g frass in the same period.

Stage	Consumption rate (mg/day/worm)	Consumption lower limit (5 days) (g/worm)	Consumption upper limit (7 days) (g/worm)	Frass production (mg/worm/day)	Frass production lower limit (5 days) (g/worm)	Frass production upper limit (7 days) (g/worm)
Instar 2	49.80	0.25	0.35	23.92	0.12	0.17
Instar 3	194.93	0.97	1.36	91.10	0.46	0.64
Instar 4	1517.79	7.59	10.62	629.27	3.15	4.40
Instar 5	3405.44	17.03	23.84	1400.00	7.00	9.80
Total		25.84	36.18		10.72	15.01

To estimate the number of worms per hectare, several variables were considered, such as the number of trees per hectare, the number of egg clutches per tree, the number of eggs per clutch, the estimated survival rate of larvae, and the proportion of trees with eggs clutches (Table 4-2). Some values, such as the number of eggs per clutch (ranging from 20-200 or 30-355 eggs) (Ditlhogo,

1996; Gondo et al., 2010), have wide ranges. Due to these uncertainties, rounded estimates were used, resulting in values with high variability. The calculations are presented in equations 1, 2 and 3:

$$\text{Density estimates, worms per hectare} = (\text{Nr of egg clutches per tree}) * (\text{number of eggs per clutch}) * (\text{Success rate of eggs hatching}) * (\text{Nr of trees per hectare}) * (\text{Portion of trees with egg clutches per hectare}) \quad (1)$$

$$\text{Lower estimate, worms per hectare} = 1.9 * 20 * (60/100) * 391 * (44/100) = 5,883.77 \quad (2)$$

$$\text{Upper estimate, worms per hectare} = 8.9 * 355 * (60/100) * 391 * (44/100) = 275,608.08 \quad (3)$$

Density estimates for mopane worms per hectare during outbreak periods widely ranged between 5,884 and 275,608. These values were used to estimate the consumption rate of mopane leaves and the production of frass per hectare (equations 4, 5, 6, 7 and 8).

$$\text{Rate (kg/ha)} = (\text{Consumption rate and frass production in mg/worm} * \text{worm density})/1000 \quad (4)$$

$$\text{Consumption rate, lower limit} = (25.84 * 5,883.77)/1000 = 152.04 \text{ kg/ha} \quad (5)$$

$$\text{Consumption rate, upper limit} = (36.18 * 275,608.08)/1000 = 9,970.33 \text{ kg/ha} \quad (6)$$

$$\text{Frass production, lower limit} = (10.72 * 5,883.77)/1000 = 63.08 \text{ kg/ha} \quad (7)$$

$$\text{Frass production, upper limit} = (15.01 * 275,608.08)/1000 = 4,136.89 \text{ kg/ha} \quad (8)$$

Consumption of mopane leaves thus varied between 152 and 9,970 kg per hectare, while frass production varied between 63-4,137 kg per hectare.

4.5 Discussion

The role of mopane worms in nutrient cycling through leaf consumption and frass production is significant in savanna ecosystems, particularly within mopane woodlands. Mopane worms are known for their high biomass consumption during their feeding phases, and this activity has both direct and indirect effects on the ecosystem's nutrient dynamics. This study examined the extent of leaf consumption and frass production by mopane worms and their potential contribution to nutrient cycling at a landscape level.

The findings of this study show that mopane worms can consume substantial amounts of foliage, ranging from 152 to 9,970 kg of leaves per hectare during their developmental stages. This range includes consumption rates of 1,645 kg/ha reported by De Swart et al. (2018). The estimates in this study were based on consumption rates of 25.84-36.18 g of dry leaf consumption across the life stage of one worm. This is lower than rates reported by Styles (1996), who measured 45 g of dry leaf per worm. There is therefore confidence that the higher estimate of 9,970 kg is a conservative estimate, which might even be exceeded. This broad range reflects the variability in population density, food availability, and the specific growth conditions within different parts of mopane woodland (Siddiqui et al., 2024). High levels of defoliation can significantly affect the host trees' growth and survival (Wiggins, 1997; Ditlhogo, 1996). The relationship between tree survival and mopane worm herbivory proves to be complex. It was shown that heavy pruning can impact host tree regrowth (Hrabar et al., 2009) and reduce the next season's egg masses (Hrabar & Du Toit, 2014). However, mopane worms typically consume most of the older, less nutritional leaves, thereby stimulating new and palatable leaf flush and rejuvenating the system in outbreak areas (Styles, 1996). Mopane trees showed no visible trade-off in investment between tolerance and resistance, as they are adapted to both (Hrabar et al., 2009).

Frass production ranges from 63 to 4,137 kg per hectare and represents a critical pathway through which mopane worms contribute to the nutrient cycle (De Swart et al., 2018). Frass is rich in nitrogen, phosphorus, and other essential nutrients, which can enhance soil fertility. The decomposition of frass enriches the soil organic matter, promoting nutrient availability for plants and microorganisms. For example, nitrogen mineralization studies found that frass decomposes faster and releases more inorganic N when compared to mopane leaves (Reynolds & Hunter, 2004; Netshanzhe et al., 2022). Additionally, the large volume of frass deposited during periods of high

mopane worm activity can facilitate the cycling of nutrients that are otherwise locked in the plant biomass.

The mopane worms' ability to consume and process such large quantities of biomass suggests they play a keystone role in nutrient cycling within mopane woodlands. By converting leaf material into frass, mopane worms contribute to soil nutrient pools, facilitating the redistribution of nutrients from tree canopies to the soil surface. This can enhance soil structure, increase microbial activity, and promote plant growth, especially during periods of rapid decomposition following rainfall events.

4.6 Conclusion

Mopane worms play a vital role in nutrient cycling within mopane woodlands through their extensive leaf consumption and frass production. This study shows that their consumption of foliage, ranging from 152 to 9,970 kg per hectare, significantly impacts nutrient flow within these ecosystems. Frass production, ranging from 63 to 4,137 kg per hectare, contributes crucial nutrients, particularly nitrogen and phosphorus, to the soil, enhancing fertility and promoting plant regrowth. The mopane worm's role in redistributing nutrients from the canopy to the soil underscores its importance in maintaining ecosystem balance. While the results of this study highlighted the need to monitor and manage extensive harvesting of mopane worms, as local extinction of worms due to overharvesting and land use changes can negatively impact mopane woodlands, there is still need for more future research with aims to quantify more accurate consumption rate and frass production for different instar stages of development, as well as a better understanding of outbreak dynamics, to improve the understanding of ecosystem service by mopane worms and the long-term impacts of mopane worm outbreaks on tree health, nutrient cycling, and overall ecosystem resilience. This study faced challenges of low sample size and high variations due to field conditions and thus more studies with high sample size, improved monitoring such as use of camera traps, where all the instar stages are monitored could improve the accuracy of the quantification of the worms importance in their ecosystem.

4.7 References

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4.8 Appendix

Table A1. Average amount of MWs, leaf mass consumed (wet and dry) and frass produced per MW per day, sorted by instar and cluster of caterpillars.

Instar	Average # of MWs	Wet Leaf Mass (mg)	Dry Leaf Mass/Worm/Day (mg)	Frass Produced/Worm/Day (mg)
1	88.5	-5.32854	-0.01074	0.002938
1	57.5	-2.9221	-0.00907	0.003362
1	42	3.265876	0.013871	0.004444
1	57.5	-24.7519	-0.07679	0.004
1	75	-0.85944	-0.00204	0.007244
2	49.5	11.51651	0.041501	0.019933
3	57	39.87807	0.124797	0.024737
3	44	5.500424	0.022299	0.036591
3	64	136.4793	0.380393	0.132344
3	58	94.19475	0.289698	0.131954
3	86.5	-38.503	-0.0794	0.064509
3	35	22.68925	0.115637	0.03981
3	32	55.69179	0.310447	0.123958
3	50	80.44369	0.286991	0.070267
3	73.5	76.66215	0.186054	0.074649
3	91	37.81541	0.074127	0.069963
3	68	5.500424	0.014429	0.047451
3	29.5	49.84759	0.301418	0.114689
3	43	22.68925	0.094123	0.091395
3	36.5	55.00424	0.268813	0.11516
3	29.5	16.50127	0.09978	0.14791
3	44.5	52.94158	0.212218	0.120749
3	50	33.34632	0.118966	0.074733
3	55	54.14479	0.175606	0.06103
3	20.5	12.37595	0.107689	0.061626
3	41	8.250635	0.035896	0.04374
3	33.5	86.28789	0.459464	0.040796
3	54	23.3768	0.077221	0.075309
3	55.5	60.16088	0.19336	0.026907
3	57	9.110076	0.02851	0.031754

Table A2. All GPS coordinates for every bag (tag number) in the three sites, as well as the coordinates for all available plots that were done in two of the sites. Coordinates for plots in the first site could not be retrieved, as they were prematurely deleted from the GPS.

Site	Tag Number	GPS Coordinates
1	J1A	S22.23827 E029.37520
1	J2A	S22.23828 E029.37506
1	J3A	S22.23823 E029.37490
1	J4A	S22.23826 E029.37490
1	T1A	S22.23800 E029.37532
1	T2A	S22.23808 E029.37531
1	T3A	S22.23808 E029.37523
1	Z1A	S22.23835 E029.37521
1	Z2A	S22.23818 E029.37525
1	Z3A	S22.23817 E029.37522
2	Z4A	S22.23984 E029.37788
2	Z4B	S22.23984 E029.37788
2	Z5A	S22.24009 E029.37774
2	Z6A	S22.24000 E029.37769
2	Z7A	S22.23944 E029.37758
2	Z8A	S22.23927 E029.37736
2	J5A	S22.24007 E029.37783
2	J6A	S22.23973 E029.37770
2	J7A	S22.23971 E029.37778
2	J7B	S22.23971 E029.37779
2	J8A	S22.23940 E029.37787
2	J9A	S22.23941 E029.37754
2	J10A	S22.23940 E029.37753
2	T4A	S22.24003 E029.37775
2	T4B	S22.24003 E029.37776
2	T5A	S22.23961 E029.37775
2	T6A	S22.23950 E029.37756
2	T7A	S22.23951 E029.37753
3	T8A	S22.21708 E029.36405
3	J11A	S22.21719 E029.36386
1	Plot 1	----
1	Plot 2	----
1	Plot 3	----
1	Plot 4	----
1	Plot 5	----
1	Plot 6	----
1	Plot 7	----
1	Plot 8	----
1	Plot 9	----
1	Plot 10	----
2	Plot 1	S22.23976 E029.37706
2	Plot 2	S22.23976 E029.37706
2	Plot 3	S22.23995 E029.37674
2	Plot 4	S22.23995 E029.37674
2	Plot 5	S22.24025 E029.37656
2	Plot 6	S22.24025 E029.37656
2	Plot 7	S22.24052 E029.37636
2	Plot 8	S22.24052 E029.37636
2	Plot 9	S22.24046 E029.37599
2	Plot 10	S22.24046 E029.37599

Table A3. Average dry leaf mass consumed, and frass mass produced per mopane worm per day from first through fourth instar. The fourth instar leaf mass was not recorded.

	First Instar	Second Instar	Third Instar	Fourth Instar
Dry Leaf Mass/Worm/Day (g)	0.0139	0.0415	0.1730	-----
Frass Mass/Worm/Day (g)	0.0044	0.0110	0.0759	0.6175

5 CHAPTER 5: The role of mopane worms in nutrient cycling: Insights from nitrogen incubation studies



Mopane worms hanging on a mopane tree in Ga-Selwane communal land, Phalaborwa.

The role of mopane worms in nutrient cycling: Insights from nitrogen incubation studies

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5.1 Abstract

The significance of edible insects has been greatly quantified by studies exploring their nutritional content, contribution to food security, and economic value. Apart from contributing to the food and economic sector, herbivorous edible insects have been reported to influence ecosystem functions such as biogeochemical cycling, amongst others, with contributions that seem to exceed that of herbivorous mammals. Herbivorous insects feed on leaves and release fast-decomposing frass in large values to the ground, thus enhancing the nutrient pool and accelerating nutrient cycling. Mopane worms are an example of edible herbivorous insects that are reported to defoliate whole trees and release vast amounts of frass. Mineralization studies often use N as an indicator of mineralization rate and nutrient release from nutrient sources. Considered an important food and income source, it is important to explore whether these worms have other important ecosystem services to aid the Nitrogen cycling process, as indicated by a release in N-NH₄ and N-NO₃.

This study aimed to compare the N mineralisation rate as indicated by N-NH₄ and N-NO₃ release from frass from mopane worms and leaves from mopane trees to establish whether the presence of mopane worms' frass led to faster nutrient release for plant uptake. It was found that frass from mopane worms mineralise faster when compared to leaves of mopane trees after application to soil,

releasing both N-NH_4 and N-NO_3 faster than in leaves, while the size of the organic material applied to the soil does not affect the mineralisation rate.

Keywords: mopane leaves, frass, mineralisation rate, N-NH_4 , N-NO_3

5.2 Introduction

Mopane worms, the caterpillar of the mopane moth *Gonimbrasia belina*, have a wide distribution in southern and central Africa (Makhado et al., 2012). They are known and used widely in these countries as a food and income source (Nemadodzi et al., 2023). Additionally, they play an important ecological role by forming part of the animal food chain, providing nutrients to both domestic and wild animals (Gaston et al., 1997), as well as facilitating nutrient cycling by consuming mopane leaves and converting it into fertile frass (De Swardt et al., 2018).

Mopane worms depend largely on mopane trees (*Colophospermum mopane* (J. Kirk ex Benth.) J Leonard Kirk J. (1860)) (Nemadodzi et al., 2023), but also feed from other trees such as *Terminalia sericea*, *Sclerocarya birrea* (Ditlhogo et al., 1996), *Brachystegia spiciformis* and *Julbernardia paniculata* (Mughogho & Munthali, 1993).

Mopane worms are heavy leaf defoliators and can defoliate entire tracts of trees (Ditlhogo et al., 1996), producing large quantities of frass, which decomposes rapidly, releasing nutrients into the soil and facilitating natural nutrient cycling (Netshanzhe et al., 2022). However, limited studies such as (De Swardt et al., 2018) have investigated or quantified the extent to which nutrients are returned from frass compared to undigested leaves.

This study aims to quantify decomposition (mineralization) rates in soil enriched with mopane worm frass versus soil containing only leaf litter, using nitrogen (N) mineralization as an indicator. Thus, to determine whether the addition of frass enhances the conversion of organic material into plant-available nutrients, potentially accelerating nutrient cycling. This research will provide insight into mopane worms' role in improving nutrient cycling and soil fertility in mopane woodland ecosystems.

5.3 Materials & Methods

A laboratory incubation trial was done under controlled conditions to contrast N mineralization rates from mopane leaves and mopane worm frass. Since mopane leaves have a different surface area compared to frass, a 'fine leaf' and 'fine frass' treatment was also included to include a range of particle sizes to eliminate any difference in mineralization due to particle size.

5.3.1 Field capacity

The experiment was done at field capacity (FC). Two 50 ml open-ended tubes (the other end covered with a fibre that allows water movement) were filled with soil up to 30 ml to resemble bulk density. The tubes were placed in a 250 ml beaker filled with water and left for 5 hours to absorb water. The tubes were removed and left to stand overnight, allowing excess water drainage. Samples were taken from the centre of the tubes, weighed, and placed in an oven at 105°C for 24 hours, after which the samples were weighed again. The difference between the wet weight and the dry weight is the field capacity, calculated as follows:

Weight of container (a)

Weight of wet soil + container (b)

Weight of wet soil = (b) – (a) (c)

Weight of dry soil + container (d)

Dry soil = (d) – (a) (e)

Soil water content = (c) – (e) (f)

Field capacity = $f/e * 100$

Based on gravimetric estimation, 1.82 ml of water is needed to bring 10 g of soil to FC (Hillel, 2004).

5.3.2 *Treatments: Frass, leaves, and soil*

Five treatments were used: i) soil-only (control), ii) whole leaves and soil, iii) whole frass and soil, iv) fine leaves and soil and v) fine frass and soil.

Mopane worm frass, mopane leaves and soil (0-15 cm of topsoil) were collected in mopane outbreak regions in Giyani (23°25'22.1"S, 30°54'30.3"E) and Letaba Ranch (23°42'18.1" S, 30°54'36.4"E), Limpopo Province, South Africa. Topsoil samples were selected since mopane worms commonly burrow into the top 15 cm of soil. The soil was air-dried and passed through a 2 mm sieve. The leaves were over-dried at 60°C until completely dry.

Both the soil, leaves and frass were analysed for chemical composition (Table 5-1)

5.3.3 *Incubation trial*

The incubation trial followed a randomized block design over 100 days at a constant temperature of 24°C. One hundred and eighty centrifuge tubes of 50 ml volume were filled with 10 g of soil and 1.82 ml of deionized water, and the tubes were incubated for seven days to allow the microbes to adjust to the water and temperature. During the pre-incubation period and for the duration of the experiment, the water content was kept constant by weighing the tubes and adding water to keep the soil at FC.

The 7th day of pre-incubation was marked as day 0 of sample extraction. Treatments were added to the tubes. Each treatment was replicated four times. Both the whole leaf and frass treatments received 0.25 g of mopane leaves or frass, respectively. The fine leaf and frass treatments were made up of ground leaves and frass and were also applied at 0.25 g per vial of soil. A set of 20 samples (5 treatments x 4 replicates) was analysed on days 0, 1, 4, 7, 14, 21, 54, 71, and 100 (Van Niekerk et al., 2005) and analysed for N-NH₄ and N-NO₃.

5.3.4 *Sample Analysis*

A total of 30 ml of 2 M potassium chloride (KCl) was added to the sample tubes. The tubes were shaken at 190 osc/min for 60 minutes. Samples of supernatants were then filtered into 15 ml conical centrifuge tubes using 150 mm grade 292a Sartorius filter paper. Samples were analysed using a

Pharmacia Biotech Ultrospec III spectrophotometer. N-NO₃ analyses were read at a wavelength of 540 nm while N-NH₄ were read at 650 nm wavelength.

N-NO₃

For N-NO₃ analysis, the method outlined by García-Robledo et al. (2014) was applied. A subsample of 0.1 ml was pipetted into 2.5 ml cuvettes, to which a 1.2 ml NO₂ mix (Appendix for mix make-up) was added. The samples were left to stand for 20 minutes before analysing.

Thereafter, a volume of 0.1 ml of NO₃ mix (1.0g Vanadium (III) Chloride + 50 ml H₂O) was added to the above NO₂ samples, placed in an oven at 60°C for 25 minutes, which were then cooled for 10 minutes before analysis.

N-NH₄

N-NH₄ analysis followed methods from Baethgen & Alley (1989). Filtered samples (1 ml), were pipetted into 4 ml cuvettes. A 1 ml buffer mix, 0.84 ml nitro mix (Appendix for mix make-up), and 0.42 ml of Jik were added and left to stand for 45 minutes before analysis.

Total inorganic N estimation

Total inorganic N was estimated by adding together the values of N-NH₄ and N-NO₃ on each sampled day.

5.3.5 Data analysis

The aim of this study was to investigate the effects of treatment factors (Control, Fine Frass, Frass, Leaves, Whole Leaves) on the release of N-NH₄ and N-NO₃ over time (from Day 0 to Day 100). Given that mineralization rates exhibited a non-linear trend, treatments were first grouped by day, and separate linear models were fitted for each day to evaluate the effect of treatment on mineralization. For each day, a treatment model was fitted, and significance was assessed using one-way ANOVA (Type I: Wahid et al., 2017; Dudek, 2023). If significance was detected, a Tukey post hoc test was

done to evaluate mean differences between treatments (Agbangba et al., 2024). The linear model assumptions were evaluated using the Shapiro-Wilk test for normality of residuals (Razali and Wah, 2011) and Levene's test for homogeneity of variance across treatment groups (Nordstokke & Zumbo, 2010). If model assumptions were violated, the non-parametric Kruskal-Wallis test (Ramachandran and Tsokos (Eds), 2015) was used to evaluate treatments and Dunn's test for post hoc analysis (Dinnio, 2015). All the statistical analyses were done on R (R core team, 2023).

5.4 Results

5.4.1 Chemical composition of soil, leaves, and frass

The chemical analysis of the frass, leaves and soil is listed in Table 5-1. Frass (54.72% C) and leaves (54.46% C) had high carbon as compared to soil (0.94% Ash). Frass (1.38%) and leaves (1.82%) had low nitrogen contents as compared to soil (18.1%). The C: N ratio for frass, leaves and soil is listed in Table 5-1.

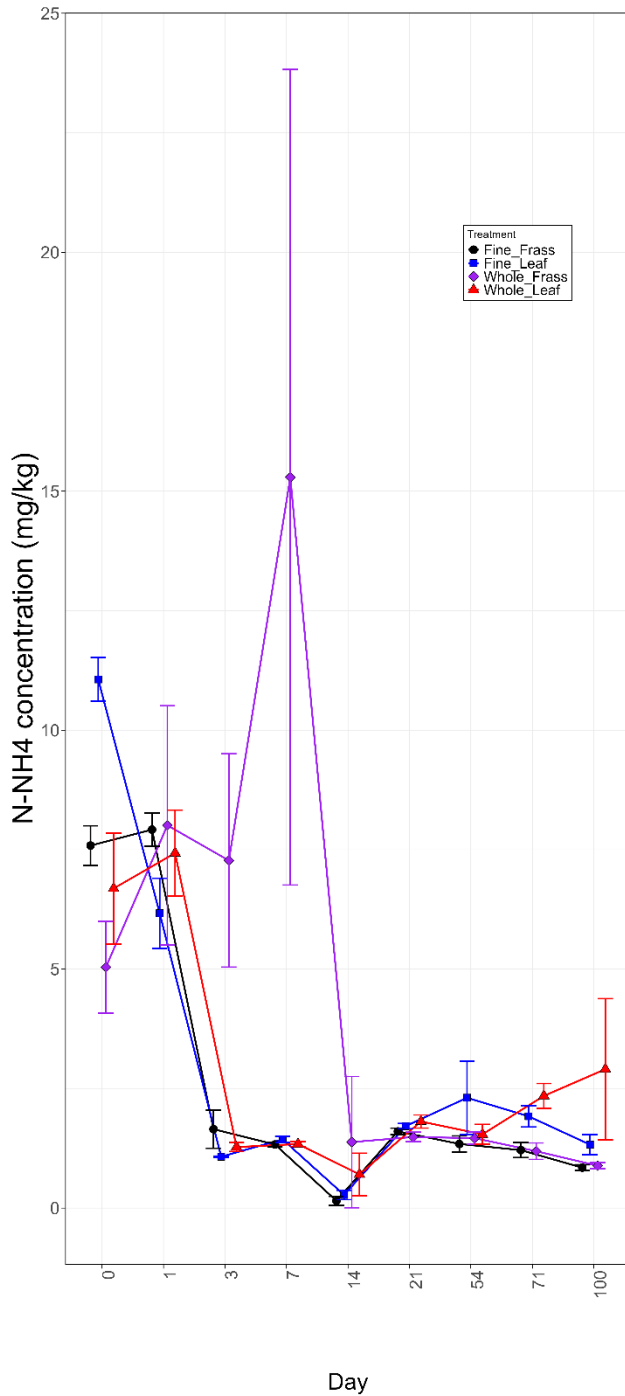
Table 5-1: Chemical composition of the soil, leaves and frass used in the incubation experiment.

Element	Unit	Frass	Leaves	Soil
C	%	54.72	54.46	0.94 (Ash)
N	%	1.38	1.82	18.10
B	mg/kg	71	112	0.25
Ca	mg/kg	13 100	19 400	618
Cu	mg/kg	6	8	0.86
Fe	mg/kg	124	65	23.68
K	mg/kg	9 100	8 100	160
Mg	mg/kg	2 300	1 400	111
Mn	mg/kg	28	51	12.11
Na	mg/kg	99	100	15
P	mg/kg	1500	1 400	8
Zn	mg/kg	13	30	0.4
C:N ratio		39.65	29.92	0.05

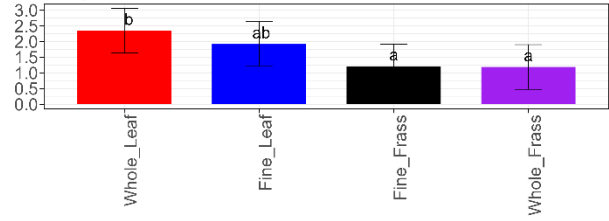
5.4.2 Ammonium

Detailed results are given in Table 5-2 and show under what treatments a parametric (ANOVA) or non-parametric (Kruskal-Wallis) test was applied to test the treatment effect per day. Treatments and analysis were applied on Day 0, and analysis showed that treatments significantly affected N-NH₄ mineralization, where N-NH₄ was highest in the Fine and Whole Leaf treatments (Figures 5-1A & B; Table 5-2) and lowest in the frass treatments (Figures 1A & B; Table 5-2). An increase in N-NH₄ concentrations was observed after 24 hours (day 1), although no significant differences existed between treatments (Figures 5-1A & C; Table 5-2). On days 3 and 7, N-NH₄ concentrations in Whole Frass treatment were significantly higher than the other treatments (Figures 5-1 A & D & E; Table 5-2), where production peaked on Day 7 for Whole Frass (Figures 5-1A & E; Table 5-2). There was no treatment effect on days 14, 21 and 54, and mean production was similar across all treatments (Figure 5-1A). On days 54 and 100, N-NH₄ production rates were significantly higher in the leaf treatments, while the frass treatments had the lowest rates (Figures 5-1A & F & G; Table 5-2). All treatments follow a similar pattern where all rates decline post-day 1 and stay similar for the rest of the treatment periods (Figure 5-1A). However, the Whole Frass treatment N-NH₄ rates stayed higher and declined to similar levels on day 7 (Figure 5-1A).

A

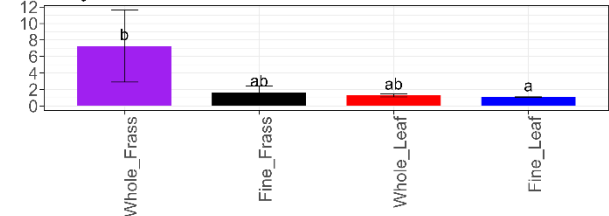


Day 0 Im



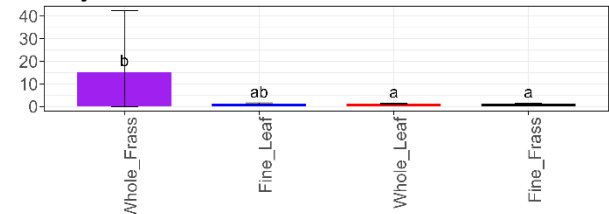
C

Day 3 Dunn's Test



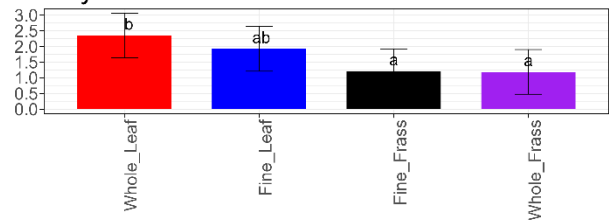
D

Day 7 Dunn's Test



E

Day 71 Im



F

Day 100 Dunn's Test

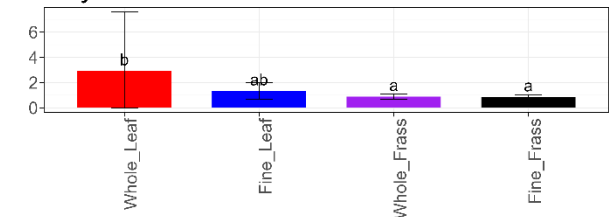


Figure 5-1: Daily N-NH₄ release from fine frass, fine leaf, whole frass and whole leaf. Significant differences in the groups are shown by different letters.

Table 5-2: N-NH₄ release from treatments over time (0 days). Significance over time is shown by non-bold numbers. Significant differences in the groups are shown by different letters.

Test	Significance	Day	Treatment	Mean	SE	lower.CL	upper.CL	.group
ANOVA	0.002	0	Fine Frass	7.59	0.81	4.79	10.38	a
ANOVA	0.002	0	Fine Leaf	11.06	0.81	8.27	13.85	b
ANOVA	0.002	0	Whole Frass	5.04	0.81	2.25	7.83	a
ANOVA	0.002	0	Whole Leaf	6.69	0.81	3.90	9.48	a
ANOVA	0.774	1	Fine Frass	7.92	1.39	3.15	12.68	a
ANOVA	0.774	1	Fine Leaf	6.17	1.39	1.40	10.93	a
ANOVA	0.774	1	Whole Frass	8.01	1.39	3.24	12.77	a
ANOVA	0.774	1	Whole Leaf	7.43	1.39	2.66	12.19	a
ANOVA	0.774	1	Mean	7.38	0.65	6.11	8.65	
Kruskal-Wallis	0.010	3	Fine Leaf	1.07	0.01	1.05	1.10	a
Kruskal-Wallis	0.010	3	Whole Leaf	1.28	0.09	1.09	1.46	ab
Kruskal-Wallis	0.010	3	Fine Frass	1.65	0.40	0.87	2.43	ab
Kruskal-Wallis	0.010	3	Whole Frass	7.27	2.23	2.90	11.60	b
Kruskal-Wallis	0.018	7	Fine Leaf	1.44	0.13	1.24	1.64	ab
Kruskal-Wallis	0.018	7	Whole Leaf	1.34	0.10	1.18	1.50	a
Kruskal-Wallis	0.018	7	Fine Frass	1.33	0.10	1.18	1.48	a
Kruskal-Wallis	0.018	7	Whole Frass	15.30	17.10	0.00	42.40	b
Kruskal-Wallis	0.472	14	Fine Leaf	0.27	0.20	0.00	0.59	a
Kruskal-Wallis	0.472	14	Whole Leaf	0.70	0.89	0.00	2.11	a
Kruskal-Wallis	0.472	14	Fine Frass	0.15	0.19	0.00	0.45	a
Kruskal-Wallis	0.472	14	Whole Frass	1.38	2.75	0.00	5.75	a
ANOVA	0.149	21	Fine Frass	1.60	0.10	1.28	1.93	a
ANOVA	0.149	21	Fine Leaf	1.71	0.10	1.39	2.04	a
ANOVA	0.149	21	Whole Frass	1.49	0.10	1.16	1.81	a
ANOVA	0.149	21	Whole Leaf	1.81	0.10	1.48	2.14	a
Kruskal-Wallis	0.422	54	Fine Leaf	2.31	1.53	0.00	4.74	a
Kruskal-Wallis	0.422	54	Whole Leaf	1.54	0.42	0.88	2.20	a
Kruskal-Wallis	0.422	54	Fine Frass	1.34	0.34	0.80	1.88	a
Kruskal-Wallis	0.422	54	Whole Frass	1.46	0.26	1.05	1.87	a
ANOVA	0.005	71	Fine Frass	1.21	0.21	0.50	1.92	a
ANOVA	0.005	71	Fine Leaf	1.92	0.21	1.21	2.63	ab
ANOVA	0.005	71	Whole Frass	1.19	0.21	0.48	1.90	a
ANOVA	0.005	71	Whole Leaf	2.35	0.21	1.63	3.06	b
Kruskal-Wallis	0.020	100	Fine Leaf	1.33	0.42	0.66	1.99	ab
Kruskal-Wallis	0.020	100	Whole Leaf	2.91	2.95	0.00	7.60	b
Kruskal-Wallis	0.020	100	Fine Frass	0.85	0.12	0.65	1.04	a
Kruskal-Wallis	0.020	100	Whole Frass	0.89	0.12	0.69	1.08	a

5.4.3 Nitrate

Detailed results and statistics are available in Table 5-3. All treatments had an initial N-NO₃ concentration of between 6.65 - 7.17 mg/kg (Figure 5-2A, Table 5-3). After 24 hours, the N-NO₃ concentration from Fine Leaf treatment declined, while it increased in other treatments (Figure 5-2A). Even though treatment effects were non-significant on day 1 (Table 5-3), non-overlapping confidence intervals indicate that the Fine Leaf had the lowest rate (Figures 5-2A & B; Table 5-3). From day 3 to day 100, Fine Frass and Fine Leaves consistently showed a lower N-NO₃ rates (Figure 5-2A) compared to Whole Frass and Whole Leaves (Figure 5-2A; Table 5-3). From day 3, Whole Frass consistently had the highest N-NO₃ rates (Figure 5-2A), with significantly different rates from Fine Frass and Fine Leaf on Days 7, 14, 21, 54 (Figures 5-2A, B, C, D, E & F; Table 5-3). Both leaf treatments had higher N-NO₃ rates on day 100, which is again highlighted by the non-overlapping confidence intervals (Figures 5-2A & G & H).

A

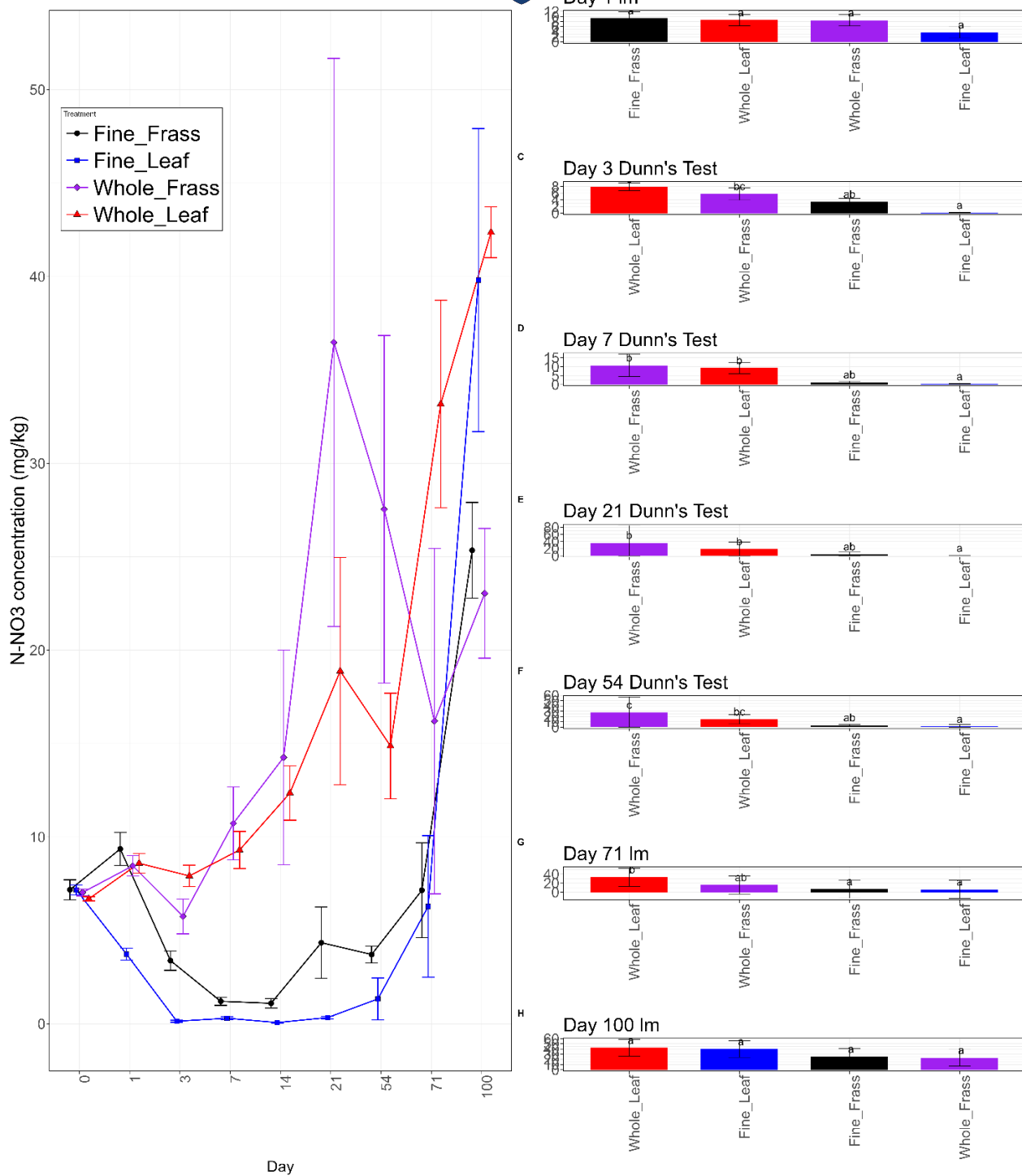


Figure 5-2: Daily N-NO₃ release from fine frass, fine leaf, whole frass and whole leaf. Significant differences in the groups are shown by different letters.

Table 5-3: N-NO₃ release from treatments over time (0 days). Significance over time is shown by non-bold numbers. Significant differences in the groups are shown by different letters.

Test	Significance	Day	Treatment	Mean	SE	lower.CL	upper.CL	.group
Kruskal-Wallis	0.372	0	Fine Leaf	7.17	0.26	6.65	7.69	a
Kruskal-Wallis	0.372	0	Whole Leaf	6.69	0.11	6.47	6.91	a
Kruskal-Wallis	0.372	0	Fine Frass	7.17	0.54	6.12	8.23	a
Kruskal-Wallis	0.372	0	Whole Frass	7.03	0.18	6.68	7.38	a
ANOVA	0.000	1	Fine Frass	9.36	0.73	6.81	11.91	a
ANOVA	0.000	1	Fine Leaf	3.73	0.63	1.52	5.94	a
ANOVA	0.000	1	Whole Frass	8.46	0.63	6.25	10.67	a
ANOVA	0.000	1	Whole Leaf	8.59	0.63	6.38	10.80	a
Kruskal-Wallis	0.005	3	Fine Leaf	0.14	0.05	0.04	0.24	a
Kruskal-Wallis	0.005	3	Whole Leaf	7.92	0.57	6.79	9.04	c
Kruskal-Wallis	0.005	3	Fine Frass	3.38	0.52	2.37	4.40	ab
Kruskal-Wallis	0.005	3	Whole Frass	5.75	0.93	3.93	7.57	bc
Kruskal-Wallis	0.005	7	Fine Leaf	0.30	0.16	0.04	0.55	a
Kruskal-Wallis	0.005	7	Whole Leaf	9.31	1.99	6.15	12.50	b
Kruskal-Wallis	0.005	7	Fine Frass	1.20	0.43	0.52	1.89	ab
Kruskal-Wallis	0.005	7	Whole Frass	10.70	3.91	4.50	17.00	b
Kruskal-Wallis	0.010	14	Fine Leaf	0.07	0.06	0.00	0.16	a
Kruskal-Wallis	0.010	14	Whole Leaf	12.40	2.91	7.72	17.00	a
Kruskal-Wallis	0.010	14	Fine Frass	1.10	0.51	0.30	1.90	a
Kruskal-Wallis	0.010	14	Whole Frass	14.30	11.50	0.00	32.50	a
Kruskal-Wallis	0.006	21	Fine Leaf	0.33	0.14	0.11	0.56	a
Kruskal-Wallis	0.006	21	Whole Leaf	18.90	12.20	0.00	38.20	b
Kruskal-Wallis	0.006	21	Fine Frass	4.34	3.82	0.00	10.40	ab
Kruskal-Wallis	0.006	21	Whole Frass	36.50	30.40	0.00	84.90	b
Kruskal-Wallis	0.007	54	Fine Frass	3.71	0.91	2.27	5.15	ab
Kruskal-Wallis	0.007	54	Fine Leaf	1.33	2.25	0.00	4.91	a
Kruskal-Wallis	0.007	54	Whole Frass	27.50	18.60	0.00	57.10	c
Kruskal-Wallis	0.007	54	Whole Leaf	14.90	5.65	5.88	23.90	bc
ANOVA	0.024	71	Fine Frass	7.15	5.85	0.00	27.22	a
ANOVA	0.024	71	Fine Leaf	6.28	5.85	0.00	26.35	a
ANOVA	0.024	71	Whole Frass	16.20	5.85	0.00	36.27	ab
ANOVA	0.024	71	Whole Leaf	33.17	5.85	13.11	53.24	b
ANOVA	0.025	100	Fine Frass	25.34	4.64	9.42	41.26	a
ANOVA	0.025	100	Fine Leaf	39.81	4.64	23.89	55.72	a
ANOVA	0.025	100	Whole Frass	23.04	4.64	7.12	38.96	a
ANOVA	0.025	100	Whole Leaf	42.37	4.64	26.45	58.29	a

5.4.4 Total inorganic N release

Total inorganic N ($\text{N-NH}_4 + \text{N-NO}_3$) for all treatments indicated a steady increase in N release over time. The Whole Frass treatment significantly outperformed all other treatments up to day 54 (Figure 5-3A; Table 5-4; Appendix Table A1). Whole Leaf and Whole Frass treatments had higher rates up to day 54 (Figure 5-3A); however, at the end of the trial (day 100), leaf treatments resulted in the highest total inorganic N release (Figures 5-3A & H; Appendix Table A1), while the frass treatments resulted in the lowest inorganic N release (Figure 5-3A).

Table 5-4. Inorganic N release from treatments after 100 days

Treatment	Total inorganic N (mg/kg)
Whole Leaves	20.06
Fine Leaves	9.68
Fine Frass	9.40
Whole Frass	21.34

A

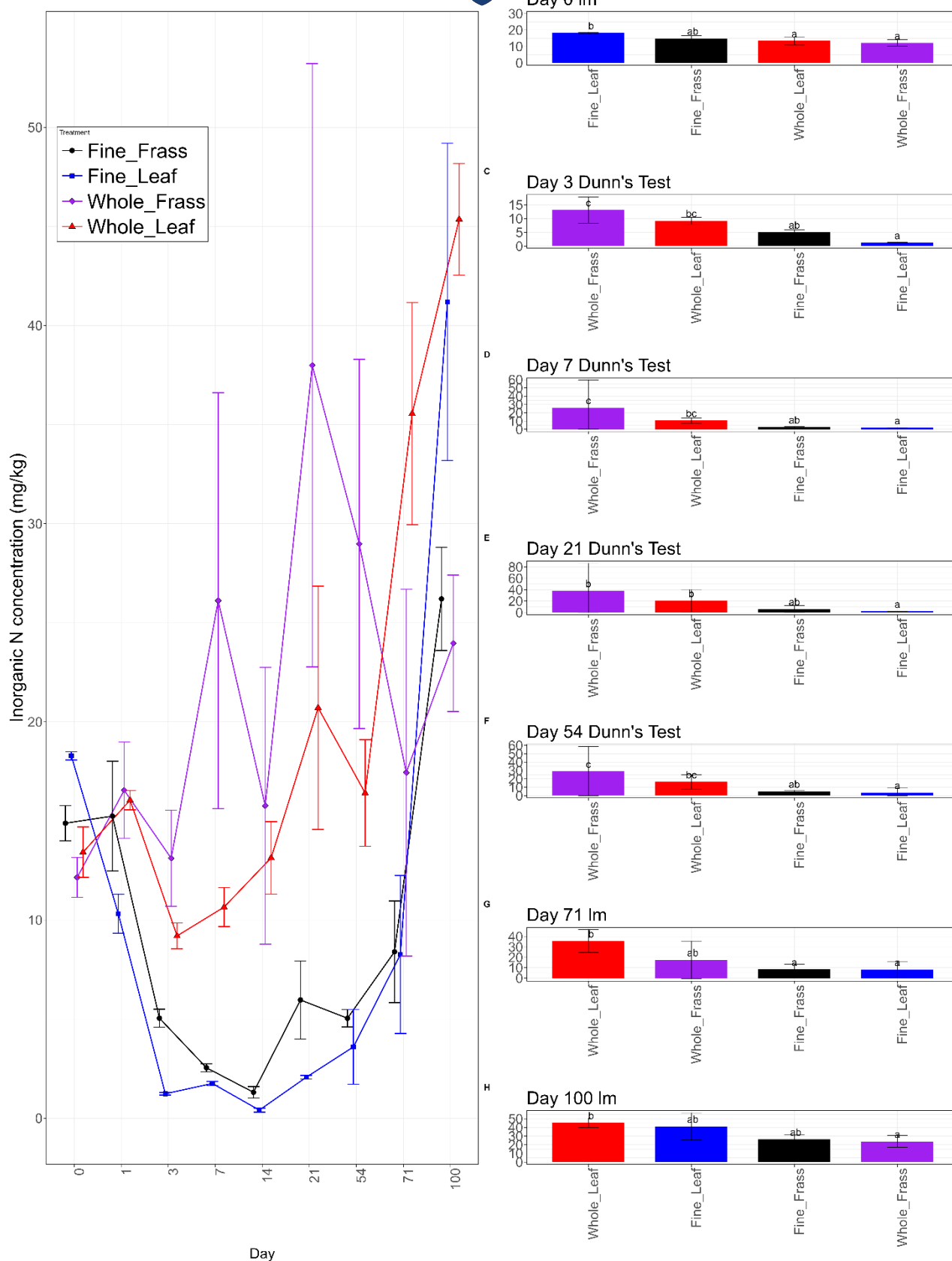


Figure 5-3: Total daily inorganic N (N-NH₄ + N-NO₃) production from fine frass, fine leaf, whole frass and whole leaf on selected sampling days. Significant differences in the groups are shown by different letters.

5.5 Discussion

5.5.1 Ammonium

N-NH₄ is the first form of inorganic N released from organic materials due to mineralization (Grzyb et al., 2020). Immediately after adding the organic matter into the soil, N-NH₄ concentrations were noticeable in all treatments. Organic components with easily mineralizable N can release a flush of N-NH₄ immediately after application to the soil (Stevenson, 1986; Drinkwater & Snapp, 2007), which explains the presence of N-NH₄ on day 0 immediately following treatment application. After 24 hours of treatment application (Day 1), N-NH₄ in the Whole Frass treatment increased to 15 mg/kg and peaked on Day 7. After Day 7, it started to decline, as N-NH₄ is converted into N-NO₃, as seen by the corresponding rise in N-NO₃ concentration. By day 54, N-NH₄ production from the Whole Leaves treatment increased again, possibly due to the slow decomposition of leaf litter (Belovsky & Slade, 2000), releasing a new flush of N-NH₄. This could be an advantageous process for plants as they get N-NH₄ faster from the fast-decomposing frass, and then later on, when the N-NH₄ from the frass has undergone nitrification (Johnson et al., 2005), leaves begin to release N-NH₄ for plants uptake.

In contrast, all treatments except Whole Frass showed an immediate decrease in nitrogen levels within 24 hours of application. This N immobilization likely resulted from high labile C content, which stimulated microbial activity and led to N sequestration (Lovett & Ruesink, 1995). It was hypothesized that the N-negative response in all treatments except Whole Frass may be due to more effective contact with the soil surface. Fine Frass and Fine Leaves, in particular, can easily integrate with the soil, promoting direct interaction and a quick microbial response (Garnier et al., 2008). Whole Leaves also have a larger contact area with the soil. In contrast, Whole Frass has most of its organic material encapsulated in frass, limiting soil contact and thus reducing immediate microbial activity.

The high C: N ratio of the frass and leaves (39.65 and 29.92, respectively) could result in slow N-NH₄ mineralization, explaining the otherwise slow response and low N-NH₄ production. In the latter days, N-NH₄ concentrations for all treatments remained relatively low; this could be because most of the easily mineralizable N fractions in the frass and leaves were already consumed, thus reducing N production (Van Niekerk et al., 2005).

5.5.2 Nitrate

N-NO₃ daily production varied between treatments. The treatment effect on N-NO₃ significantly differed between treatments from Day 1 to the end of the trial. After the initial high N-NO₃ levels in all treatments due to the free N in frass and leaves applied, an N-negative, or N immobilisation can be observed due to the high C: N ratios of both frass and leaves. The Whole Frass treatment reacted to the N-NH₄ production, and a corresponding increase was observed as N-NH₄ was oxidised into N-NO₃ (Van Niekerk et al., 2005). The Whole Leaf treatment also increased, similar to the Whole Frass treatment, while the other treatments responded slower, but all treatments produced a gradual increase of N-NO₃ as the organic products were mineralized, and the inorganic N was released (Poffenbarger et al., 2018; Grzyb et al., 2020). N-NO₃ production from whole leaves fluctuated throughout the incubation period.

5.5.3 Total inorganic N release

The total inorganic N was highest in Whole Frass, followed by Whole Leaf until day 54 when Whole Leaf outperformed the Whole Frass treatment. Regarding treatments, the difference was significant for all days except day 1. Total inorganic N for all treatments followed the trend of an initial high, followed by a decline and then a gradual increase. This could be due to the fast mineralization of frass, followed by nitrification and immobilization because when the easily mineralizable N components are mineralized, N production is reduced (Van Niekerk et al., 2005).

Frass is known for its rapid decomposition (Belovsky & Slade, 2000; Reynolds & Hunter, 2004). While the frass treatments initially decomposed faster than the leaf treatments, after 100 days, there was no significant difference in final NO₃ levels. However, NH₄ concentrations were notably higher in the leaf treatments, especially in Whole Leaves. The slower mineralization in Whole Leaves may be attributed to their smaller surface area relative to finer fractions or the presence of more complex organic compounds compared to frass, which slows down decomposition.

Other factors not explored in this study, such as soil pH, soil temperature (Thorup-Kristensen, 1994), duration of the incubation trial (Guan et al., 2022), and size and activity of the microbial communities, quality of organic matter, its quantity (Grzyb et al., 2020) could have influenced the process.

5.6 Conclusion

This study establishes the complementary roles of frass and mopane leaves in providing nitrogen to soils over time. Frass rapidly mineralizes, releasing high N-NH_4 concentrations shortly after application and then high N-NO_3 concentrations following nitrification. In contrast, mopane leaves release inorganic nitrogen more gradually, with a significant release occurring later in the incubation period, once the mineralizable components of frass have been depleted. This balance between fast-decomposing frass and slow-decomposing leaves suggests a beneficial synergy for plant nitrogen availability: frass supplies an immediate nutrient boost, while leaves sustain nitrogen release over a longer term. However, the variation experienced in the data motivates for more repetition of this study to accurately qualify this service of mopane worms frass in the woodland.

5.7 References

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5.8 Appendix

NO₂ mix

NO₂ mix was made up of 50 ml solution A + 50 ml solution B

Solution A: dissolve 0.25 g Sulphanilamide (C₆H₈N₂O₂S) + 2.5 ml Hydrochloric Acid (HCL) 32% to make up to 50 ml with H₂O.

Solution B: dissolve 0.025 g N-(1-Naphthyle)-Ethylen-Diammoniumdichlorid (C₁₂H₁₆CL₂N₂) and make up to 50 ml with H₂O.

NO₃ mix

NO₃ mix was made up of Vanadium (III) Chloride. Dissolve Vanadium (III) Chloride (VCL₃) and make up to 50 ml with H₂O.

NH₄ mix

Buffer mix

Dissolve 25 g of Potassium Sodium Tartrate (KNaC₄H₄O₆·4H₂O), 7.1 g of Di-Sodium hydrogen Orthophosphate (Na₂HPO₄), 8.9 g of Di-Sodium hydrogen Orthophosphate (Na₂HPO₄·2H₂O) and 17.9 g of Di-sodium hydrogen Orthophosphate (Na₂HPO₄·12H₂O) in 300 ml H₂O in a 500 ml volumetric flask. Mix 54 ml of 50% Sodium hydroxide (50% NaOH) in H₂O to make up to 500 ml on the volumetric flask.

Nitro mix

Mix 75 g Sodium Salicylate (C₆H₄(OH). COONa) and 0.15 g Nitroprusside Natrium (Na₂[Fe (CN)₅NO]·2H₂O) in H₂O to make up to 500 ml in a volumetric flask.

Jik (500 ml)

Mix 4.5 ml of Jik (3.5 ml) Sodium Hypochlorite (NaOCL) with H₂O to make up to 100 ml.

Appendix Table A1: Comparison of Total daily inorganic N ($\text{N-NH}_4 + \text{N-NO}_3$) production from treatments over time (100 days). Significance over time is shown by non-bold numbers. Significant differences in the groups are shown by different letters.

Test	Significance	Day	Treatment	Mean	SE	lower.CL	upper.CL	.group
ANOVA	0.003	0	Fine Leaf	18.30	0.20	17.90	18.70	b
ANOVA	0.003	0	Whole Leaf	13.40	1.27	10.90	15.90	a
ANOVA	0.003	0	Fine Frass	14.90	0.89	13.10	16.60	ab
ANOVA	0.003	0	Whole Frass	12.20	1.00	10.20	14.10	a
ANOVA	0.138	1	Fine Frass	15.24	2.77	9.82	20.67	a
ANOVA	0.138	1	Fine Leaf	10.32	0.99	8.39	12.25	a
ANOVA	0.138	1	Whole Frass	16.55	2.43	11.79	21.32	a
ANOVA	0.138	1	Whole Leaf	16.05	0.48	15.10	17.00	a
Kruskal-Wallis	0.004	3	Fine Leaf	1.23	0.06	1.11	1.35	a
Kruskal-Wallis	0.004	3	Whole Leaf	9.20	0.65	7.93	10.50	bc
Kruskal-Wallis	0.004	3	Fine Frass	5.04	0.46	4.15	5.94	ab
Kruskal-Wallis	0.004	3	Whole Frass	13.10	2.43	8.36	17.90	c
Kruskal-Wallis	0.005	7	Fine Leaf	1.75	0.20	1.43	2.08	a
Kruskal-Wallis	0.005	7	Whole Leaf	10.70	1.97	7.52	13.80	bc
Kruskal-Wallis	0.005	7	Fine Frass	2.54	0.40	1.91	3.18	ab
Kruskal-Wallis	0.005	7	Whole Frass	26.10	21.00	0.00	59.50	c
Kruskal-Wallis	0.010	14	Fine Leaf	0.41	0.20	0.09	0.73	a
Kruskal-Wallis	0.010	14	Whole Leaf	13.10	3.65	7.32	19.00	b
Kruskal-Wallis	0.010	14	Fine Frass	1.31	0.58	0.38	2.23	ab
Kruskal-Wallis	0.010	14	Whole Frass	15.80	14.00	0.00	38.00	b
Kruskal-Wallis	0.008	21	Fine Frass	5.97	3.93	0.00	12.20	ab
Kruskal-Wallis	0.008	21	Fine Leaf	2.07	0.19	1.77	2.37	a
Kruskal-Wallis	0.008	21	Whole Frass	38.00	30.40	0.00	86.40	b
Kruskal-Wallis	0.008	21	Whole Leaf	20.70	12.30	1.18	40.20	b
Kruskal-Wallis	0.007	54	Fine Leaf	3.60	3.77	0.00	9.60	a
Kruskal-Wallis	0.007	54	Whole Leaf	16.40	5.38	7.86	25.00	bc
Kruskal-Wallis	0.007	54	Fine Frass	5.04	0.88	3.64	6.45	ab
Kruskal-Wallis	0.007	54	Whole Frass	29.00	18.60	0.00	58.60	c
ANOVA	0.021	71	Fine Frass	8.40	2.56	3.38	13.42	a
ANOVA	0.021	71	Fine Leaf	8.26	3.98	0.45	16.07	a
ANOVA	0.021	71	Whole Frass	17.44	9.25	-0.70	35.57	ab
ANOVA	0.021	71	Whole Leaf	35.56	5.61	24.57	46.55	b
ANOVA	0.025	100	Fine Frass	26.21	2.60	21.11	31.30	ab
ANOVA	0.025	100	Fine Leaf	41.19	8.00	25.51	56.87	ab
ANOVA	0.025	100	Whole Frass	23.97	3.44	17.22	30.71	a
ANOVA	0.025	100	Whole Leaf	45.36	2.81	39.85	50.88	b

6 CHAPTER 6: SYNTHESIS AND CONCLUSION



A mopane worm as it crawls down the trunk of a mopane tree, getting ready for pupation.

This study highlights the ecological importance of mopane worms, by evaluating current research and existing knowledge around mopane worms, assessing their roles in nutrient cycling and release, and examining their distribution and conservation status in southern Africa. The comprehensive review (Chapter 2) identifies critical gaps in ecological understanding and emphasize the need for further research to ensure sustainable management and conservation of mopane worm populations amidst growing demand and environmental pressures. Most published research on mopane worms focusses on utilisation, including aspects such as nutritional value and income sources. Only 22% of studies since 1960 investigated ecology and biology aspects. Research efforts are also not equally distributed across southern Africa, with most of the research efforts occurring in South Africa, Botswana and Zimbabwe. With this skewness in research studies future mopane worms studies should focus more on their biology and ecology, including topics such as the effect of the changing climate, how their environment influences them, how they influence their environment, the different factors affecting their life cycle and availability and how they interact with other host trees. A better representation across countries will ensure a clear understanding of the sustainability and influence of mopane worms in the subcontinent.

Sustainability concerns due to overharvesting, habitat destruction and climate change prompted Chapter 3, where protected area coverage and factors influencing mopane worm populations were evaluated. By using advanced modelling methods, this chapter identified 15.1% - 40% of the landscape as suitable habitat, with 63.2% outside protected areas, where very little regulation about harvesting of worms or host trees exists. Considering that the demand and commercialization of mopane worms is increasing, this implies that they are being harvested in areas with less regulations. These suitable habitats outside protected areas could be communal, which is mostly under the governance of local chiefs, and thus not in protected areas. Because these lands are under local Chiefs, there are issues of non-compliance with the harvesting rules set by the Chiefs (Thomas, 2013; Sekonya et al., 2020). However, local communities have started establishing traditional guidelines to manage sustainability, though these vary and are often informal. While some areas have implemented stricter management practices, the study highlights the need for regional cooperation between local authorities, governments, and conservation organizations to formalise and enforce sustainable harvesting strategies. Protected areas alone may not be sufficient, as most suitable habitat remain unprotected.

Mopane worms contribute substantially to nutrient cycling through significant leaf consumption and frass production, which facilitate biomass turnover and nitrogen release. The findings of Chapter 4

indicate the need for more detailed studies across all instar stages to accurately quantify their ecological contributions. The calculations indicate that mopane worms are consuming between 152 and 9,970 kg of dry leaf mass per hectare and producing between 63 and 4,137 kg of frass per outbreak, thereby facilitating nutrient redistribution from the canopy to the soil, enhancing soil fertility. However, extensive harvesting and habitat changes could threaten these worms, risking disruptions to nutrient flow and ecosystem balance, underscoring the need for sustainable harvesting practices.

Lastly, the mineralization rate of frass compared to mopane leaves was investigated (Chapter 5), to understand the impact of mopane leave conversion for nutrient release, both in terms of temporal variation and nutrient value. While there were a lot of variation in the data, frass treatments reacted rapidly after applied to the soil, while the leaf treatments responded much slower. This demonstrates the complementary roles of frass and mopane leaves in providing nitrogen to soils over time. Frass rapidly mineralizes, releasing high N-NH_4 concentrations shortly after application and then high N-NO_3 concentrations following nitrification. In contrast, mopane leaves release inorganic nitrogen more gradually, with a significant release occurring later in the incubation period, once the mineralizable components of frass have been depleted. This balance between fast-decomposing frass and slow-decomposing leaves suggests a beneficial synergy for plant nitrogen availability: frass supplies an immediate nutrient boost, while leaves sustain nitrogen release over a longer term.

6.1 Sustainable mopane worms utilisation recommendations

This study highlighted that the main studies that have been done on mopane worms, which were more on the use of mopane worms as a food and income source, emphasizing their importance to rural livelihoods. This further highlights the need to harvest the worms in a sustainable manner as they are importance for rural sustenance. From the distribution study, it was found that more of the harvesting seem to occur outside of the protected areas, which are areas where the land is under local chief governance, and studies (Thomas, 2013), have shown that there is little to no regulatory laws governing harvesting here. The findings of chapter 4 and 5, revealed that mopane worms are important to the ecological function of their habitat and hence there is need to conserve them and further study them to quantify their ecological services accurately. All this drives the need to develop

strategies that foster a sustainable use of mopane worms while ensuring a balance with conservation. These could include but not be limited to:

- Education - educating harvesters on the life cycle of the worms and the importance of selective harvesting to discourage the harvesting of young instar stages worms and overharvesting of the last instar stage as a way to allow enough worms to pupate for the next season and thus continual existence. Locals can also be informed on the findings of this study on the influence mopane worms have on the ecological functioning of the mopane woodland so they understand the importance of conserving the worms for ecological services..
- Regulatory laws - while mopane worms are harvested in lands with different management regimes such as privately owned lands, public-governed lands and communal lands, studies (Thomas, 2013; Mufandaedza et al., 2015) have shown that harvesting done in communal lands, which contributes the majority is done with little or no regulatory laws (Sekonya et al., 2020). This lack of regulatory laws to govern harvesting results in excessive harvesting/ damage to host trees. There is need for the establishment of regulations and rules in addition to the education to control harvesting of both the worms and the host trees. These regulations can either be at the community level or self-organised governance (Ndeinoma and Wiersum, 2017), allocating woodlots to members of the community for easy management (Dube and Dube, 2010), restricted harvest periods, a tax to account for undervaluation of the value of mopane worms (Akpalu et al., 2007) or properly enforced permit system (Mufandaedza et al., 2015), where harvesters need permits to harvest the worms.
- Community co-developed harvest regulations - which would influence harvesters compliance as they would be involved in the development of these regulations. IPBES (2019) found that Community-based conservation institutions and local governance regimes seemed to be more effective in preventing habitat loss, at times than formally established protected areas. A sense of ownership towards the resource could foster more commitment to the regulations and enhance accountability. Mbata & Chidumayo (2003) and Musundire et al. (2021) highlighted instances where local community members were the ones to develop and uphold regulatory measures to edible insects harvesting. For example, in

midwestern Zambia, harvesting of *Gynandrus maja* and *Gonimbrasia zambesina* caterpillars observe temporal restrictions to ensure sustainability (Mbata et al. 2003), while the cutting down of caterpillar host plants, is prohibited except under special permission from traditional leaders (Mbata & Chidumayo 2003).

- Domestication - although mopane worms are host exclusive, mopane worms domestication pivot projects have shown success in countries such as South Africa and Zimbabwe, where this has led to a commercial farm selling mopane worms from this production (Mopane Worms Enterprises, 2025). Insect rearing has been reported to be successful in producing food and feed in Europe, with frass, which can be used as fertilisers as a by-product (Safitri et al., 2024). If adopted this could help alleviate the pressure on the mopane woodlands and also help improve rural livelihoods by producing the worms throughout instead of waiting for the two generations per year.

6.2 Study constraints

Mopane worms are seasonal species dependent on the climatic conditions of the environment. This greatly affected the fieldwork of this project because, during the times of field observation, there was a massive heatwave in South Africa that affected the worms' development, which resulted in no worms to study in the field.

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