THE VALUE OF MOPANE WORMS: QUANTIFYING ECOSYSTEM SERVICES IN TERMS OF NUTRIENT CYCLING IN SEMI-ARID REGIONS OF LIMPOPO

by

Netshanzhe Mutali Winnie

16006731

Submitted in partial fulfillment of the requirement for the degree of Master of Science in Biological Sciences (Zoology)

in the

Department of Biological Sciences

Faculty of Sciences, Engineering and Agriculture

University of Venda

Thohoyandou, Limpopo

South Africa

December 2021

Supervisor:

Dr L Swanepoel:

Co-supervisors:

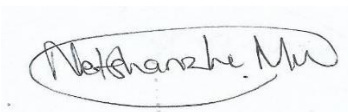
Dr C Swanepoel

Prof S Foord

DECLARATION

I, Mutali Winnie Netshanzhe hereby declare that this thesis submitted to the University of Venda for the degree of Msc in Biological 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 has been duly acknowledged.

Signature   Date



16 December 2021

ACKNOWLEDGEMENT

I would like to extend my sincere gratitude to the following organisations:

- The University of Venda and Department of Biological Sciences for the opportunity to pursue my Msc degree.
- The National Research Foundation (NRF) and SARChi Chair in Biodiversity Value and Change for the financial support.
- The African Institute for Conservation Ecology (AICE) for all the logistical and academic support.

My sincere, deepest gratitude to the following individuals:

- My supervisors: Dr Corrie Swanepoel, Dr Lourens Swanepoel and Prof Stefan Foord
- Dr Corrie Swanepoel, for her interest in the project right from the start, the consistent discussions that helped steered me in the right direction, her guidance and encouragement through the experimental trial and statistical data analysis.
- Dr Lourens Swanepoel, for his immense involvement in the entire project from the review, the experiment, right through to the statistical analysis. I am grateful.
- Prof Stefan Foord, for the valuable comments and corrections he gave especially toward the review section and his help with statistical analysis.
- Dr Veldtman for his contribution towards the review section.
- Consol Kubayi for his tremendous help with my experimental trial set-up.
- Dr Madala’s laboratory for the glassware for my experiment.
- My family for their love and kindness.
- I thank the Lord, for His grace is sufficient for me.

ABSTRACT

Mopane worms are the late-instar stage caterpillars of the mopane moth, *Imbrasia belina*/ *Gonimbrasia belina*, found in southern Africa virtually following their hosts' trees, *Colophospermum mopane*, *Terminalia seriscea*, and *Sclerocarya birrea*, among others. The caterpillars are harvested for consumption and sale, thus crucial for rural livelihoods. Due to their importance to the livelihood of people and the rise in the human population, coupled with poverty, there is growing concern about harvest rates which might pose a risk to their sustainability. It is thus essential that research focused on aspects related to sustainable harvest rates is done alongside research focusing on the general species ecology. This study has two parts; firstly, the study aimed to do a systematic review to determine the current state of knowledge and research on mopane caterpillars in southern Africa. Secondly, the study aimed to assess the contribution of mopane caterpillars' frass to nutrient cycling by determining the nitrogen mineralization rates of frass compared to that of mopane leaves. We used search engines such as African Journals Online (AJOL), Google Scholar, and Web of Knowledge. The online databases were searched using the following keywords, 'mopane worms', 'mopane caterpillar', 'Imbrasia belina', 'Imbrasia belina caterpillars', and 'Gonimbrasia belina'. All the publications were compiled and evaluated based on title, keywords, and abstract to select only studies focusing directly on mopane caterpillars. Only peer-reviewed publications were included and did not include grey literature, such as reports, presentations, dissertations, or book chapters. A total of 104 publications were found that fit the assessment criteria. The temporal distribution showed that studies on mopane caterpillars began in the 1960s, and there was a steady increase from 1991 onwards. The spatial distribution of publications closely followed the mopane caterpillars distribution in southern Africa. Two main themes emerged from the papers: resource use and ecology. Most papers (79 papers; 76%) focused on resource use, while only 25 papers (24%) focused on the species' ecology. Furthermore, spatially there was a lack of studies in many countries where mopane caterpillars occur, highlighting the spatial bias in studies. The review highlighted that research on mopane caterpillars is biased to resource use, with little attention toward the ecological role the species play in the ecosystem.

We conducted an incubation experiment to measure mineralization rates between mopane caterpillars' frass and mopane leaves. The results showed that frass had high mineralizable N due to the high NH_4^+ and NO_3^- concentration compared to the whole and fine (ground) leaves. Overall, frass released more inorganic N than leaves; thus, it seems that mopane caterpillars' frass has high mineralization potential and is essential in aiding nutrient cycling in the ecosystem. From this, we recommend more field and lab studies to establish the mineralization potential of mopane caterpillar frass and the rate at which the frass release nutrients. We also recommend more studies that will focus on consumption of leaves and the release of frass by mopane caterpillars to determine the biomass consumed and consequently returned to the ecosystem by mopane caterpillars.

Keyword: mopane caterpillar, mopane leaves, frass, mineralization, NH_4^+, NO_3^-

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.1. Background

Insects have long been a part of the diet for the people in Sub-Saharan Africa (Van Huis, 2003); this is because they are high in protein (Siulapwa et al., 2014; Chagwena et al., 2019) and are accessible to the rural communities. Insects are collected to use for food and are also sold to generate household income (Dube et al., 2013). In southern Africa, mopane caterpillars (*Imbrasia belina*) are an example of insects that form part of both the household diet and income (Baiyegunhi & Oppong, 2016). In addition to food and financial supplementation to local communities, mopane caterpillars are also culturally important to communities. According to Mbata and Chidumayo (2003) caterpillars have social significance deeply embedded in different cultures and traditional beliefs of different ethnic groups harvesting the caterpillars in Africa.

The importance of mopane caterpillars as it relates to human resources has been studied intensively; however, their influence on the natural ecosystem has seldom been studied (De Swardt et al., 2018). However, all aspects of mopane caterpillars must be studied as there is emerging evidence that current harvest rates might be unsustainable (Makhado et al., 2012). For example, in many harvesting areas, it appears that current harvests yields are declining (Togarepi et al., 2020), exacerbated by habitat destruction of mopane woodland (Makhado et al., 2012). Therefore, it is essential to study and quantify their contribution to the natural ecosystem before they face an even greater danger of extinction and thus conserve them.

1.2. Problem statement

In many landscapes, invertebrates are the major defoliators of leaves, releasing large amounts of frass to the ground, enhancing the nutrients pool in the environments and thus accelerating nutrient cycling (Reynolds & Hunter, 2004; Jankielsohn, 2018). The frass is often fast decomposing, thus contributing to nutrients input into the soil (Reynolds & Hunter, 2004). For example, *Periclista sp.* (sawflies) in Canada increased NO_3^--N levels in soils and streams after only one short seasonal defoliation (Reynolds et al. 2000).

In southern Africa, mopane caterpillars are not just an essential defoliator of mopane woodland; they are also an important food source for communities and wildlife. However, like all other species, mopane caterpillars face challenges due to climate change, habitat destruction, and overuse of as a food source, consequently influencing mopane caterpillars population. However, the contribution of mopane caterpillars to nutrient cycling is not well understood. As such, the loss of mopane caterpillars could negatively affect the ecosystem. Therefore, it is important to review the current literature on mopane caterpillars to establish research themes to enable future research trajectories. Secondly, it is essential to lay the groundwork on the importance of mopane caterpillars for ecosystems.

1.3. Aims

- To determine the current state of knowledge and research on mopane caterpillars in southern Africa.
- To determine the contribution of mopane caterpillars to nutrients cycling in semi-arid regions of southern Africa.

1.4. Objectives

- Determine the current state of knowledge and research done on mopane caterpillars in southern Africa, by collating all relevant published research.
- Compare the nutrient content of mopane leaves with that of frass of mopane caterpillars.
- Compare the NH_4^+ and NO_3^- mineralization rates between caterpillar frass and mopane leaves to indicate the rate of nutrient return between mopane leaves with and without mopane caterpillars (which convert leaves into frass).

1.5. Significance of study

The mopane caterpillar is an iconic species in southern Africa known to many people who consume it. However, with the increase in the human population, resulting in more pressure on natural ecosystems and species, there is concern about the long-term conservation of the mopane caterpillars. In terms of the study's specific aims, the significance is as follows:

Aim 1: The review is important because it will reveal gaps in knowledge in mopane caterpillar studies, thus revealing areas that need research. It will show which aspects of mopane caterpillars have not been studied and which have been understudied because it looked at the temporal trend of studies and the scope covered in the studies. Previous reviews have highlighted that the impact of invertebrates, particularly insects, on the African savanna has not been adequately addressed (De Swardt et al., 2018). Thus, this study will reveal whether it is also the same for mopane caterpillars.

Aim 2: Studies have shown that as herbivorous insects feed on leaves, they convert the leaves into easily mineralizable frass, increasing nutrient availability and facilitating nutrient cycling at the landscape level (Reynolds & Hunter, 2004). This study aims to assess the mineralization rates of mopane frass in a semi-arid region of Limpopo and thereby lay a foundation for further ecological studies investigating the broader ecological role of mopane caterpillars (De Swardt et al., 2018).

1.6. Thesis layout

This thesis is divided into two parts; the first chapter is a systematic literature review (Chapter 2), where we reviewed all papers published on mopane caterpillars. The second part is the incubation trial (Chapter 3), where we explore the ecosystem service of mopane caterpillars in terms of nutrient cycling in semi-arid regions of Limpopo province. Each data chapter is prepared as a standalone paper that will be submitted for publication (or is currently under review).

1.7. References

Baiyegunhi, L.J.S. & Oppong, B.B. 2016. Commercialisation of Mopane Worm (*Imbrasia belina*) in Rural Households in Limpopo Province, South Africa. *Forest Policy and Economics*, 62:141-148. http://dx.doi.org/10.1016/j.forpol.2015.08.012.

Chagwena, D.T., Matanhire, G.T., Jombo, T.Z. & Maponga, C.C. 2019. Protein Quality of Commonly Consumed Edible Insects in Zimbabwe. *African Journal of Food, Agriculture, Nutrition and Development*, 19 (3): 14674-14689. DOI: 10.18697/ajfand.85.17645.

De Swardt, D. B., Wigley-Coetsee, C. & O'Connor, T.G. 2018. Insect Outbreaks Alter Nutrient Dynamics in a Southern African Savanna: Patchy Defoliation of Colophospermum Mopane Savanna by *Imbrasia belina* Larvae. *Biotropica*, 50(5): 789–796.

Dube, S., Dlamini, N.R., Mafunga, A., Mukai, M. & Dhlamini, Z. 2013. A Survey on Entomophagy Prevalence in Zimbabwe. *African Journal of Food, Agriculture, Nutrition and Development*,13 (1):7242-7253.

Jankielsohn, A. 2018. Insects: the answer to sustainable agriculture. In Coleman, A, Crops: Insects and Biodiversity. *Farmers Weekly*, 44-46.

Makhado, R.A, Potgieter, M.J, Wessels, D.C.J, Saidi, A.T, & Masehela, K.K. 2012. Use of mopane woodland resources and associated woodland management challenges in rural areas of South Africa. *Ethnobotany Research & Applications*, 10:369-379. www.ethnobotanyjournal.org/vol10/i1547-3465-10-369.

Mbata, K.J. Chidumayo, K.J. 2003. Traditional Values of Caterpillars (Insecta: Lepidoptera) among the Bisa People of Zambia. *Insect Sci. Applic.*, 23 (4): 341-354.

Reynolds, B.C., Hunter, M.D. & Crossley, D.A. 2000. Effects of Canopy Herbivory on Nutrient Cycling in Northern Hardwood Forest in Western North Carolina. *Selbyana*, 21 (1,2):74-78.

Reynolds, B.C. & Hunter, M. D. 2004. Nutrient Cycling. Chapter 19, 387-396.

Siulapwa, N., Mwambungu, A., Lungu, E. & Sichilima, W. 2014. Nutritional Value of Four Common Edible Insects in Zambia. *International Journal of Science and Research (IJSR)*, 3 (6): 876-884. www.ijsr.net.

Togarepi, C., Nashidengo, E. & Siyambango, N. 2020. Effects of Climatic Variability and Non-Climatic Factors on Mopane Worms' (*Gonimbrasia Belina*) Distribution and Livelihood Options in North Central Namibia. *Environment and Natural Resources Research*, 10(2):14-25. https://doi.org/10.5539/enrr.v10n2p14.

Van Huis, A. 2003. Insects as Food in Sub-Saharan Africa. *Insect Sci. Applic*., 23 (3):163–185.

CHAPTER 2: LITERATURE REVIEW: An Iconic Edible Insect in southern Africa: State of knowledge on the mopane caterpillar *Gonimbrasia belina*

Mutali Winnie Netshanzhe[1], Corrie Swanepoel[2], Stefan Foord[3], Ruan Veldman[4], Lourens Swanepoel[1,5]

[1]Department of Biological Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou, 0950

[2]Agricultural Research Council, Pretoria, South Africa

[3]SARChI chair in Biodiversity Value and Change, University of Venda, South Africa

[4]South African National Biodiversity Institute, Pretoria, South Africa

[5]African Institution for Conservation Ecology, Levubu, South Africa

Prepared for: Bothalia - African Biodiversity Conservation

2.1. Background

Mopane worms are late-instar stage caterpillars of the mopane moth, *Imbrasia belina/ Gonimbrasia belina*, belonging to order Lepidoptera and family Saturniidae (Ditlhogo et al., 1996; Gondo et al., 2010). They are distributed across southern Angola, northern Namibia, northern Botswana into Zimbabwe, central and northern Mozambique, southern Zambia, Malawi, and northern South Africa (Makhado et al., 2012), which closely follow their major host tree, *Colophospermum mopane* (mopane tree) (Stack et al., 2003) (Fig. 2.1). Even though there seems to be host specificity towards *C. mopane*, mopane caterpillars have also been recorded to feed on *Terminalia seriscea* and *Sclerocarya birrea* (Ditlhogo et al., 1996). However, where both *C. mopane* and *T. seriscea* occurred, mopane moths preferred to lay eggs on *C. mopane* (Ditlhogo et al., 1996). Under laboratory conditions, it took *I. belina* 56 to 62 days to develop from egg, larvae (5 instars) to pupation while feeding on *C. mopane* leaves, while it took 72 days on *S. birrea* leaves (Teferra et al., 2000). Population breakouts have been reported in *Brachystegia spiciformis* and *Julbernadia paniculata*; however, they occur in highest numbers on *B. spiciformis* below 3 m in height, while on *J. paniculata* it is more abundant from 3 m above to 10 m in height (Potgieter et al., 2012).

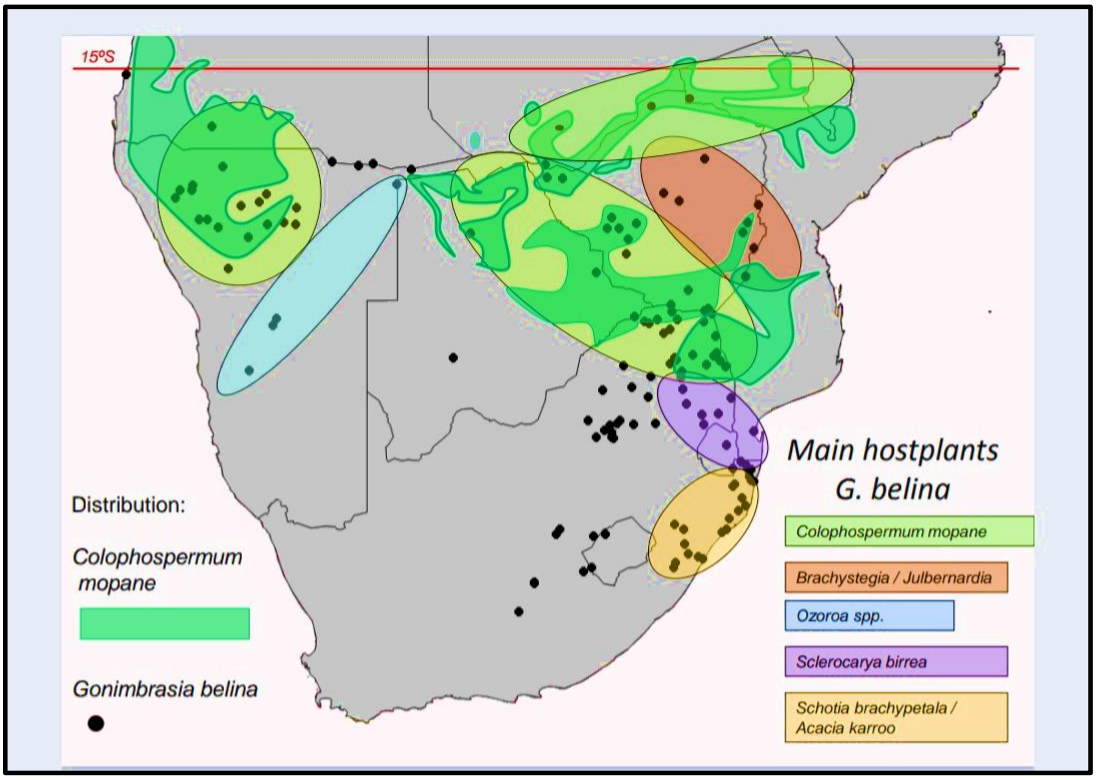


Figure 2. 1. Distribution of mopane caterpillars (source: Gardiner et al., 2012)

2.1.1. Life cycle of a mopane caterpillar

Mopane caterpillars are bivoltine, emerging twice a year, from December to January and April to May (Kozanayi & Frost, 2002; Makhado et al., 2014). During the October-November period, the adult moth of *I. belina* emerges for a few days to mate, lay eggs, and die without feeding (Gondo et al., 2010). The female oviposit eggs in clusters of 50-200 on twigs/leaves of mopane trees (Gondo et al., 2010; Makhado et al., 2014). After 10 days of oviposition, the eggs hatch, releasing tiny black larvae/caterpillars, which metamorphoses through five instars (I-V) of development for 4-6 weeks (Gondo et al., 2010; Makhado et al., 2014) (Fig 2.2). Early-stage (instar stages I-III) caterpillars live in cluster groups of 20-200; however, during instar stage IV, the caterpillars disband and disperse. When the caterpillars reach instar stages IV and V, they are harvested (Gondo et al., 2010; Makhado et al., 2014). At the end of instar stage V, the caterpillars crawl down to the base of their host tree and burrow into the ground and pupate to emerge as a moth to lay eggs for the February-March generation. When the second generation's caterpillar burrows at the end of the outbreak, it enters a state of diapause for 6 -7 months until emerging at the start of the next rainy season in October (Gondo et al., 2010; De Swardt et al., 2018) (Fig. 2.2).

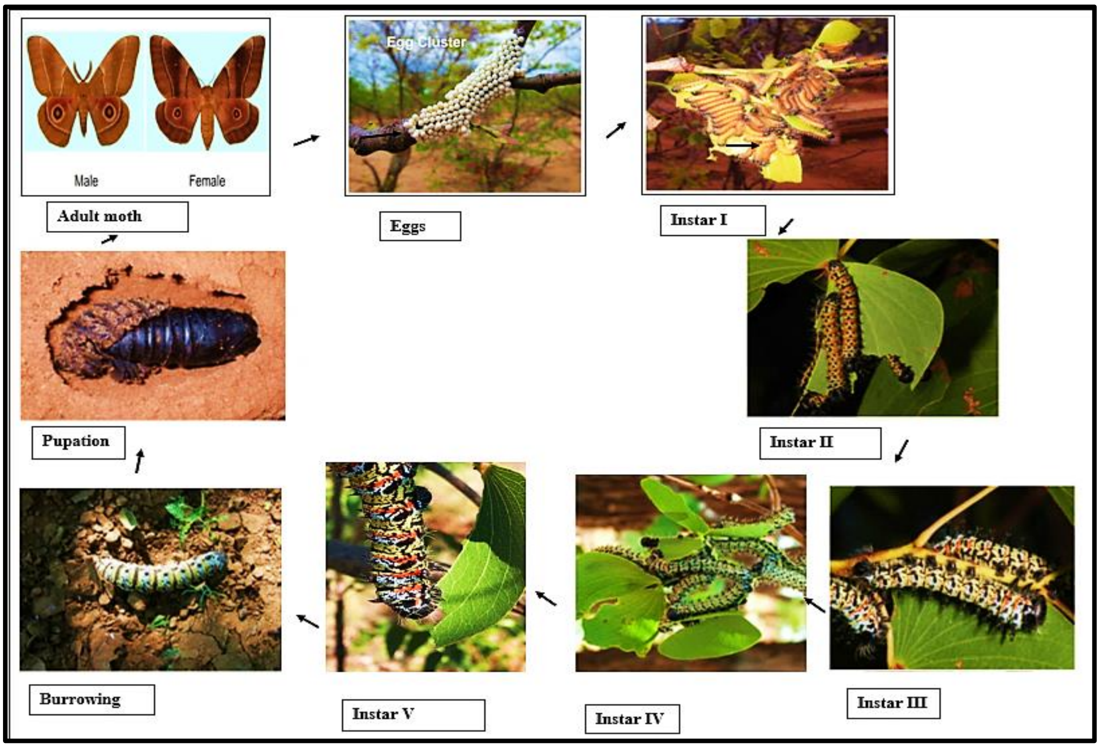


Figure 2. 2. Life cycle of a mopane caterpillar (Image source: Gardiner et al., 2012)

2.1.2. Importance as a resource for human consumption.

Mopane woodlands are found in areas of low natural resource potential, with similar low cultivation potential (Stack et al., 2003). As such, local communities in these areas often depend on subsistence farming for their livelihood (Baiyegunhi et al., 2016). In such areas, mopane caterpillars are valued as an important source of nutrition to supplement food produced from subsistence farming (Stack et al., 2003).

Mopane caterpillars are an excellent protein source, with their protein value exceeding other usual protein sources such as beef and chicken by up to three times unit weight (Potgieter et al., 2012). They are also rich in unsaturated fatty acids, phosphorus, iron, and calcium, which are essential for human nutrition (Potgieter et al., 2012). Mopane caterpillars are also food sources to animals, with some exported from Botswana to South Africa for animal feed (Madibela et al., 2007). Several studies have also looked at the potential of mopane caterpillars as food sources for fish (Mwimanzi & Musuka, 2014; Rapatsa & Moyo, 2017; Rapatsa & Moyo, 2019; Moyo et al., 2019) and birds (Chiripasi et al., 2013; Marareni & Mnisi, 2020; Mogwase et al., 2018; Nobo et al., 2012(a); Nobo et al., 2012(b); Moyo et al., 2019).

Apart from being a source of nutrition, they also provide financial support as they create employment opportunities during their seasonal availability (Makhado et al., 2014), through harvest period (short-term employment on harvesting days), and through selling (medium-term employment throughout the selling months). Trading of mopane caterpillars provides income for the poor rural women and children (Illgner & Nel, 2000), financing things like school fees (Stack et al., 2003). Kozanayi et al. (2002) found that prices of mopane caterpillars fluctuate amongst localities and selling points. During periods when mopane caterpillars are available, their trade resulted in an average monthly income of R1,775 (US$161) in rural areas, which constitutes about 30% of a household's total monthly income (Baiyegunhi et al., 2016).

2.1.3. Mopane caterpillar herbivory as an important ecological function in mopane woodlands

In mopane woodlands, the primary nutrient cycling process seems to be modulated by mammal herbivory (De Swardt et al., 2018). Grazing mammals increase foliar N in the mopane woodland ecosystem by excreting N in forms that plants can easily take up (De Swardt et al., 2018). However, leaf fall and decomposition also play an essential role in mopane woodland nutrient cycling. For example, although *C. mopane* is leguminous (Makhado et al., 2014), it does not "nodulate" (Swardt et al., 2018), and as such, one of the main mopane trees' main nutrient cycles pathways is through leaf flush, leaf fall, and decomposition. However, plant litter/litterfall is not readily available as nutrients for plant uptake since decomposition and subsequent release of plant-available nutrients are lengthy processes (Belovsky & Slade, 2000; Reynolds & Hunter, 2004). As such, processes that can facilitate the breakdown of mopane leaves to readily available plant nutrients would be an essential process in mopane woodland nutrient cycling.

Arthropod herbivory can be an important ecosystem process in woodland and forests. For example, Reynolds et al. (2000) studied sawflies in Canada and found that "a short-term, one season defoliation" resulted in an increase in NO_3^-- N levels in soil and stream draining the watershed at the centre of the outbreak due to frass and cadaver production, by 60 ug/l as opposed to 35 ug/l in control. Similarly, Belovsky and Slade (2000) found that increased grasshopper (*Melanoplus sanguinipes*) density increased frass production, which led to increase in N and NH_4+. Frost and Hunter (2004) found that frass from the eastern tent caterpillar, *Malacosoma americanum* increased total soil N, however frass deposition did not increase NO_3^--N concentration as reported in (Reynolds et al., 2000). These opposing results thus make the quantifying role of herbivore insects on nutrient cycling difficult.

When mopane caterpillars feed on leaves of mopane tree, they release frass into the ecosystem, and such a process can be a key component in mopane woodland nutrient cycling. This is because, in mopane woodlands, mopane caterpillars are one of the key leaf defoliators of mopane trees (Ditlhogo, 1996; De Swardt et al., 2018). Mopane caterpillar herbivory releases a large amount of "fast-decomposing frass" (Reynolds & Hunter, 2004) into the soil, which aids with plant nutrient uptake and nutrient cycling (De Swardt et al., 2018). According to Styles (1994), during the fifth instar stages, a larva can consume >40g of dry leaf material. The fourth/fifth instars release an average of 1.42g of frass per day (De Swardt et al., 2018). Mopane caterpillars' frass were also found to influence the cycling of potassium (K), nitrogen (N), and phosphorus (P), returning more K and P into the soil (De Swardt et al. 2018). Thus, highlighting their potential in nutrient cycling, nonetheless, the role of arthropods herbivores in biogeochemical cycles in the African Savannas is not well documented (De Swardt et a., 2018), and the role of mopane caterpillars is no exception.

2.2. Objective and importance of the review

The mopane caterpillar is an iconic species in southern Africa, that is known to many people that consume them. The species plays an important role in human nutrition, food security and income generation. Sustainable harvest of the species will require thorough understanding of their life cycles as well as their ecological role in the mopane woodlands. It is, therefore, important to assess the current and historical research to enable researchers to evaluate harvest rates as well as develop new research ideas. Therefore, the aim of this review is to establish the current state of knowledge regarding mopane caterpillar research, its temporal and spatial trends and identify future opportunities or research needs.

2.3. Materials and Methods

2.3.1. Selection of studies

To assess the spatial, temporal, and thematic research on mopane caterpillars, we conducted a systematic literature review covering 1961 to 2020. In terms of reproducible research, we developed a prior defined set of criteria to allow for replication of the review process (Fig. 2.3), which followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement and Checklist) guidelines when including or excluding publications during screening stages (Fig. 2.3). We used several search engines to find research papers, including African Journals Online (AJOL), Google Scholar, and Web of Knowledge. The online databases were searched using the following keywords, 'mopane worms', 'mopane caterpillar', '*Imbrasia belina*', '*Imbrasia belina* caterpillars' and *Gonimbrasia belina*'. All the publications were compiled and evaluated based on title, keywords, and abstract to select only studies focusing directly on mopane caterpillars. Only peer-reviewed publications were used. Grey literature was not included, such as reports, presentations, dissertations, or book chapters. For the Google Scholar search, which resulted in over 1000 papers, we stopped evaluating papers 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 that were already captured. After removing the duplicates, 104 papers were retained (Fig. 2.3).

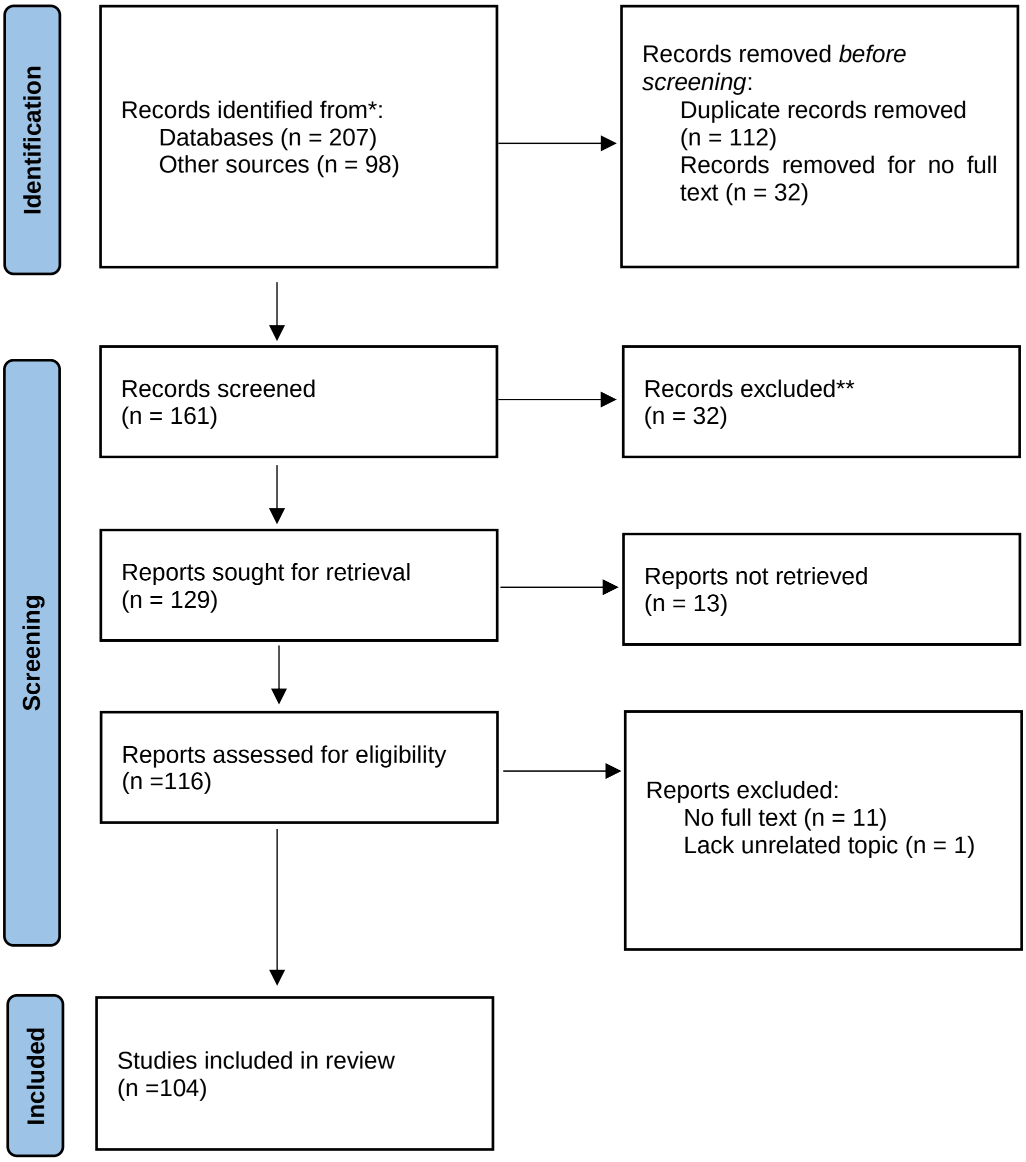


Figure 2. 3. PRISMA guideline (Source: Page et al., 2021)

To adequately evaluate the types of studies and identify the knowledge generated and the gaps in mopane caterpillars' research, we categorized the papers into two broad themes, namely 'Ecology' and 'resource use'. Ecology studies focused directly on mopane caterpillars themselves, including papers on genetics, reproduction, interaction with other species, impact on mopane trees, and nutrient cycling. Resource use studies focused on the use of mopane caterpillars in human and animal nutrition, impact on the local economy, post-harvest processes or policies, and sustainable use.

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

Table 2. 1. Themes and subthemes used in categorizing publications

Theme	**Ecology**	**Resource use**
subthemes	Autecology	Human nutrition
	Synecology	Animal nutrition
		Social impact
		Economics
		Sustainable use

2.3.2. Data analysis

The Chi-Square test (Franke et al., 2012) was used to investigate the effect of country on research theme, research theme per country as well as the number of studies per country. The Mann-Kendall test was used to test for a significant temporal trend in research publications (Pettitt, 1979). Analysis was done in R (R Development Core Team, 2012) with the 'Kendall' package for trend analysis (McLeod, 2011).

2.4. Results

2.4.1. Temporal

A total of 104 papers fit the selection criteria (Fig. 2.4). There was a significant positive publication trend (Mann-Kendall test: tau = 0.601, $p < 0.0001$; Fig 2.4) with an average of 1.99 publications per year. However, the study themes affected the positive trend (Fig. 2.5a&b). Papers focusing on mopane caterpillar ecology showed a slight significant trend (Mann-Kendall test: tau = 0.229, p= 0.028; Fig. 2.5a) over the review period with an average of 0.41 papers per year. In contrast, papers focusing on resource use had a significant positive trend (Mann-Kendall test: tau = 0.692, $p < 0.0001$; Fig. 2.5b) with an average of 1.33 papers per year (Fig. 2.5b). The publication rate focusing on resource use were 3.02 times higher than paper focusing on ecology (Fig. 2.5a&b).

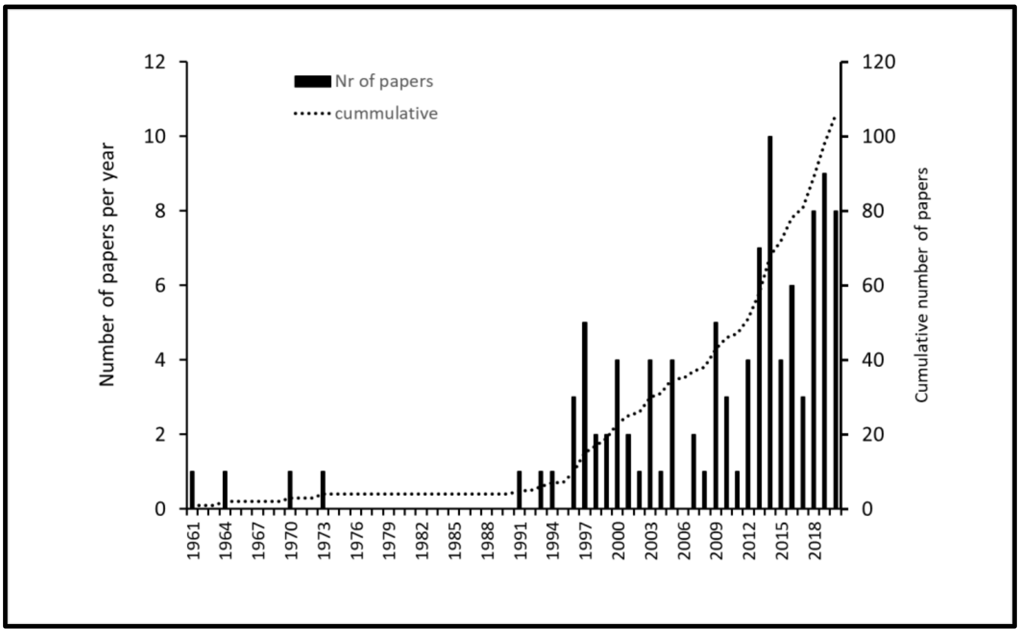


Figure 2. 4. Number of research papers on mopane caterpillars published per year

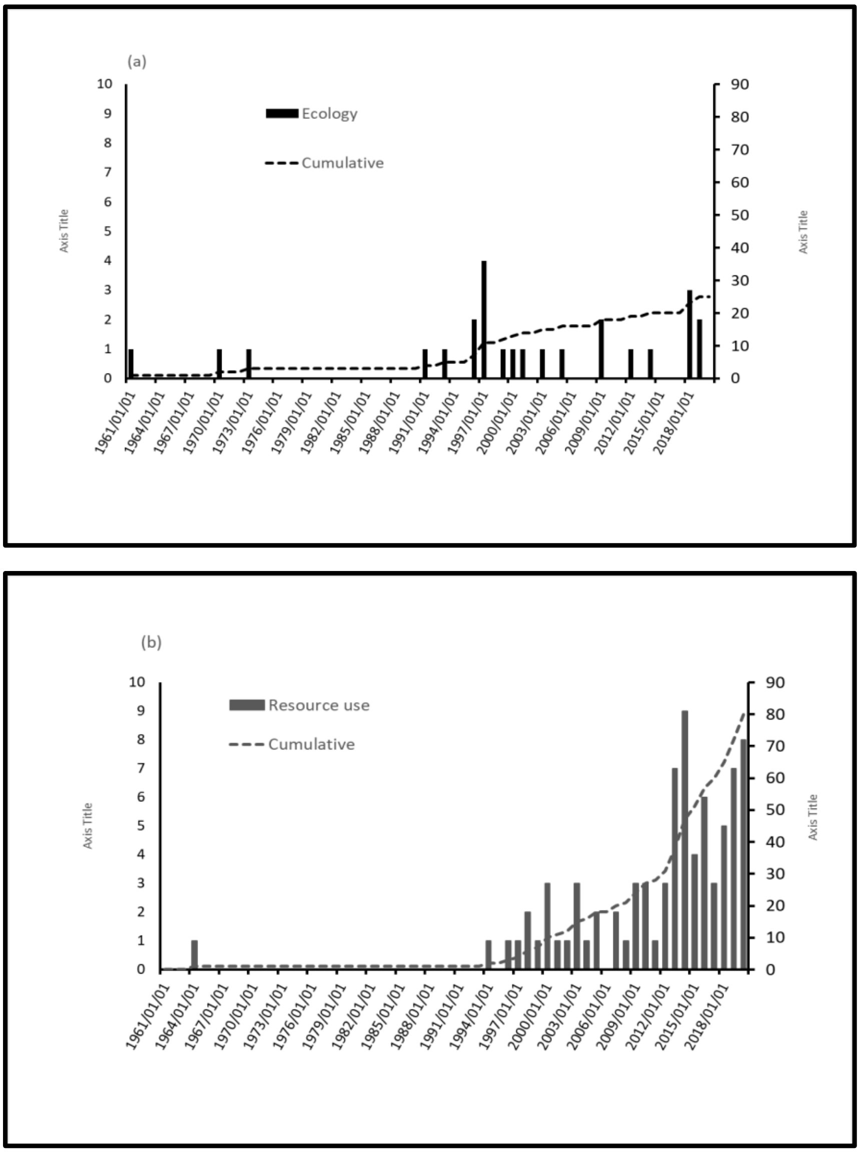


Figure 2. 5. Number of studies published per main theme per each year (a. Ecology, b. Resource use)

2.4.2. Spatial representation of studies

There were 104 papers from which 176 location points were extracted, from 6 different countries (Fig 2.6). Many of these points were in the same location. While the study locations closely followed the distribution of mopane caterpillars, there was a disparity in the number of studies per country ($X^2_6 = 60.186$, $p < 0.0001$; Fig 2.6). The highest number of studies was done in South Africa (30%) followed by Botswana (27%), Zimbabwe (22%), Namibia (9%), Zambia (4%), Malawi (1%) and Nigeria (1%) as well as a combination of the above countries, that were labeled southern Africa (6%) (Fig. 2.6). Even though there are mopane caterpillars and mopane trees in southern Angola and central and northern Mozambique (Makhado et al., 2012), no studies were found in these countries.

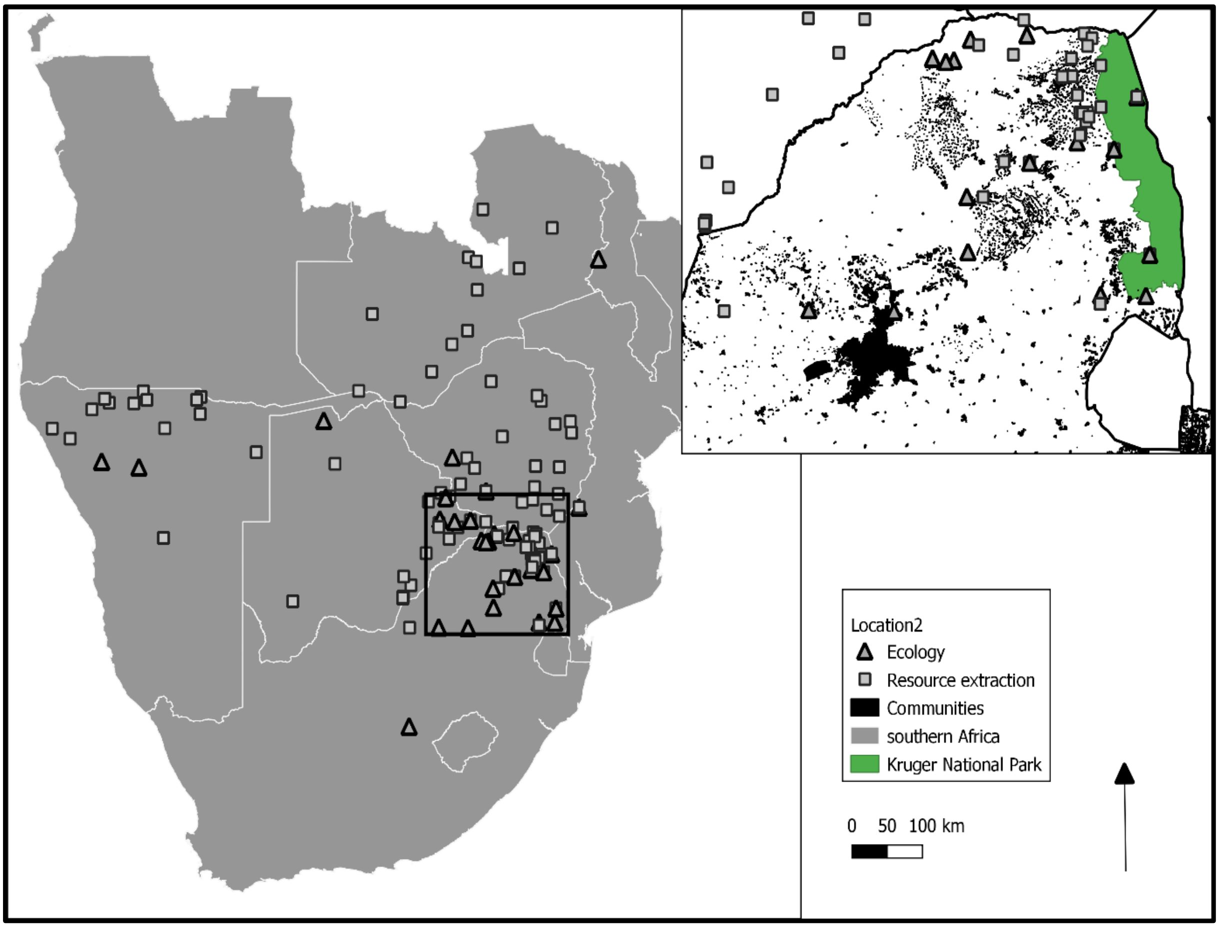


Figure 2. 6. Spatial distribution of mopane caterpillar studies in southern Africa

2.4.3. Study themes

There was a significant difference ($X^2_1 = 28.038$, $p < 0.001$) in the number of publications among the broad themes, with most papers (76 %) focusing on resource use (Fig. 2.7). Similarly, in terms of resource use, there was a significant difference in the sub themes ($X^2_4 = 52.625$, $p < 0.001$), where most papers focused on studies related to the nutritional value of mopane caterpillars to humans (Fig. 2.7). Papers focusing on the sustainable use of mopane caterpillars were limited to 6 % of total research effort (Fig. 2.7). Papers on mopane caterpillars' ecology are almost equally divided between synecology and autecology (Fig. 2.7).

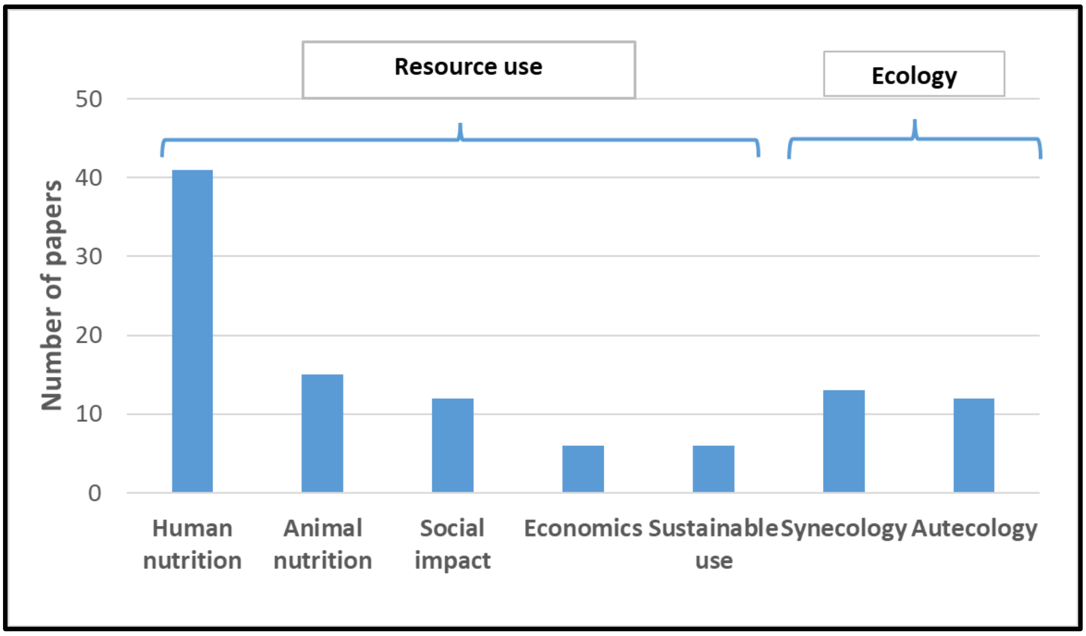


Figure 2. 7. Number of papers published within different themes (broad themes: resource use and ecology)

The discrepancy between papers focusing on resource use and ecology was also evident in the different countries, even though non-significant ($X^2_7 = 10.112$, $p = 0.18$) the pattern suggests that countries focus more on resource use than ecology of mopane caterpillars (Fig. 2.8). Two countries, South Africa (30 % of papers) and Botswana (27 %) had the highest number of papers related to mopane caterpillar ecology.

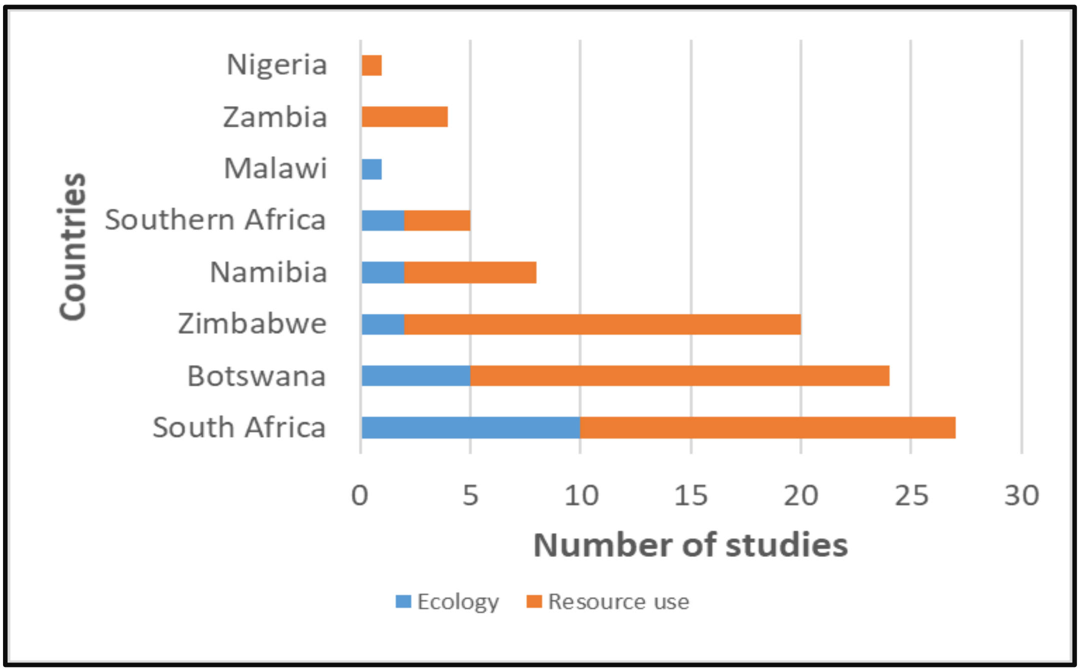


Figure 2. 8. Number of studies per theme per country

2.5. Discussion

2.5.1. Spatial and temporal distribution of papers

As expected, mopane caterpillar studies overlap with mopane tree distribution in southern Africa. However, a spatial discrepancy still emerged. Recourse use studies are more often in private land where other forms of resource extraction like wood harvesting also occurs. In contrast, ecological studies were mostly done in 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 caterpillars. For example, density of mopane caterpillars in private land can be affected by factors not necessary present in protected areas, which can include wood harvesting, variation in livestock grazing/browsing densities and land clearing activities. By extrapolating caterpillar densities from protected areas, we can over estimate abundance in private areas and lead to overharvesting.

Since 1991 studies focusing on mopane caterpillars gradually increased, while prior to this, very few studies were found, these earlier studies primarily investigated ecological aspects of mopane caterpillars, such as the distribution and seasonal occurrence of caterpillars in the Transvaal (Van den Berg et al., 1973) or chemical composition of the mopane caterpillar's gut (Cmelik, 1970; Glew et al., 1999). The increase in caterpillar-related studies might be a response to the increase in pressure on natural resources in dry and semi-arid regions. Amongst other factors, increased population growth and poverty in these areas result in increased pressure on natural resources, such as wood and wildlife (Makhado et al., 2012). As pressure on natural resources increases, some of these might become locally extinct, increasing food insecurity for local communities that depend thereon (Kamwi et al., 2020). The increased pressure on food sources can highlight the importance of food security and local economics and the ecological impact of extinction on the environment. In response, a possible increase in research interest resulted in increased publications on this topic (Makhado et al., 2012, Kamwi et al., 2020).

2.5.2. Types of studies

The high number of studies on resource use could probably be because mopane caterpillars are valued more for being a source of food and income (Baiyegunhi et al., 2016). The ecological importance of insects is often overlooked as they are mostly focused on as pests (Jankielsohn, 2018). The importance of invertebrates has often been overlooked in research and society. Therefore, they are also overlooked in terms of conservation (Reid, 1999), with conservation energy mainly focusing on big animals like the Big Five (Potgieter et al., 2012).

(i)Ecology

Synecology

Studies focusing on habitat distribution and life cycles of mopane caterpillars found that mopane moths start emerging from October until the beginning of April. Up to three generations could occur, from the end of October to the end of November, between mid-January to the beginning of March, and from the end of April to the end of May (Van den Berg et al., 1973). Mopane caterpillars are common in semi-arid regions of southern Africa and overlap well with the mopane veld (Douglas, 1991). However, mopane caterpillars are not entirely restricted to mopane veld only, as they can also feed on several other trees, such as the *Salix bebbiana* (willow), *Searsia lancea* (karee), *Protorhus longifolia* (red beech), *Sclerocarya birrea* (marula), *Terminalia sericea* and *Ficus craterostoma* (wild fig) (Douglas, 1991). However, larvae 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 might explain why mopane moths prefer to lay their eggs on *C. mopane*. However, this could be co-evolution rather than preference, i.e., the presence of more mopane trees resulted in caterpillars co-evolving and becoming more adapted to mopane leaves.

Wiggins (1997a) found that eggs are more likely to be deposited in areas of clusters on conspecific trees, while small trees or solitary trees rarely receive clutches. The choice of host trees is further based on the measure of resource abundance, namely tree size, as expected for an outbreak species, since resources availability rather than nutritional quality is likely to be determinant of larval survival (Hrabar et al., 2009b; Ditlhogo et al., 1996; Hrabar & Du Toit 2014). Survival, impact on habitat, wellbeing and health of mopane caterpillars have been studied on different levels. The following aspects has been investigated:

- Impact on habitat:

The tree-grass relationship in the African savanna affects mopane trees' survival, and subsequently, mopane caterpillars. Apart from fire, invertebrate herbivores can also affect the tree-grass dynamic (Duffy et al., 2018). Complete defoliation of trees resulted in altered competitive relations between trees and grasses. Models run by Duffy et al. (2018) showed that the tree-grass relationship could change under high caterpillar abundance (complete defoliation). High worm abundance resulted in a two-fold increase in grass biomass density, whereas low caterpillar abundance resulted in 80% tree biomass density (Duffy et al., 2018).

Defoliation activity of mopane caterpillars not only removes leaves but also impacts the regrowth of host trees. When defoliation (from mopane caterpillar) was compared to natural pruning (e.g., elephants), the natural pruning resulted in longer shoots and leaves, while defoliation produced shorter shoots and leaves (Hrabar et al., 2009a). Despite the difference between pruning and defoliation, mopane trees showed no visible trade-off in investment, as mopane trees are adapted to both (Hrabar et al., 2009a). Heavy pruning resulted in egg masses almost halved in the next season, resulting from lower leaf biomass (Hrabar & Du Toit., 2014). The caterpillars' defoliation pressure affects trees' fecundity (Ditlhogo et al., 1996). Only 14% of defoliated trees produced seeds whereas 84% of the undefoliated trees produced seeds (Ditlhogo et al., 1996).

Burning influences the preference of certain forage-tree species. Early burning enhanced the preference of mopane caterpillars for *Brachystegia spiciformis*, *Terminalia sericea* and *D. condylocarpon* whilst late burning changed the preference to *Brachystegia manga* (DeWild). (Mughogho & Munthali, 1993). While mopane trees have been associated with the presence of mopane caterpillars, not all mopane veld were found to have mopane caterpillars. Local absence was investigated by Styles and Skinner (1996). Extensive utilization of mopane veld by mammalian herbivores had a negative impact on the caterpillars' presence and the preponderance of ants, which could act as an effective anti-herbivory mechanism (Styles & Skinner, 1996).

The only paper studying the mapping of caterpillar defoliation using remote sensing (Adelabu et al., 2012) showed that detecting defoliation by remote sensing is not always effective due to understory of grasses and herbs that may be present, because the reflectance of heavily defoliated mopane tree may be mistaken to that of grasses and herbs, and in addition, field monitoring to map is often intensive (Adelabu et al., 2012)

Mopane caterpillars' defoliation and frass release may affect nutrient cycling, especially potassium (K) and to some extent, phosphorus (P), in their environment. Leave litter together with frass resulted in higher K and P values in the soil under mopane caterpillars' infected areas (De Swardt et al., 2018).

- Tree properties

Research from the reviewed papers indicated that several relationships between mopane caterpillars' occurrence and mopane trees were investigated. Leaf nutrition (Nunu et al., 2019) or tannin levels (Mufandaedza et al., 2018) did not influence caterpillars' distribution. Similarly, Hrabar et al. (2009b) found no relationship between oviposition by females and leaf nutritional value. It therefore seems that leaf chemistry do not influence egg mass density, suggesting that mopane moths choose resource quantity, rather than quality.

Wiggins (1997b) looked at fluctuating asymmetry in leaf length and mean leaf size and found no difference in terms of 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 utilized had greater leaf size. A study of moths' preference in laying eggs on mopane trees in the Venetia Nature Reserve (Hrabar et al., 2009b) showed that utilized trees were significantly taller, had significantly greater shoot weight, and had a larger shoot biomass, than proximate non-utilized trees. Egg mass abundance showed an increase with an increase in tree height. Host selection regarding canopy volume showed that larger canopy trees were preferred instead of trees of smaller canopy (Hrabar et al., 2009b). Thus, tree size seems to be the primary factor influencing oviposition at the individual tree level. However, the degree to which ovipositing females select host trees concerning resource availability/individual level is unclear and still needs further research (Hrabar et al., 2009b).

The age of larvae influences forage patterns, as older larvae have a higher assimilation efficiency than younger larvae (Teferra, 2003). Leaves of both *C. mopane* and *S. birrea* contains indigestible cellulose thus limiting assimilation and making it difficult for the larvae to utilize the plant material because the larvae do not have the cellulase for cell wall break down (Teferra, 2003).

- Climate

The climate was found to influence the caterpillars’ presence and functioning. Frears et al. (1997) tested whether *I. belina* has physiological mechanisms of thermoregulation and found that they have no behavioral mechanisms to elevate temperature but hang upside down to reduce high temperatures. While a study (Frears et al.,1999) on the influence of temperature on the foraging activity of caterpillars reported that temperature is not the only factor influencing foraging, but factors such as predation also have an influence.

Heavy rains during harvesting periods can interfere with the harvesting and drying process, reducing the number of caterpillars harvested and causing caterpillar spoiling due to cloudy periods (Mataboge et al., 2016).

- Predation

Gaston et al. (1997) expressed patterns of predation faced by the caterpillars in the environment. There is a strong positive correlation between the body mass of the predator birds species and the body mass of the successive instars they are feeding on (Gaston et al., 1997). As successive instars develop, different bird species with a broader range of body sizes feed on them (Gaston et al., 1997); thus, caterpillars rarely outgrow predation because as they advance in size, they also get preyed on by different birds species of bigger sizes.

- Soil properties

Styles and Skinner (1996) suggested that unsuitable burrowing substrate or soil could limit caterpillars' activity in a region. They found that the soil was of fine texture in areas (entire Tuli block) where caterpillars' exoskeletons were encountered. In contrast, in the Northern Tuli Game Reserve (NTGR, which is within the Tuli block), where the caterpillars had been absent for two decades, the soil had a very coarse texture and a rocky surface ground (Styles & Skinner, 1996). Mufandaedza et al. (2018) studied how soil parameters (soil texture, soil pH) influenced the distribution of *Imbrasia belina* in the southeastern Lowveld of Zimbabwe and found that soil texture only influences the caterpillars' distribution indirectly through the distribution of mopane trees. Soil chemical properties such as N and P did not influence the caterpillars' distribution (Mufandaedza et al., 2018).

- Competition

Only one study investigated the interaction between the two edible caterpillars, *G. belina* and *G. maia* in Kasungu National Park, Malawi (Mughogho & Munthali, 1993). Both caterpillars have similar food requirements, but they circumvent competition by feeding at different heights in the same tree. This divergence may have evolved due to interspecific competition for the same food. There were differences in the abundance of the two caterpillar species by forage species and tree height. *G. belina* dominated *Brachystegia spiciformis*, at heights more than 3 meters, while *G. maia* occurred abundantly in the lower height classes. Both fed on mature leaves rather than on tender leaves (Mughogho & Munthali, 1993); however, there were no records for differences in temporal exploitation.

Autecology

- Biochemistry

Cmelik (1970) studied the sterols in the diet and some organs of the caterpillar and found that the main sterol from the mopane tree was B-sitosterol, while the sterol fractions from various organs of the mopane caterpillars were identified as mixtures. The gut and fat bodies contained cholesterol as a major component and lesser qualities of B-sitosterol, campesterol, and di-unsaturated sterol (Cmelik, 1970). He also studied laboratory processes and measuring of fat bodies of insects (Cmelik, 1969). No significant difference was found in the nutritional content of caterpillars in different regions (Jennings et al., 2001). Mopane caterpillars have a comparatively high nutritive value; with a protein content of 38-74% (Motshegwe et al., 1998; Moyo et al 2019), or more specific, 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, Zn (Glew et al., 1999), as well as a significantly higher content of Na (42.1 mg/100g) and Fe (26.7 mg/100g) (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 compared to cowpeas (Nantanga & Amakali, 2020)

The caterpillar contained 10 essential amino acids in varying amounts (Amadi & Kiin-Kabari 2016). The predominant amino acid was glutamic, and the predominant essential amino acid was arginine (Siulapwa et al., 2014). The major 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), but there is dramatic variation in the composition of palmitic and linoleic acids in the lipid content from early III to late V instar of the larvae (Pharithi et al., 2004). The oil profiles for mopane caterpillar's oil were like those of seed oil (Motshegwe et al., 1998) and compared better to cow's milk than animal fat (Zinzombe & George, 1994). It is thus a better source of essential fatty acids than many other animal food sources (Motshegwe et al., 1998).

The cholesterol content of the mopane caterpillar was relatively high compared to other animals, such as ox, pig, or chicken meat. In general, it is considered that the relatively high cholesterol could be mitigated by the presence of a substantial amount of B-sitosterol and campesterol which are known to be blood plasma cholesterol-lowering phytosterols (Yeboah & Mitei, 2009).

- Genetics

Greyling et al. (2001) found that two geographically distant (approximately 170 km apart) I. *belina* populations had high levels of polymorphism (42% and 47%) and heterozygosity (0.182 to 0.210). There was a high amount of gene flow between these two *I. belina* populations and a large amount of variation, and overall genetic divergence between the groups were therefore relatively small. The different generations in a year were genetically similar, suggesting that two generations are not sub-species and that pupae from the first generation are the moths that emerge in the second generation (Greyling et al., 2001).

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

(ii)Resource use

***Human* nutrition**

Alternative protein sources are commonly investigated with ever-increasing pressure on food sources and a growing population. 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 caterpillars could be an ideal protein replacement (Illgner & Nel, 2000; Obopile & Seeletso, 2013; Nantanga & Amakali, 2020). Mopane caterpillars are a significant food source for many rural communities, and most studies in the review focused on the value of these caterpillars as human nutrition. These caterpillars are a significant source of protein in rural areas where it is expensive for the marginalized poor to afford other protein sources like beef or chicken. However, even affluent people purchase the caterpillars from markets and/or rural harvesters as they also enjoy the caterpillars as a delicacy or snack.

Mopane caterpillar is one of the 250 edible insects in Africa (Van Huis, 2003). Early studies on entomophagy in southern Africa found that insects are delicacies, of which mopane caterpillars are a favourite (Quin, 1964; Dube et al., 2013). In some communities, mopane caterpillars are still popular; a study done in the Limpopo Province at Lemana College showed that 60% of students included mopane caterpillars in their diet (Mbhenyane et al., 2005). Similarly, Baiyegunhi et al. (2016) found that 61.7% of sampled households in the Mopani district consumed mopane caterpillars at least 3 to 5 times a week. Another Zimbabwe study showed that only 10% of the study sample did not participate in entomophagy due to religion, stigma, or repulsion (Dube et al., 2013). However, in other communities, the popularity of mopane caterpillars has declined, for example, in Botswana (Obopile & Seeletso, 2013). This could be due to fear of recent cases of allergic reactions to the caterpillars (Kung et al., 2013) or due to religious reasons such as the Zion Christian Church (ZCC), which forbids its members from consuming insects (Baiyegunhi et al., 2015). A study on socio-economic factors influencing mopane caterpillars harvesting in Limpopo province in the Mopani district reported that about 26% of participants' households belonged to religious groups that forbid mopane caterpillar collection.

Pollution, especially heavy metal contamination from industries and mines, could influence the safety of mopane caterpillar consumption. A study in Botswana, found that caterpillars found closer to a smelter had higher heavy metals concentration (Ca, Cu, Al, Zn, Co, Cd & Mn) than those in the control sample site, 56km away, possibly due to the heavy metals deposited in the leaves consumed by the caterpillars (Ekosse et al., 2005). It is recommended that food safety regulation should be in place as these have potential health risks to those consuming the caterpillars (Greenfield et al., 2014). However, no studies are showing clear health risks faced due to consuming caterpillars contaminated with heavy metals, highlighting that it is of no serious concern.

When using mopane caterpillar meal as nutritional additives, it was found that smaller particle size increases the meal functionality (Ekpo et al., 2008). Including mopane caterpillar meal in fermented cereal could increase the bioavailability of Fe and Zn (Gabaza et al., 2018).

Post-harvest processing and storage of mopane caterpillars impact the quality or safety of consuming the caterpillars (Kachapulula et al., 2018; Dangwa et al., 2014). A review on the safety of edible insects showed that processing methods that include boiling, frying, and roasting greatly affect the safety of edible insects (Murefu et al., 2019; Potgieter &

Ramalivhana, 2020). When mopane caterpillars that were field dried and laboratory dried were tested for microorganisms, both types showed contamination with fungi and bacteria, including *Escherichia coli, Klebsiella pneumoniae, Aspergilli flavus, Penicillium sp.* and *Fusarium sp.* (Gashe et al., 1997; Simpanya et al., 2000). During storage, dried caterpillars were subject to pest infestation, including bacteria, molds, insects (Mpuchane et al., 2000), and fungi (Nawases et al., 2018).

Ramashia et al. (2020) studied the microbiological quality of different dried insects sold at the Thohoyandou open market. The quality of food products at street vendors suggests that food safety and hygiene standards are low. All dried insects were sold from open containers, with insects being handled with bare hands. Vendors also lacked appropriate shelters and clean, suitable utensils (Ramashia et al., 2020). Yeast and mold 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). Degutting, roasting, and boiling in saltwater, coupled with open pan roasting, reduced bacterial count in dried caterpillars (Mujuru et al., 2014).

However, there are also negative aspects associated with mopane caterpillar consumption. The caterpillar was 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). The presence of fungi, such as *Aspergillus*, which occurred in 47% of samples, reached toxic levels under post-harvest conditions (Mpuchane et al., 1996), impeding consumption. Another negative impact reported in the literature is mopane caterpillar's allergy. A few cases were described by Okezie et al. (2010), Kung et al. (2013), Potter (2013), and Ribeiro et al. (2017). Some of the symptoms included headache, dyspnoea, cough, wheeze, tongue, and lip swelling within 10 minutes after consuming mopane caterpillars (Kung et al., 2011).

Animal nutrition

Apart from humans, mopane caterpillars also provide food for different animals. Mopane caterpillar meal was found to be an adequate protein replacement for ruminants (Madibela et al., 2009), fish, broilers, layers, and guinea fowl (Moyo et al., 2019) and has the potential to replace soybean products in quail diets without compromising their performance, health, or meat quality (Marareni & Mnisi, 2020). Degutting influences chemical attributes and improves the quality of caterpillars for consumption by animals, as it increases the crude protein, consequently improving rumen undegraded protein (Madibela et al., 2007; Madibela et al., 2009). Compared to fish meal and soybean, *I. belina* caterpillars contain greater concentrations of some essential amino acids, such as threonine, valine, tryptophan, and phenylalanine. Its inclusion into the diet may balance the indispensable amino acids level. Mopane caterpillars are composed of all essential amino acids required by *Oreochromis mossambicus* (Tilapia; Rapatsa & Moyo, 2017). *I. belina* caterpillar is a lipid source that provides essential fatty acid (Moyo et al., 2019) as well as unsaturated fatty acids that can improve the concentration on conjugated linoleic acids (Madibela et al., 2007). It was found that there is no significant difference in dietary fat levels of *I. belina* caterpillar meal and that of fish meal (Moyo et al., 2019).

Inclusion of 4.5% (Nobo et al., 2012a) to 9% (Chiripasi et al., 2013) and 13% (Mogwase et al., 2018) of mopane caterpillar meal for guinea fowl, did not affect growth patterns, mineral intake, or taste of meat products. Similarly, mopane caterpillar inclusion did affect the growth rate or eggs production in Tswana hens (Manyeula et al., 2018) or the taste of meat in Tswana hens (Manyeula et al., 2019). However, including too much mopane caterpillar 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). Including mopane caterpillar meal to feed tilapia is a good source of protein. Up to 60% inclusion was optimally profitable (Rapatsa and Moyo, 2017), while others suggested 50% inclusion yielded the highest growth performance (Mwimanzi & Musuka, 2014). Nonetheless, it seems that the inclusion of 40-60% of caterpillars in fishmeal could lead to liver degradation (Rapatsa & Moyo, 2019).

Regardless of the caterpillars' potential as poultry and fish meal, factors such as chitin, the risk of being overexploited, their unpalatability, and a chance of indigestions at higher inclusion levels in diets limit the use of mopane caterpillars as protein substitutes in animal feed (Moyo et al 2019). Rasengwatshe and Madibela (2005) also found that mopane caterpillar has a higher effective degradability than carcass meal. Thus, more mopane caterpillars would be fermented in the rumen than carcass meal. Roasting or basking were recommended to protect mopane caterpillars from rumen fermentation (Rasengwatshe & Madibela, 2005).

Social impact

Mopane caterpillar harvesting has real monetary, economic, and social benefits (Swemmer et al 2015). Consumption and commercialization are crucial in supporting rural livelihoods. These caterpillars supplement nutritional requirements and are sold to generate income. Consequently, their conservation is affected, and thus there is a need to improve rural livelihood to forge a balance between utilization and conservation (Baiyegunhi et al., 2016). Although poorer households tend to harvest the caterpillars more, they are collected by households from all income groups (Stack et al., 2003), and 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). Most of the harvesters are female (96%) (Stack et al., 2003) and are also more dependent on mopane caterpillars for a source of income (Togarepi et al., 2020).

During their seasonal availability, mopane caterpillars create both short-term (a few days) and medium-term (a few months) employment opportunities for the rural unemployed people, providing up to 10,000 seasonal jobs in southern Africa (Makhado et al., 2014), thus aiding household livelihood.

Several household socio-economic factors influence mopane caterpillar harvesting (Hope et al., 2009; Baiyegunhi et al., 2015). These 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).

Economics

Mopane caterpillars have been traditionally harvested as subsistence food, however, there has been a shift to trade, e.g., in some sites in Zimbabwe, 59% of mopane caterpillars' stocks were sold for cash, 19% exchanged for goods, while only 9% were consumed at home (Stack et al., 2003). In Zambia, the sales of mopane caterpillars generated income higher than that of agricultural produce, i.e., the study estimated that an average family of 6 can produce 552.6 medas of sun-dried caterpillars per harvesting season (Mbata & Chidumayo, 2003).

Mopane caterpillars have a high level of commercialization, as 63% of harvested caterpillars in Mopani district are sold within a production year (Baiyegunhi & Oppong, 2016). The product's utility is a function of its form, location, concentration, and time of availability. 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 that influenced commercialization decision and commercialization intensity of mopane caterpillars in Mopani district of Limpopo Province were household head, household size, income, association, the average distance to the nearest market, transporting, communication assets, and lack of institutions/laws regulating the use and management of the woodland resources (Baiyegunhi & Oppong, 2016). Export prices highly influenced local availability, especially close to the border of South Africa, where the price for mopane caterpillars is higher than local prices in Botswana or Zimbabwe. This can create local shortages (Mufandaedza et al., 2014).

Mbata and Chidumayo (2003) found that even in instances where mopane caterpillars' harvest volumes are relatively small (an average of 19.28 Liters per household), it can contribute positively to households in terms of consumption and income generation in Zambia. A study by Swemmer et al. (2020) in the Kruger National Park, reported that most of the respondents (75%) consumed all their harvested caterpillars at home, while those that sold their harvest gained an average amount of R517.14 per person (Swemmer et al., 2020).

Sustainable use

Studies suggest that mopane caterpillars are being used unsustainable (Gondo, 2010; Thomas, 2013). Several factors were identified that affect the sustainable use of mopane caterpillars. These include a growing demand both locally and in urban populations, rising prices in urban markets [which increase financial incentives), maximizing harvest volume (which can lead to overexploitation) Roberts, 1998; Thomas, 2013; Baiyegunhi & Oppong, 2016], and damage to mopane trees during harvesting process (Roberts, 1998). Furthermore, growth in settlements results in a reduction of vegetation cover and mopane woodlands and consequently a decline in caterpillar harvests (Ndlovu et al., 2019). A descriptive study to identify the factors that determine the abundance and distribution of mopane caterpillars in north-central Namibia, found that non-climatic factors that influence caterpillar abundance and distribution included human activities such as deforestation, overharvesting, urbanization and burning of mopane veld. Deforestation was concluded to be the major contributor to mopane caterpillars' decline, i.e., the majority of participants (57.5%) from Okahao and 36.7% from Tsandi, indicated that deforestation is the major contributing factor to the decline of mopane caterpillars (Togarepi et al., 2020). A review showed that climatic factors affect the preservation of mopane trees, which affects the sustenance of mopane caterpillars (Mataboge et al., 2016).

Lack of regulatory laws to govern caterpillar extraction which result in excess harvesting, damage to mopane trees, and harvesting of younger caterpillars which subsequently reduce the emergence of subsequent generations affects mopane caterpillars' sustainability. There is thus a need for government to establish regulations and laws to control harvesting (Moyo et al., 2019, Baiyegunhi & Oppong, 2016), especially in localized harvest areas, as this is where harvesters tend to be excessive. However, larger areas in Limpopo, such as Venetia Nature Reserve, seem to be unaffected by these factors. A harvesting governance study in the Vhembe and Mopani districts showed that private and public governed land exercised more control and monitored caterpillars 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 big commercial companies.

To improve the sustainable use of mopane caterpillars, several practices have been suggested in various publications (Dube & Dube, 2010; Thomas, 2013; Ndeinoma & Wiersum, 2017; Akpalu et al., 2007; Mufandaedza et al., 2015), these include: improving property rights and institutional arrangements that govern the use of mopane caterpillars, by supporting communities to establish indigenous natural resource management systems (Thomas, 2013), allocating woodlots can help instigate responsible and sustainable harvesting and protect the environment (Dube and Dube, 2010), and developing policies and regulations, either on a community level or self-organized governance (Ndeinoma and Wiersum, 2017), such as restrictive harvest periods, optimal tax that corrects for undervaluation of the scarcity value of mopane stock (Akpalu et al., 2007) or properly enforced permit system (Mufandaedza et al., 2015). Domestication and mopane caterpillar farming, which is the artificial rearing of mopane caterpillars, independent of the wild (Gardiner, 2003; Hope et al., 2009), is another method to ensure the sustainability of the caterpillars in their natural environment. A three-year domestication experiment in Botswana showed that domestication can results in production of fresh caterpillars, in high numbers and at any time of the year (Gardiner, 2003).

The results showed very few papers (six) on sustainable use, while 41 papers were focused on human nutrition, with greater emphasis on the use of mopane caterpillars as food and fewer concerns around sustainability. The implication of few studies on the sustainable use of mopane caterpillars is that the danger of them going extinct is overlooked. Their conservation is not considered as there is little knowledge on their conservation status and ways to best conserve them. Not knowing their ecological importance and conservation status will hinder early measures that could conserve them for the foreseeable future.

2.6. Conclusion

This literature review highlighted the spatial and temporal trend in mopane caterpillar research. If further highlighted, the discrepancy in research topics, as most research focused on social-economic impacts of harvesting. In contrast, ecological research and topics related to the ecological role of the caterpillars in the ecosystem received less attention.

In general, the published research shows that the caterpillars provide a readily available and cheaper source of animal protein, containing comparatively higher quantities of protein, fat, carbohydrate, and valuable minerals (Kwiri et al., 2014). This provisioning extends from humans (adults, pregnant women, and children; Jennings et al., 2001; Siulapwa et al., 2014; Quin, 1964; Musundire et al., 2016; Glew et al., 1999; Mushaphi et al., 2017) to livestock (Madibela et al., 2007), poultry (Nobo et al., 2012b; Chiripasi et al., 2013) and fish (Mwimanzi & Musuka, 2014; Rapatsa & Moyo, 2017). Several researchers suggested and emphasized that effort should be made to retain the tradition of entomophagy (Van Huis, 2003).

Commercial utilization of caterpillars depends on the correct postharvest practices and educating harvesters could improve the cleanliness and safety of mopane caterpillar consumption (Simpanya et al., 2000). Additionally, harvesters can be taught about semi-domestication and domestication to reduce pressure on the natural environment and increase the availability of caterpillars outside of the wild.

While this review highlighted the limited research on ecology, several important topics emerged. Mopane caterpillars stand out as a key mopane tree defoliator and thus place a significant role in nutrient cycling, tree-grass dynamics, tree recruitment and ecosystem stability. However, several of these impacts remains unexplored and provide a great starting point for research on mopane caterpillar ecology and its impact on the ecosystem.

2.7. References

Adelabu, S., Mutanga, O. & Cho, M.A. 2012. A Review of Remote Sensing of Insect Defoliation and its Implications for the Detection and Mapping of *Imbrasia belina* Defoliation of Mopane Woodland. *The African Journal of Plant Science and Biotechnology*, 6 (1): 1-13.

Akpalu, W., Muchapondwa, E. & Zikhali, P. 2007. Can the Restrictive Harvest Period Policy Conserve Mopane Worms in Southern Africa? A bio-economic modelling approach. Working papers, no 65.

Amadi, E.N. & Kiin-Kabari, D.B. 2016. Nutritional Composition and Microbiology of Some Edible Insects Commonly Eaten in Africa, Hurdles and Future Prospects: A critical review. *Journal of Food: Microbiology, Safety & Hygiene*, 1 (1):1-7. doi:10.4172/2476-2059.1000107.

Baiyegunhi, L.J.S. & Oppong, B.B. 2016. Commercialisation of Mopane Worm (*Imbrasia belina*) in Rural Households in Limpopo Province, South Africa. *Forest Policy and Economics*, 62:141-148. http://dx.doi.org/10.1016/j.forpol.2015.08.012.

Baiyegunhi, L.J.S., Oppong, B.B. & Senyolo, G.M. 2015. Socio-Economic Factors Influencing Mopane Worm (*Imbrasia belina*) Harvesting in Limpopo Province, South Africa. *J. For. Res*. DOI 10.1007/s11676-015-0168-z.

Baiyegunhi, L.J.S., Oppong., B.B. & Senyolo, G.M. 2016. Mopane Worm (*Imbrasia belina*) and Rural Household Food Security in Limpopo province, South Africa. *Food Security*, 8:153–165. DOI:10.1007/s12571-015-0536-8.

Chagwena, D.T., Matanhire, G.T., Jombo, T.Z. & Maponga, C.C. 2019. Protein Quality of Commonly Consumed Edible Insects in Zimbabwe. *African Journal of Food, Agriculture, Nutrition and Development*, 19 (3): 14674-14689. DOI: 10.18697/ajfand.85.17645.

Chiripasi, S.C., Moreki, J.C., Nsoso, S.J. & Letso, M. 2013. Effect of Feeding Mopane Worm Meal on Mineral Intake, Retention and Utilization in Guinea Fowl under Intensive System. *International Journal of Poultry Science*, 12 (1): 19-28.

Cmelik, S.H.W. 1969. Carotenoids from the Haemolymph of the Mopane Moth Caterpillars *Gonimbrasia belina*. *Experientia*, 25(11).

Cmelik, S.H.W. 1970. Sterols in the Diet and Various Organs of the Caterpillars of the Mopane Moth *Gonimbrasia belina*. 351: 360-372.

Dangwa. N., Mwenje, E., Dhlamini, Z. & Siwela. A.H. 2014. Molecular Characterization of Aflatoxigenic *Aspergillus* species in dried Traditional foods in Zimbabwe. *Advances in Bioresearch*, 5 (1): 29-36. http://www.soeagra.com/abr.html.

De Swardt, D. B., Wigley-Coetsee, C. & O'Connor, T.G. 2018. Insect Outbreaks Alter Nutrient Dynamics in a Southern African Savanna: Patchy Defoliation of Colophospermum Mopane Savanna by *Imbrasia belina* Larvae. *Biotropica*, 50 (5): 789–796.

Ditlhogo, M., Allotey, J., Mpuchane, S., Teferra, G., Gashe, B.A. and Siame, B.A. 1996, November. Interactions between the mopane caterpillar, *Imbrasia belina*, and its host, Colophospermum mopane in Botswana. *Management of Mopane in Southern Africa. Proceedings of a conference held at Ogongo Agricultural College, Namibia*, 26-29.

Douglas, R.M. 1991. Mopane Worms in Bloemfontein? *Culna*, 41:20.

Dube, S., Dlamini, N.R., Mafunga, A., Mukai, M. & Dhlamini, Z. 2013. A Survey on Entomophagy Prevalence in Zimbabwe. *African Journal of Food, Agriculture, Nutrition and Development*,13 (1):7242-7253.

Dube, S & Dube, C. 2010. Towards improved utilization of macimbi *Imbrasia belina* Linnaeus, 1758 as food and financial resource for people in the Gwanda district of Zimbabwe. *ZJST*, 5: 28-36.

Duffy, K.J., O'Connor, T.G. & Collins, O.C. 2018. A Lepidopteran (*Imbrasia belina*) might Influence Tree-Grass Balance of *Colophospermum mopane* Savanna. *Theoretical Ecology*. https://doi.org/10.1007/s12080-018-0379-y.

Ekosse, G.E., Van den Heever, D.J., De Jager, L. & Totolo, O. 2005. Heavy Metals Concentrations in *Imbrasia belina* (Phane Caterpillar) around the Selebi Phikwe Ni-Cu Mine and Smelter/Concentrator Plant, Botswana. *Global Journal of Pure and Applied Sciences*, 11 (4): 553-559.

Ekpo, K.E., Azeke, M.A. & Ugbenyen, A.M. 2008. Functional Properties of Dried Imbrasia Belina Larvae Flour as Affected by Mesh Size and pH. *Nigerian Annals of Natural Sciences*, 8 (1): 6-9. www.nansjournal.com.

Franke, T. M., Ho, T. & Christie, C.A. 2012. The Chi-Square Test Often Used and More Often Misinterpreted. *American Journal of Evaluation*, 33(3):448±58.

Frears, S.L., Chown, S.L. & Webb, P.I. 1997. Behavioural Thermoregulation in the Mopane Worm (Lepidoptera). *Journal of Thermal Biology*, 22 (4/5): 325-330.

Frears, S.L., Chown, S.L. & Webb, P.I. 1999. Temperature Dependence of Feeding Behaviour in the Mopane Worm (Lepidoptera). *Journal of Thermal Biology*, 24:241-244.

Frost, C.J. & Hunter, M.D. 2004. Insect Canopy Herbivory and Frass Deposition Affect Soil Nutrient Dynamics and Export in Oak Mesocosms. *Ecology*, 85(12): 3335–3347.

Gabaza, M., Shumoy, H., Muchuweti, M., Vandamme, P. & Raes, K. 2018. Baobab Fruit Pulp and Mopane Worm as Potential Functional Ingredients to Improve the Iron and Zinc Content and Bioaccessibility of Fermented Cereals. *Innovative Food Science and Emerging Technologies*, doi: 10.1016/j.ifset.2018.04.005.

Gardiner, A, 2003. The domestication of mopane worms (*Imbrasia belina*). Annual report for mopane woodlands and the mopane worm: enhancing rural livelihoods and resource sustainability. DFID R7822 – Forestry Research Programme. Gaborone, Botswana: Veld Products Research & Development.

Gardiner A., Sitole, H., Sowry, R. & Midzi, S. n.d. Some Aspects of the Ecology & Sustainable Utilization of the Mopane Worm.

Gashe, B.A., Mpuchane, S.F., Siame, B.A., Allotey, J. & Teferra, G. 1997. The Microbiology of Phane, an Edible Caterpillar of the Emperor Moth, *Imbrasia belina. Journal of Food Protection* (11):1376-1380.

Gaston, K.J., Chown, S.L. & Styles, C.V. 1997. Changing Size and Changing Enemies: The case of the mopane worm. *Acta Ogcologica*, 18 (1):21-26.

Glew, R.H., Jackson, D., Sena, L., VanderJagt, D.J., Pastuszyn, A. & Millson, M. 1999. *Gonimbrasia belina* (Lepidoptera: Saturniidae): A nutritional food source rich in protein, fatty acids, and minerals. *American Entomologist*, 45 (4): 250- 253.

Gondo, T., Frost, P., Kozanayi, W., Stack, J. & Mushongahande, M. 2010. Linking Knowledge and Practice: Assessing Options for Sustainable Use of Mopane Worms (*Imbrasia Belina*) In Southern Zimbabwe. *Journal of Sustainable Development in Africa*, 12(4): 127-145.

Greenfield, R., Akala, N. & Van der Bank, F.H. 2014. Heavy Metal Concentrations in Two Populations of Mopane Worms (*Imbrasia belina*) in the Kruger National Park Pose a Potential Human Health Risk. *Bull. Environ. Contam. Toxicol.*, 93:316–321. DOI:10.1007/s00128-014-1324-4.

Greyling, M., van der Bank, F.H., Grobler, J.P. & Wessels, D.C.J. 2001. Allozyme Variation in Two Populations of the Mopane Worm, *Imbrasia belina* (Saturniidae), and the Effect of Developmental Stage and Staggered Generations. *South African Journal of Animal Science*, 31 (1): 15-24. http://www.sasas.co.za/Sajas.html.

Hope, R.A., Frost, P.G.H., Gardiner, A. & Ghazoul, J. 2009. Experimental Analysis of Adoption of Domestic Mopane Worm Farming Technology in Zimbabwe. *Development Southern Africa*, 26 (1):29-46, DOI: 10.1080/03768350802640065.

Hrabar, H., Hattas, D. & Du Toit, J.T. 2009 (a). Differential Effects of Defoliation by Mopane Caterpillars and Pruning by African elephants on the regrowth of *Colophospermum mopane* Foliage. *Journal of Tropical Ecology*, 25:301–309. doi:10.1017/S0266467409005872.

Hrabar, H., Hattas, D. & Du Toit, J.T. 2009 (b). Intraspecific Host Preferences of Mopane Moths (*Imbrasia belina*) in Mopane (*Colophospermum mopane*) Woodland. *African Zoology*, 44(2):131–140.

Hrabar, H. & Du Toit, J.T. 2014. Interactions between Megaherbivores and Microherbivores: Elephant Browsing Reduces Host Plant Quality for Caterpillars. *Ecosphere*, 5 (1): 1-6. www.esajournals.org.

Illgner, P & Nel, E. 2000. The Geography of Edible Insects in Sub-Saharan Africa: A Study of the Mopane Caterpillar. The Geographical Journal, 166 (4):336-351. The Royal Geographical Society: Grahamstown. https://www.jstor.org/stable/823035.

Jennings, G., Mc Geoch, M.A., Rebe, M., Jackson, S., Roberts, J., Styles, C. & Scholtz, C.H. 2001. The Nutritive Value of *Imbrasia belina* Caterpillars and their Role in the Diet of Human Consumers in South Africa. *Entomological Society of Southern Africa.*

Kachapulula, P.W., Akello, J., Bandyopadhyay, Y. R. & Cotty, P.J. 2018. Aflatoxin Contamination of Dried Insects and Fish in Zambia. *Journal of Food Protection*, 81 (9):1508-1518. doi:10.4315/0362-028X.JFP-17-527.

Kamwi, J.M., Endjala, J. & Siyambango, N. 2020. Dependency of Rural Communities on Non-timber Forest Products in the Dry Lands of Southern Africa: A case of Mukwe Constituency, Kavango East Region, Namibia. *Trees, Forests and People 2*. https://doi.org/10.1016/j.tfp.2020.100022.

Kozanayi, W. & Frost, P. 2002. Marketing of Mopane Worm in Southern Zimbabwe. *Mopane Worm Market Survey: Southern Zimbabwe.*

Kung, S., Fenemore, B. & Potter, P.C. 2011. Anaphylaxis to Mopane Worm (*Imbrasia Belina*) *Annals of Allergy, Asthma & Immunology*, 106: 538-540.

Kung, S., Mazhani, L. & Steenhoff, A.P. 2013. Allergy in Botswana. Current Allergy & Clinical Immunology, 26 (4): 202-209.

Kwiri, R., Winini, C., Muredzi, P., Tongonya, J., Gwala, W., Mujuru, F. & Gwala, S.T. 2014. Mopane Worm (*Gonimbrasia belina*) Utilisation, a Potential Source of Protein in Fortified Blended Foods in Zimbabwe: A Review. *Global Journal of Science Frontier Research: D Agriculture and Veterinary*, 14 (10): 55-67.

Langley, J., Van der Westhuizen, S., Morland, G., & van Asch, B. 2020. Mitochondrial Genomes and Polymorphic Regions of *Gonimbrasia belina* and *Gynanisa maja* (Lepidoptera: Saturniidae), Two Important Edible caterpillars of Southern Africa. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.12.055.

Madibela, O.R., Mokwena, K.K., Nsoso, S.J. & Thema, T.F. 2009. Chemical Composition of Mopane Worm Sampled at Three Sites in Botswana and Subjected to Different Processing. *Trop Anim Health Prod*, 41:935–942. DOI 10.1007/s11250-008-9282-7.

Madibela, O.R., Seitiso, T K., Thema, T.F. & Letso, M. 2007. Effects of Traditional Processing Methods on Chemical Composition and In Vitro True Dry Matter Digestibility of Mophane

Worm (*Imbrasia belina*). *Journal of Arid Environments*, 68:492-500. Doi: 10.1016/j.jaridenv.2006.06.002.

Makhado, R.A, Potgieter, M.J, Wessels, D.C.J, Saidi, A.T, & Masehela, K.K. 2012. Use of mopane woodland resources and associated woodland management challenges in rural areas of South Africa. *Ethnobotany Research & Applications*, 10:369-379. www.ethnobotanyjournal.org/vol10/i1547-3465-10-369.

Makhado, R., Potgieter, M., Timberlake, J. & Gumbo, D.A. 2014. Review of the Significance of Mopane Products to Rural People's Livelihoods in Southern Africa. *Transactions of the Royal Society of South Africa*, 69 (2): 117–122. DOI: 10.1080/0035919X.2014.922512. http://dx.doi.org/10.1080/0035919X.2014.922512.

Manyeula, F., Tsopito, C. M., Kamau, J. M., Mogotsi, K. K., Nsoso, S. J. & Moreki, J. C. 2018. Effect of *Imbrasia belina* (Westwood) or *Vigna subterranea* (L) Verde or *Tylosema esculentum* (Burchell) Schreiber as Protein Sources in Diets Fed to Tswana Hens on Egg Quality. *Journal of Animal Science and Veterinary Medicine*, 3 (6):190-196. https://doi.org/10.31248/JASVM2018.118.

Manyeula, F., Tsopito, C.M., Mogotsi, K., Nsoso S.J., Kamau, J.M. & Moreki, J.C. 2019. Chemical Composition and Sensory Evaluation of Meat of Tswana Hens Fed Different Protein Sources: Mopane Worm, Bambara Groundnut and Morama Bean. *Online Journal of Animal and Feed Research*, 9 (2): 92-102. www.ojafr.ir.

Marareni, M. & Mnisi, C.M. 2020. Growth Performance, Serum Biochemistry and Meat Quality Traits of Jumbo Quails Fed with Mopane Worm (*Imbrasia belina*) Meal-Containing Diets. *Veterinary and Animal Science,*10:1-6. https://doi.org/10.1016/j.vas.2020.100141.

Mataboge, M.S, Mulaudzi, F.M., Wenhold, F.A.M., Nkunika, P.O.Y., Dlamini, N.R. & Alders, R.G. 2016. Effects of Climate Variability on the Harvesting and Preservation of Mopane Worms. *Indilinga - African Journal of Indigenous Knowledge Systems*, 15 (2): 34-48.

Mbata, K.J. Chidumayo, K.J. 2003. Traditional Values of Caterpillars (Insecta: Lepidoptera) among the Bisa People of Zambia. *Insect Sci. Applic.*, 23 (4): 341-354.

Mbhenyane, X.G., Venter, C.S., Vorster, H.H. & Steyn, H.S. 2005. Nutrient Intake and Consumption of Indigenous Foods Among College Students in Limpopo Province. *SAJCN*, 18 (1):32-38.

McLeod, A. I.2011. Kendall: Kendall rank correlation and Mann-Kendall trend test. R package version 2.2. https://CRAN.R-project.org/package=Kendall.

Mogwase, S.S., Moreki, J.C. & Nsoso, S.J. 2018. Analysis of Drumsticks and Offal of Guinea Fowl (*Numida Meleagris*) fed Mophane Worm (*Imbrasia Belina*) as Protein Source and Raised Up to 13 Weeks Under Intensive Management System. *International Journal of Innovative Science and Research Technology*, 3 (4): 741-746. www.ijisrt.com.

Motshegwe, S.M., Holmbackb, J. & Yeboah, S.O. 1998. General Properties and the Fatty Acid Composition of the Oil from the Mophane Caterpillar, *Imbrasia belina. JAOCS*, 75 (6): 725-728.

Moyo, S., Masika, P.J. &. Muchenje, V. 2019. The potential of Imbrasia belina worm as a poultry and fish feed. A review. *Journal of Animal and Feed Sciences*, 28: 209–2019 https://doi.org/10.22358/jafs/112156/2019.

Mpuchane, S., Gashe, B.A., Allotey, J., Siame, B., Teferra, G. & Ditlhogo, M. 2000. Quality deterioration of phane, the edible caterpillar of an emperor moth *Imbrasia belina. Food Control*, 11: 453-458.

Mpuchane, S., Taligoola, H.K. & Gashe, B.A. 1996. Fungi Associated with *Imbrasia belina*, an Edible Caterpillar. *Botswana Notes and Records*, 28: 193-197. http://www.jstor.com/stable/40980141.

Mufandaedza, E., Moyo, D.Z. & Makoni, P. 2014. Management of Non-timber Forest Products Harvesting: Rules and regulations governing (Imbrasia belina) access in South-Eastern in Lowveld Zimbabwe. *African Journal of Agricultural Research*, 10 (12):1521-1530. Doi:10.5897/AJAR2013.7720. http://www.academicjournals.org/AJAR.

Mufandaedza, E., Moyo, D.Z. & Makoni, P. 2015. Models of Valuation of Non-Timber Forest Products: Study of *Imbrasia belina* dynamics in the South East Lowveld, Zimbabwe. *Agricultural Journal*, 9 (2): 132-147.

Mufandaedza, E., Moyo, D.Z., Makoni, P. 2018. Effect of Soil Type and Foliar Factors on the Distribution of *Imbrasia belina* in the Southeastern Lowveld of Zimbabwe. *Scientifica*, 1-8. https://doi.org/10.1155/2018/9273184.

Mughogho, D.E.C. & Munthali, S.M. 1993. Ecological Interaction between *Gonimbrasia belina* (L.) and *Gynanisa maia* (L.), and the Impact of Fire on Forage Preferences in a Savanna Ecosystem. *Afr. J. Ecol.*, 33: 84-87.

Mujuru, F. M., Kwiri, R., Nyambi, C., Winini, C. & Moyo, N.D. 2014. Microbiological quality of Gonimbrasia belina processed under different traditional practices in Gwanda, Zimbabwe. *International Journal of Current Microbiology and Applied Sciences*, 3(9): 1085-1094. https://www.ijcmas.com.

Murefu, T.R., Macheka, L., Musundire, R. & Manditsera, F.A. 2019. Safety of Wild Harvested and Reared Edible insects: A review. *Food Control,101: 209-224. https://doi.org/10.1016/j.foodcont.2019.03.003*.

Mushaphi, L.F., Dannhauser, A., Walsh, C.M., Mbhenyane, X.G. & van Rooyen, F.C. 2017. The Impact of a Nutrition Education Programme on Feeding Practices of Caregivers with Children Aged 3 to 5 Years in Rural Limpopo Province, South Africa. *South African Journal of Clinical Nutrition*, 30(4):101-108. DOI: 10.1080/16070658.2017.1322823.

Musundire, R., Zvidzai, C.J., Chidewe, C., Samende, B.K. & Chemura, A. 2016. Habitats and Nutritional Composition of Selected Edible Insects in Zimbabwe. *Journal of Insects as Food and Feed*, 2 (3): 189-198. DOI: 10.3920/JIFF2015.0083.

Mwimanzi, L. & Musuka, C.G. 2014. The Potential of Mopane Worm (Gonimbrasia belina) as an Alternative Protein Source in Fish Feed. *International Journal of Aquaculture*, 4 (12):73-78. http://ija.sophiapublisher.com.

Nantanga, K.K.M. & Amakali, T. 2020. Diversification of Mopane Caterpillars (*Gonimbrasia belina*) Edible Forms for Improved Livelihoods and Food Security. *Journal of Arid Environments*, 177:1-5.

Nawases, B., Uzabakiriho, J. & Chimwamurombe, P. 2018. Identification of Fungi Associated with Processed-Food Contamination at Open Markets of Windhoek, Namibia. *Journal of*

Pure and Applied Microbiology, 12 (3): 1489-1494. http://dx.doi.org/10.22207/JPaM.12.3.53.

Ndeinoma, A. & Wiersum, K.F. 2017. Diversity of governance arrangements for indigenous natural products in communal areas of Namibia. *Forests, Trees and Livelihoods*, 26 (2):124-141, DOI: 10.1080/14728028.2016.1268545.

Ndlovu, I., Nunu, W.N., Mudonhi, N., Dube, O. & Maviza, A. 2019. Land Use−Land Cover Changes and Mopane Worm Harvest in Mangwe District in Plumtree, Zimbabwe. *Environmental Systems Research*, 8 (11): 1-9. https://doi.org/10.1186/s40068-019-0141-5.

Nobo, G., Moreki, J.C. & Nsoso, S.J. 2012 (a). Growth and Carcass Characteristics of Helmeted Guinea Fowl (*Numida meleagris*) fed Varying Levels of Phane Meal (*Imbrasia belina*) as Replacement of Fishmeal Under Intensive System. *IJAVMS*, 6(4): 296-306. doi:10.5455/ijavms.164.

Nobo, G., Moreki, J.C. & Nsoso, S.J. 2012 (b). Feed Intake, Body Weight, Average Daily Gain, Feed Conversion Ratio and Carcass Characteristics of Helmeted Guinea Fowl Fed Varying Levels of Phane Meal (Imbrasia belina) as Replacement of Fishmeal under Intensive System. *International Journal of Poultry Science*, 11 (6): 378-384.

Nunu, N.W., Ncube, B., Dube, O., Mpofu, C., Ndlovu, B., Dzinomva, T. & Khumalo, N. 2019. Nutritional Factors Associated with Distribution of Mopane Worms in Mopane Woodlands in Tsholotsho and Gwanda Districts, Zimbabwe: A comparative survey. *Scientific Reports*, 9 (17320):1-7. https://doi.org/10.1038/s41598-019-53923-7.

Obopile, M. & Seeletso, T.G. 2013. Eat or Not Eat: An analysis of the status of entomophagy in Botswana. *Food Sec.*, 5:817–824. DOI 10.1007/s12571-013-0310-8.

Okezie, O. A., Kgomotso, K.K. & Letswiti, M.M. 2010. Mopane Worm Allergy in a 36-Year-Old Woman: A case report. *Journal of Medical Case Reports*, 4 (42): 1-4. http://www.jmedicalcasereports.com/content/4/1/42.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., et al. 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. doi: 10.1136/bmj.n71.

Payne, C.L.R., Umemura, M., Dube, S., Azuma, A., Takenaka, C. & Nonaka, K. 2015. The Mineral Composition of Five Insects as Sold for Human Consumption in Southern Africa. *African Journal of Biotechnology*, 14 (31): 2443-2448. DOI: 10.5897/AJB2015.14807.

Pettitt AN. A non-parametric approach to the change-point problem. *Applied statistics*. 1979:126-35.

Pharithi, M.T., Suping, S.M. & Yeboah, S.O. 2004. Variations of the Fatty Acid Composition in the Oil from the Larval Stages of the Emperor Moth Caterpillar, *Imbrasia belina. Bull. Chem. Soc. Ethiop*., 18 (1):67-72.

Potgieter, M., Makhado, R. & Potgieter, A. 2012. Mopane Worms. Assessing the potential of insects as food and feed in assuring food security. Technical consultation meeting, 23-25 January 2012, FAO, Rome, Italy.

Potgieter, M.J. & Ramalivhana, N. 2020. Data on Mopane Worm (*Imbrasia belina*) Microorganisms from Limpopo Province, South Africa. *Data in Brief*. https://doi.org/10.1016/j.dib.2020.105695.

Potter, P. 2013. An Overview of the Indigenous and Alien Allergens of Southern Africa. *Current Allergy & Clinical Immunology*, 26 (4).

Quin, P.J. 1964. Foods and Feeding Habits of the Pedi. *S.A. Medical Journal*, 969-971.

Ramashia, S.E., Tangulani, T., Mashau, M.E. & Nethathe, B. 2020. Microbiological Quality of Different Dried Insects Sold at Thohoyandou Open Market, South Africa. *Food Research*, 1-9. http://www.myfoodresearch.com.

Rapatsa, M.M. & Moyo, N.A.G. 2017. Evaluation of Imbrasia belina meal as a fishmeal substitute in *Oreochromis mossambicus* diets: Growth performance, histological analysis and enzyme activity. *Aquaculture Reports*, 5: 18–26. http://dx.doi.org/10.1016/j.aqrep.2016.11.004.

Rapatsa¸ M.M. & Moyo, N.A.G. 2019. Enzyme activity and histological analysis of *Clarias gariepinus* fed on *Imbrasia belina* meal used for partial replacement of fishmeal. *Fish Physiol. Biochem*, 45:1309–1320. https://doi.org/10.1007/s10695-019-00652-3.

Rasengwatshe, A. & Madibela, O.R. 2005. Rumen Degradability of *Imbrasia belina* (mophane worms) and Carcass Meal. *Journal of Animal and Veterinary Advances*, 4(12): 1021-1024.

R Development Core Team. 2012. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. ISBN: 3-900051-07-0. http://www.R-project.org/.

Reid, T. 1999. The other 99%. The Conservation and Biodiversity of Invertebrates. *Pacific Conservation Biology*, 6(2): 178-178.

Ribeiro, J.C., Cunha, L.M., Sousa-Pinto, B. & Fonseca, J. 2017. Allergic Risks of Consuming Edible Insects: A systematic review. *Molecular Nutrition & Food Research, 1-31*. *www.mnfjournal.com*.

Roberts, C. 1998. Long-term Costs of the Mopane Worm Harvest. *Oryx*, 32 (1).

Sekonya, J.G, McClure, N.J. & Wynberg, R.P. 2020. New Pressures, Old Foodways: Governance and Access to Edible Mopane Caterpillars, *Imbrasia (Gonimbrasia) belina*, in the Context of Commercialization and Environmental Change in South Africa. *International Journal of the Commons*, 14 (1): 139–153. DOI: https://doi.org/10.5334/ ijc.978.

Simpanya, M.F., Allotey, J. & Mpuchane, S.F. 2000. A Mycological Investigation of Phane, an Edible Caterpillar of an Emperor Moth, *Imbrasia belina. Journal of Food Protection*, 63 (1):137–140.

Siulapwa, N., Mwambungu, A., Lungu, E. & Sichilima, W. 2014. Nutritional Value of Four Common Edible Insects in Zambia. *International Journal of Science and Research (IJSR)*, 3 (6): 876-884. www.ijsr.net.

Stack, J., Dorward, A., Gondo, T., Frost, P., Taylor, F. & Kurebgaseka, N. 2003. Mopane worm utilisation and rural livelihoods in Southern Africa. Presented at The International Conference on Rural Livelihoods, Forests and Biodiversity 19-23 May 2003, Bonn, Germany.

Styles, C. V. & Skinner, J. D. 1996. Possible Factors Contributing to the Exclusion of Saturniid Caterpillars (mopane worms) from a Protected Area in Botswana. *Afr. J. Ecol*, 34: 276-283.

Swemmer, L., Grant, R., Annecke, W. & Freitag-Ronaldson, S. 2015. Toward More Effective Benefit Sharing in South African National Parks. *Society & Natural Resources: An International Journal*, 28(1): 4-20. DOI: 10.1080/08941920.2014.945055.

Swemmer, L., Landela, R., Mdungasi, P., Midzi, S., Mmatho, W., Mmethi, H. Shibambu, D. Symonds, A., Themba, S. & Twine, W. 2020. It's Not Just About the Worm: Social and Economic Impacts of Harvesting *Imbrasia Belina* larvae (Kruger National Park, South Africa). *Conservation and Society*, 18(2): 183-199. www.conservationandsociety.org.

Teferra, G. 2003. Assimilation Efficiency in Two Herbivores, *Oreochromis Niloticus* and Larvae of *Imbrasia Belina*: A comparison. *Sinet: Ethiop. J. Sci*., 26 (1):73–76.

Teferra, G., Allotey, J., Mpuchane, S., Siame, B.A. & Gashe, B.A. 2000. The nutritional status of three species of plant leaves as food for the larvae of *Imbrasia belina*, an edible caterpillar. *Botswana Notes and Records*, 32:143-150.

Thomas, B. 2013. Sustainable Harvesting and Trading of Mopane Worms (*Imbrasia belina*) in Northern Namibia: An experience from the Uukwaluudhi area. *International Journal of Environmental Studies*, 70 (4): 494-502. https://doi.org/10.1080/00207233.2013.829324.

Togarepi, C., Nashidengo, E. & Siyambango, N. 2020. Effects of Climatic Variability and Non-Climatic Factors on Mopane Worms' (*Gonimbrasia Belina*) Distribution and Livelihood Options in North Central Namibia. *Environment and Natural Resources Research*, 10 (2):14-25. https://doi.org/10.5539/enrr.v10n2p14.

Van Den Berg, M. A., Catling, H. D. & Vermeulen, J. B. 1973. The Distribution and Seasonal Occurrence of Saturniidae (Lepidoptera) in Transvaal. *Phytophylactica*, 5:111-114

Van Huis, A. 2003. Insects as Food in Sub-Saharan Africa. *Insect Sci. Applic*., 23(3):163–185.

Wiggins, D.A. 1997 (a). Clutch size and oviposition site characteristics of a nocturnal African silk moth *Imbrasia belina* (Lepidoptera: Saturniidae). *Annales Zoologici Fennici*, 34 (4): 235-240. http://www.jstor.com/stable/23735486.

Wiggins, D.A. 1997 (b). Fluctuating Asymmetry in Colophospermum Mopane Leaves and Oviposition Preference in an African Silk Moth *Imbrasia belina. Oikos*, 79(3): 484-488. http://www.jstor.com/stable/3546891.

Yeboah, S.O. & Mitei, Y.C. 2009. Further Lipid Profiling of the Oil from the Mophane Caterpillar, *Imbrasia belina*. *J Am Oil Chem Soc*, 86:1047–1055. DOI 10.1007/s11746-009-1443-y.

Zinzombe, I.M. & George, S. 1994. Larval Lipid Quality of Lepidoptera: *Gonimbrasia belina*. Botswana Notes and Records, 26: 167-173. http://www.jstor.org/stable/40959191.

CHAPTER 3: INCUBATION TRIAL: Nitrogen Mineralization of Mopane Caterpillar Frass: An ecosystem service from an iconic edible caterpillar.

Mutali Winnie Netshanzhe[1], Corrie Swanepoel[2], Stefan Foord[3], Lourens Swanepoel[1,4]

[1]Department of Biological Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou, 0950

[2]Agricultural Research Council, Pretoria, South Africa

[3]SARChI chair in Biodiversity Value and Change, University of Venda, South Africa

[4]African Institution for Conservation Ecology, Levubu, South Africa

Prepared for: African Journal of Ecology

3.1. Background

Nutrient cycling is a key driver of ecosystems (Lavelle et al., 2005), affecting their composition, structure, and functioning (Lavelle et al., 2005; Wolf et al., 2013). Nutrient cycling is the life cycles of nutrients and its movement within and between the biotic and abiotic pools over time (Lavelle et al., 2005). Nutrient cycling and availability control the productivity and growth of the biosphere (Wolf et al., 2013). Nutrient cycling requires many different organisms (Lavelle et al., 2005) with herbivory animals being one of them. While there is a rich body of research exploring the role of large mammalian grazers, mixed feeders and browsers on ecosystem functions and nutrient cycling (Sinclair, 2003; Kerley et al., 2008, Wolf et al., 2013; Lacher et al., 2019), there is growing interest on invertebrate herbivory on ecosystem functioning (Belovsky & Slade, 2000; Chapman et al., 2003; Reynolds & Hunter, 2004; De Swardt et al., 2018). This is because research has highlighted that the impact of mammalian herbivory on ecosystems can be matched or exceeded by invertebrate herbivory (Frost & Hunter, 2004; De Swardt et al., 2018). For example, *Cirina forda* larvae which mainly feed on *Burkea africana* can defoliate about a third of its leaf biomass (Scholtz, 1976). Grasshoppers in the Serengeti potentially consumes as much grass as mammalian grazers (Sinclair, 1975), while one termite colony can remove up to 15,000 kg/ha of litter per year (Dangerfield et al., 1998), with their biomass (70–100 kg/ha; Wood & Sands, 1978) exceeding that of large mammals (10–80 kg/ha; Bell, 1982).

Arthropod biomass in many ecosystems often exceeds mammalian biomass (De Swardt et al., 2018) and can thus play even more critical roles in ecosystem processes than mammals (De Swardt et al., 2018). For example, herbivorous insects feed on leaves and release frass to the ground, enhancing the nutrient pool in the environment, accelerating nutrient cycling (Reynolds & Hunter, 2004; Jankielsohn, 2018). Frass is often fast decomposing and can significantly contribute to nutrient input into the soil, aiding growth in the tree community (Reynolds & Hunter, 2004). Consequently, frass has been found to release nutrients faster than leaves, e.g., woodlice decompose frass (of *Operophthera fataga*) faster than beech leaf litter (Zimmer & Topp, 2002). Arthropod's herbivory does not just affect biogeochemical cycling but can also affect tree survival, plant chemistry, and nutrient content (De Swardt et al. 2018). It has further been suggested that insect outbreaks increase nutrients in the landscape (Reynolds & Hunter, 2004).

Insect herbivory offers a number of services to nutrient cycling and availability in soil. Herbivore insects supplement litter and soil by depositing frass in large quantities, thus returning more N into soil (Fogal & Slansky, 1985; Grace, 1986). Consequently their N return from plants to soil tend to be double (Hollinger, 1986). During outbreaks, insect cadavers accelerates litter decomposition

(Swank et al., 1981; Schowalter & Crossley, 1983; Seastedt & Crossley, 1984) because they decomposes more easily as compared to leaf litter (Schowalter et al., 1986). Insect herbivory also increases nutrients in precipitation passing through the plant canopy (Reynolds and Hunter, 2004), thus increasing nutrient deposition into soil.

3.1.1. Nutrient cycling in Mopane woodland

Colophospermum mopane (mopane) savanna covers around 555,000 km^2 of southern Africa (Mapaure, 1994). The main nutrient cycling processes in this ecosystem are modulated by mammal herbivory because grazing mammals increase foliar N in the woodland ecosystem by excreting N in forms that plants can easily take up (De Swardt et al., 2018). For example, African elephants (*Loxodonta africana*) and the mopane caterpillar are the primary herbivores of the mopane tree (Hrabar & Du Toit, 2014). Elephants drive biomass recycling through the process of wasteful feeding, tree toppling, and processing barks and roots, which are fibrous (Kerley et al., 2008). They feed by stripping, debarking, branch, and stem breaking, and uprooting mopane vegetation (Hrabar & Du Toit, 2014).

Leguminosae plants form N_2- fixing symbioses with bacteria such as *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium, Azorhizobium and Allorhizobium*. These plants form root nodules, where these rhizobial bacteria reduce atmospheric N_2 into NH_3^+ and exchange this nitrogenous solute for photosynthate from the host plant (Pule-Meuelenberg & Dakora, 2007). Although *C. mopane* is leguminous (Makhado et al., 2014), it does not "nodulate" (De Swardt et al., 2018), and as such, nutrient cycling is through leaf flush, leaf fall, and decomposition. However, plant litter/litterfall is not readily available as nutrients for plants uptake since decomposition, and subsequent release of plant-available nutrients is a lengthy process (Belovsky & Slade, 2000; Reynolds & Hunter, 2004). Thus, processes that can facilitate the breakdown of mopane leaves to readily available nutrients would be crucial to mopane woodland nutrient cycling.

C. mopane is the primary host for the lepidopteran *I. belina* (mopane caterpillars) (Ditlhogo et al., 1996). During their growing period, mopane caterpillars can completely defoliate extensive tracts of the mopane woodland (van Voorthuizen, 1976, Ditlhogo, 1996), releasing large amount of "fast-decomposing frass" (Reynolds and Hunter, 2004) into the soil, which aid with plants nutrient uptake and nutrient cycling (De Swardt et al., 2018). Styles (1996) observed that fifth instar larvae could each consume >40g of dry leaf material. In contrast, the fourth/fifth instars release an average of 1.42g of frass per day (De Swardt et al., 2018). Mopane caterpillars' frass has been found to influence the cycling of potassium (K), nitrogen (N), and phosphorus (P) (De Swardt et al., 2018). Considering that frass is fast decomposing (Reynolds & Hunter, 2004), the existence of mopane caterpillars could aid in nutrient release, especially nitrogen (N) through mineralization.

3.1.2. Nitrogen cycling

The nitrogen cycle outline the pathway of how N sources, such as manure, fertilizers and plants moves through different stages, such as soil, plants, water and air (Johnson et al., 2005) (Fig. 3.1). The N cycle is a microbial driven process, and start with mineralization or decomposition of organic matter, converting organic N into plant available N forms. This is absorbed by plants and microbes, and eventually returns to the soil when plants or microbes dies, or through animal waste (McNeill & Unkovich, 2007). Plant available N forms are converted from N_2 gas into organic N forms via biological N_2 fixation, industrial N_2 fixation, and lightning (Fig. 3.1). From there, N can undergo various processes, such as mineralization, ammonification, nitrification, before returning to the atmosphere as a gaseous N form and completing the cycle (Poffenbarger et al., 2018). Fixation, mineralization and nitrification processes increases N available for plants, whilst denitrification, volatilization, immobilization, and leaching result in N losses (Bierman & Rosen, 2005; Johnson et al., 2005).

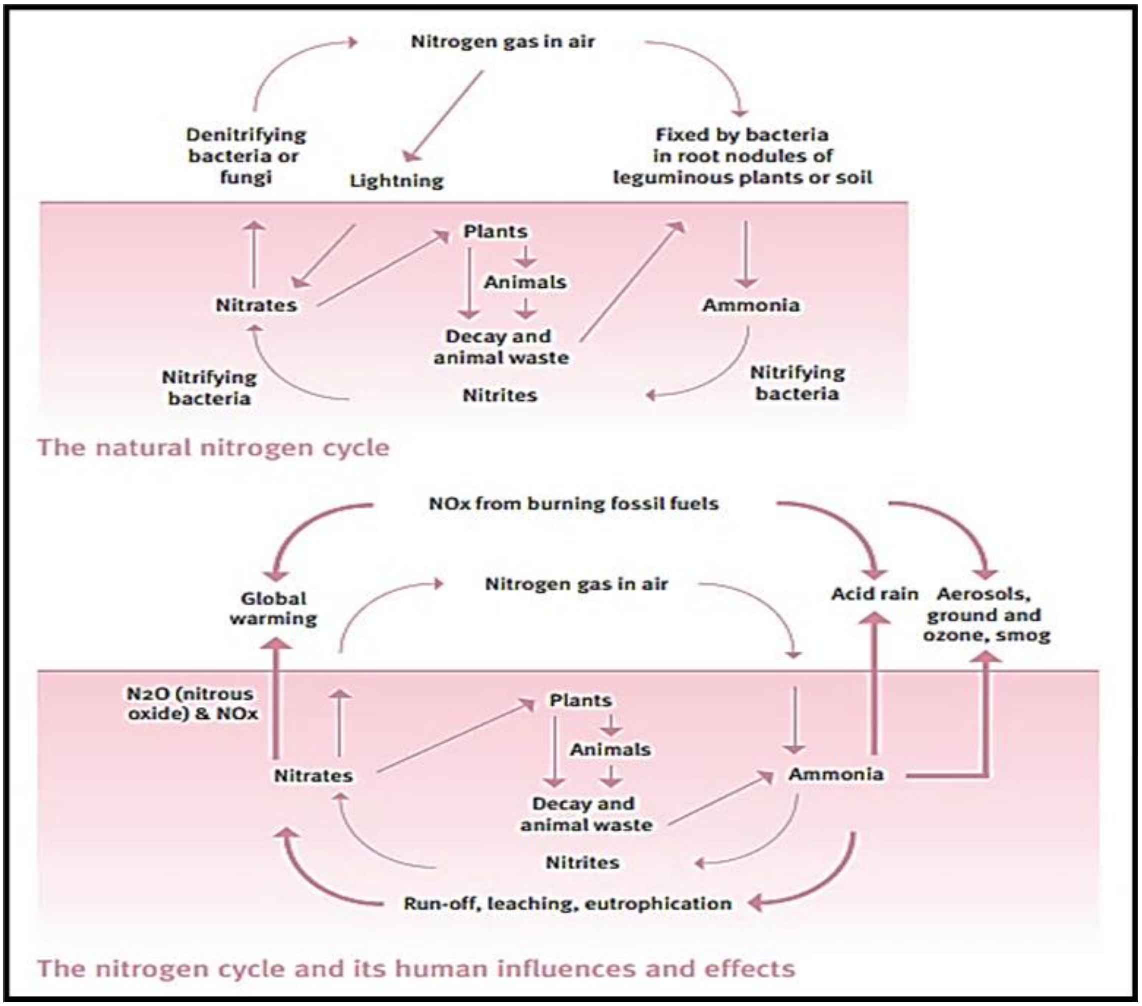


Figure 3. 1. The Nitrogen Cycle (source: Hislop, 2007)

3.1.3. N processes

i. Nitrogen fixation is when atmospheric N is converted to organic N components. This can be achieved through biological process such as nitrogen fixing bacteria as well as industrial process when producing commercial fertilizers (Johnson et al., 2005).

ii. Mineralization is microbial facilitated breakdown or decomposition of organic matter (Johnson et al., 2005). This process can be influenced by soil temperature, soil water, soil chemistry such as pH, the type of organic materials, the type of soil or the amount of oxygen in available.

iii. Nitrification is the conversion of ammonium to nitrate through oxidation. This is a microbial process to obtain energy. The rate can be increased when soil is warm (19 - 30°C), with adequate soil water and temperature. As with other N processes, this is a microbial mediated process, and will be affected by conditions that reduce microbial activity, such as high and low temperatures (Johnson et al., 2005).

iv. Denitrification is the loss of N, particularly NO_3 in anaerobic soil conditions. When the soil is saturated, bacteria use nitrate as an oxygen source (Johnson et al., 2005), and subsequently convert NO_3 into N_2O or eventually N_2 components. This is the final step to complete the cycle, as these N forms are returned to the atmosphere (Poffenbarger et al., 2018).

v. Volatilization is the loss of N through ammonia gas, which is released to the atmosphere (Kusumah, n.d.). Volatilization is more likely at higher soil pH and conditions that favor evaporation (e.g., hot and windy) and are also higher for manures and urea fertilizers that are surface applied (Johnson et al., 2005).

vi. Immobilization is the process in which N fractions are temporarily unavailable for plant uptake. NH_4^+, NO_3^-, or NO_2 can be taken up by soil organisms and therefore become unavailable to crops (Johnson et al., 2005; Poffenbarger et al., 2018). Immobilization occurs when organic materials with a high C:N ratio are applied to the soil. The microbes are stimulated due to the C-rich source but cannot satisfy their N needs from the organic supplement. Microbes will then extract N from the soil matric, resulting in a decrease of plant available N (Johnson et al., 2005).

vii. Leaching is a pathway of N loss into water. Due to the negative charge of NO_3^-, clay minerals cannot adsorb these molecules, and it is typically highly soluble. Nitrate can therefore move in the soil with the water, and in high rainfall regions, nitrate leaching is a common loss of N from the soil (Kusumah, n.d.)

The aim of this study was to compare the NH_4^+ and NO_3^- mineralization rates between mopane caterpillar frass and mopane leaves to indicate the rate of nutrient return between mopane leaves with and without mopane caterpillars (which convert leaves into frass).

3.2. Materials and Methods

3.2.1. Study area

Soil, mopane leaves and frass were collected from Venetia Nature Reserve, close to the Botswana and Zimbabwe border. Venetia Nature Reserve is located in Musina Local Municipality, Limpopo Province (22^{o}08’27” S and 29^{o}13’28” E) (Fig. 3.1.). It is situated in the semi-arid savanna and is characterized by wet, hot summers with mean monthly temperatures of 32^{o}C during October to December and dry, mild winters with mean monthly temperatures of 24.7^{o}C during June (Hrabar et al., 2009) with long-term rainfall averages of 350 mm per year. This area has a flat topography dominated by sandstone underlying deep (>2m) colluvial soils (Hrabar et al., 2009). The vegetation is woodland dominated by acacia trees and mopane veld.

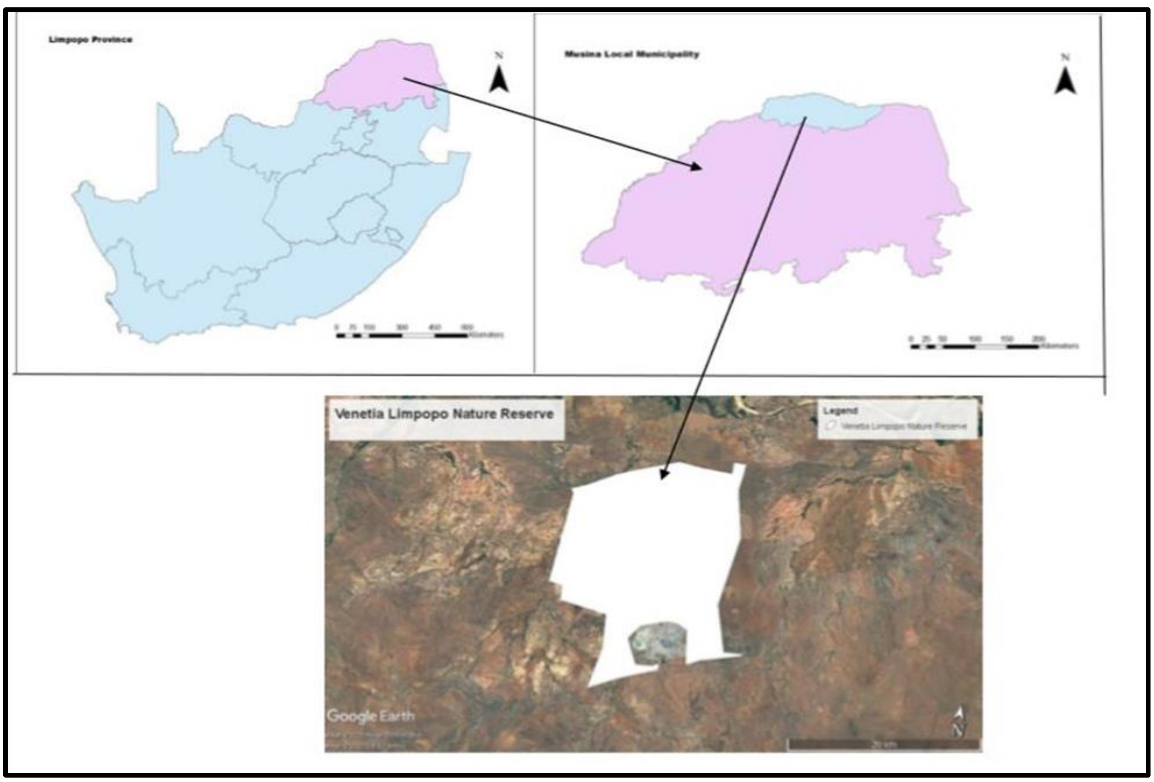


Figure 3. 2. Study area, Venetia Nature Reserve

3.2.2. Collection of frass, leaves and soil

Soil, mopane leaves and mopane caterpillars' frass (Fig. 3.2) were collected in January 2021. Frass was collected from mature caterpillars. Mature leaves were collected from the trees with mopane caterpillars, as well as surrounding trees (Fig. 3.3). Topsoil (0-20cm) was also collected from the same site as frass and leaves. Soil, leaves and frass samples were submitted to Nvirotek laboratory where standard laboratory analysis were used to determine chemical composition (Table 3.2).

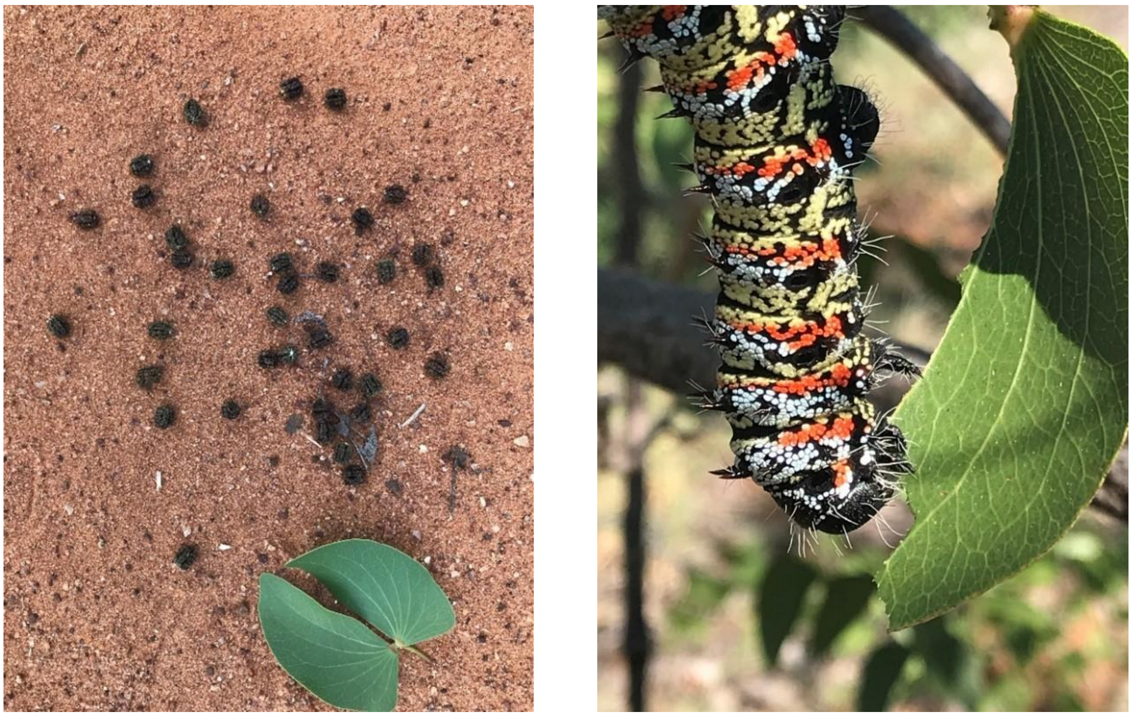


Figure 3. 3. Mature mopane caterpillar frass (left) are easily seen on the cleared soil. Mature mopane caterpillar (right)

Figure 3. 4**. Marked tree with leaf litter removed to aid in the collection of fresh frass**

3.2.3. Incubation trial

In preparation for the incubation trial the soil was prepared by drying and sieving, the leaves were dried and some of the dried leaves were finely grinded. The finely grinded leaves was included as a fine leaf treatment, to check if any difference between whole leave and frass is attributed to the size of the fraction, or additional nutrients added through mopane caterpillar digestive processes. The field capacity (FC) of the soil was also determined, and the amount of soil and treatment quantities were calculated as follows:

Field capacity

The incubation study was done at FC to determine optimal mineralization rates. To determine the FC of the soil, three freely drained cylinder containers (40cm X 3.5cm)(Van Niekerk et al., 2005). were used. One end of the container was covered with a mesh (40cm) that allow free drainage of water. The containers were filled with soil and packed to resemble natural bulk density. The containers were saturated with water, were covered with their caps to prevent evaporation, and the containers were left to drain for 24 hours. After 24 hours, soil samples (40g) were taken from each container, weight, and then oven dried at 100°C until dry. The soil was weighed again. The difference between the mass of wet soil and dried soil, is expressed as a percentage per dry soil, and represent FC of the soil (Van Niekerk et al., 2005).

Treatment rates

Treatment rates of whole leaves, fine (grinded) leaves and frass were calculated using the following parameters:

- 40 g of soil
- Contact with leaves and frass is limited to the top 5 mm of soil (soil depth)
- Soil bulk density is 1.3 Mg m^{-3}
- An average of 1,6 Mg (1600 kg) of mopane leaves per hectare (Mutakela, 2009)

In one hectare of soil, the total volume of soil (5 mm depth) is

100 m x 100 m x 0.005 m = 50 m^3

With a density of 1.3 Mg m^{-3}, the total mass of this soil is:

50 x 1.3 = 65 Mg (65000 kg) of soil

According to Mutakela (2009), an average mass of 1.6 Mg (1600 kg) of mopane leaves can be found in 1 hectare, so the application of leaves to 40 g of soil was calculated as follows:

0.04kg/65000kg x 1600kg x 1000 = 0.00098 kg = 0.98 g frass

The N content of the frass and the leaves were similar, so 1g of frass, whole and fine leaves were added respectively to the soil.

Trial design

The incubation trial was done over a period of 3 months (Table 3.1). Treatments included a control (soil only with no leaves or frass added), whole leaves, fine leaves and frass (Fig. 3.4). Each treatment was replicated four times and repeated seven times to allow for seven sampling events. Extra soil (four jars) that served as controls were added for analysis prior to the incubation.

Figure 3. 5. Mopane caterpillar frass (left), and whole mopane leaves (middle) and fine leaves (right)

Table 3. 1. Treatment allocation and sampling days

Treatments	**Day 0**	**Day 1**	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 40**	**Day 60**	**Total per treatment**
Pre-incubation	4								***4***
Control		4	4	4	4	4	4	4	***28***
Frass		4	4	4	4	4	4	4	***28***
Leave fine		4	4	4	4	4	4	4	***28***
Leave large		4	4	4	4	4	4	4	***28***
Total per sampling day	***4***	***16***	***16***	***16***	***16***	***16***	***16***	***16***	***116***

The 100 glass containers (352ml) were filled with 40g of soil, and 4ml of water was added to bring the soil to FC. The containers were slightly closed and placed in an incubator at constant temperature of 21°C for seven days as a pre-incubation step to allow the microbes to adjust to the water content and set temperature. During this pre-incubation period and for the duration of the experiment, the water content was checked at least every second day by weighing the jars and adding water to keep the soil at FC. The last day of the pre-incubation period was marked as ‘day

0’. On day 0, four glass jars were removed to be analysed for NH_4^+ and NO_3^- (see description below), which will represent the baseline NH_4^+ and NO_3^- content of the soil. Also, on day 0, the treatment was added to the glass jars: 28 glass jars each received 1 g of frass; 28 jars received 1 g of whole leaves; 28 jars received 1 g of fine leaves, and 28 jars were kept as control (Fig 3.5). The treatments were slightly mixed with the soil, to ensure better soil contact area for effective decay. Twenty-four hours later, on day 1, one set of samples were extracted to be analysed for NH_4^+ and NO_3^-. Subsequent samples were taken on days 3, 7, 14, 14, 21, 40 and 60 (Van Niekerk et al., 2005).

Figure 3. 6. Randomized block design glass jars for incubation trial

3.2.4. NH_4^+ and NO_3^- Analysis

Nitrogen was extracted from all the soil in jars by adding 100 ml of 0.01M K_2SO_4 solution and shaking the jars for half an hour. The samples supernatant were then filtered through Whatman’s 42 filter paper (Fig. 3.6 below). The extractions were kept in a fridge at 4°C and transported the next day in a cooler box with icepacks to NviroTek Laboratories. NH_4^+ and NO_3^- analysis were done on a spectrophotometer using a colour indicator.

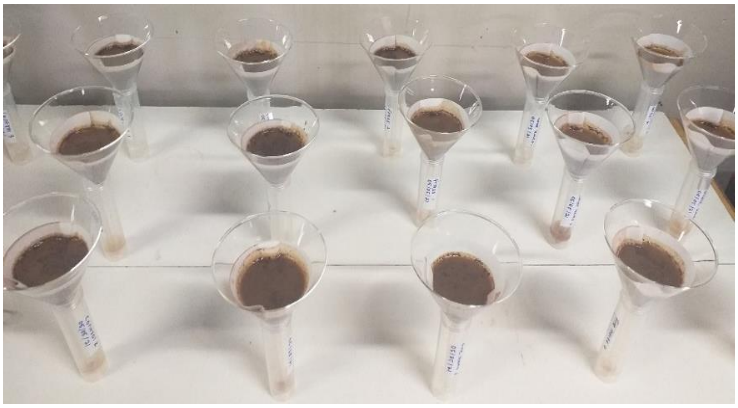


Figure 3. 7. Soil filtration

3.2.5. Total N and Cumulative N estimation

Total inorganic N was calculated by adding together the values of NH_4^+ and NO_3^- in each sampled day.

The cumulative NH_4^+ was calculated by adding together the NH_4^+ value of the present day (day 60) and NH_4^+ value of the previous day (day 40) and this was also done for the cumulative NO_3^-. The total cumulative N was calculated by adding together the cumulative NH_4^+ and NO_3^- for each sampled day.

3.2.6. Statistical analysis

The effect of treatment factors on the amounts of NH_4^+ and NO_3^- were tested using a one-way analysis of variance test (ANOVA). We ran an ANOVA for each day and used the adjusted Tukey's method for post hoc testing using "glht" function from the "multcomp" package (Hothorn et al., 2008). All the statistical analyses were done using R software version 3.6.0 (R Development Core Team., 2019).

3.3. Results

3.3.1. Chemical composition of soil, leaves and frass

Chemical analysis of the soil, leaves and frass (Table 3.2) revealed that the soil had low C content (0.43% soil organic C) compared to frass (54.72% C) and leaves (54.46 % C). Nitrogen content was highest in soil (18.10 %) compared to leaves (1.82 %) and frass (1.38%; Table 3.2) having the lowest. Chemical composition of Ca, Mg, K, Na, Mn, Cu, Fe for both soil, leaves and frass are listed in Table 3.2. The C:N ratio for both leaves and frass is also shown in Table 3.2.

Table 3. 2. Chemical analysis of soil, leaves and frass

Element	**Unit**	**Content Soil**	**Content Leaves**	**Content Frass**
C	%	0.43 (Organic C)	54.46	54.72
N	%	18.10 (Total N)	1.82	1.38
Ca	mg/kg	945	19 400	13 100
Mg	mg/kg	92	1 400	2 300
K	mg/kg	149	8 100	9 100
P	mg/kg	7	1 400	1500
Na	mg/kg	23	100	99
Fe	mg/kg	22.99	65	124
Mn	mg/kg	68.73	51	28
Cu	mg/kg	1.44	8	6
Zn	mg/kg	0.57	30	13
B	mg/kg	0.77	112	71
C:N ratio		0.02	29.92	39.65

Table 3. 3. Concentrations of daily production of ammonium (NH_4^+) from 1(g) of mopane caterpillar frass, whole mopane leaves and fine mopane leaves as a function of time.

	Day	Control	Whole leaves	Fine leaves	Frass	P-value
NH_4^+	0	3.35 ± 0.22a	3.35 ± 0.22 a	3.35 ± 0.22 a	3.35 ± 0.22 a	ns
	1	2.90 ±0.39a	2.37 ± 0.61 a	3.22 ± 1.23 a	30.95 ± 0.15 b	***
	3	3.60 ±0.49a	3.07± 0.21 a	1.52 ± 0.6 a	29.96 ± 5.9 b	***
	7	3.84 ±0.47a	1.51 ± 0.54 a	2.21 ± 0.48 a	11.43 ± 3.36 b	***
	14	4.38 ±1.0 a	4.12 ± 1.26 a	4.13 ± 1.31 a	4.15 ± 1.48 a	ns
	21	5.18 ±0.39 a	4.54 ± 0.98 b	2.47 ± 1.88 b	2.29 ± 0.59 b	**
	40	3.51 ±0.32 a	4.01 ± 0.83 a	3.64 ± 0.37 a	5.32 ± 2.07 a	ns
	60	2.94 ±0.51a	6.40 ± 0.23 b	4.26 ± 1.09a	4.29 ± 0.42a	***

***p<0.001, **p<0.01, *p<0.05, ns=not significant at p>= 0.05. Within the same row and per parameter, means (±standard error) with the same letters are not significantly different.

Table 3. 4. Concentrations of daily production of nitrate (NO_3^-) from 1(g) of mopane caterpillar frass, whole mopane leaves and fine mopane leaves as a function of time.

	Day	Control	Whole leaves	Fine leaves	Frass	P-value
NO_3^-	0	14.69 ± 0.49	14.70 ± 0.57	14.70 ± 0.57	14.70 ± 0.57	ns
	1	15.05 ± 0.24	14.80 ± 1.43 a	2.23 ± 4.46 b	0 ± 0 b	***
	3	15.30 ± 0.57	13.62 ± 1.62 a	0.50 ± 0.75 b	0 ± 0 b	***
	7	13.77 ± 0.42	2.99 ± 1.42	0.09 ± 0.10	4.27 ± 4.99	ns
	14	14.90 ± 0.92	4.01 ± 1.70 a	0.17 ± 0.34 b	0.55 ± 0.59 b	**
	21	22.37 (±0.43)	5.99 ± 2.00 a	0.12 ± 0.10 b	10.12 ± 8.15 a	*
	40	24.09 (±0.40)	1.16 ± 0.96 a	0 ± 0 a	4.09 ± 4.91 a	ns
	60	31.12 (±1.82)	1.94 ± 0.78 a	2.36 ± 1.30 a	5.40 ± 7.35 a	ns

***p<0.001, **p<0.01, *p<0.05, ns=not significant at p>= 0.05. Within the same row and per parameter, means (±standard error) with the same letters are not significantly different

3.3.2. Ammonium

NH_4^+-N concentration (Fig. 3.8) in the frass treatment drastically increased immediately after frass application and remained high during day 1 and 3, but thereafter the daily NH_4^+ production decreased, until similar to that of the other treatments. In terms of significance, only frass had significantly higher NH_4^+ values compared to control on days 1 & 3 (see Table 3.3 for significant values), whole leaves on day 1 and 3 (Table 3.3) and fine leaves on day 1 and 3 (Table 3.3). There was no significant differences between whole leaves, fine leaves or control treatments for the entire treatment period (Table 3.3), and these remained low at daily NH_4^+ production of between 1.5 mg/kg and 4.5 mg/kg. The standard deviation for frass was also higher than for the leaf treatments and control (Table 3.3). It decreased during day 14 and from there fluctuated with the whole leaves treatment and control, i.e., as NH_4^+-N concentration in frass decreased (days 14-33), the NH_4^+-N concentration in whole leaves and control increased and vice versa, whilst it stayed fairly low in the fine leaves treatment. However, in the latter days of the trial, NH_4^+-N concentration in all treatments was considerably low, i.e., below 7 mg/kg. There was a significant increase in NH_4^+ concentration on day 60 in the whole leaves treatment, with a total of 6.40 mg/kg (Table 3.3). However, as the experiment stopped on day 60, it cannot be determined if this was the start of an increase, or just normal variation.

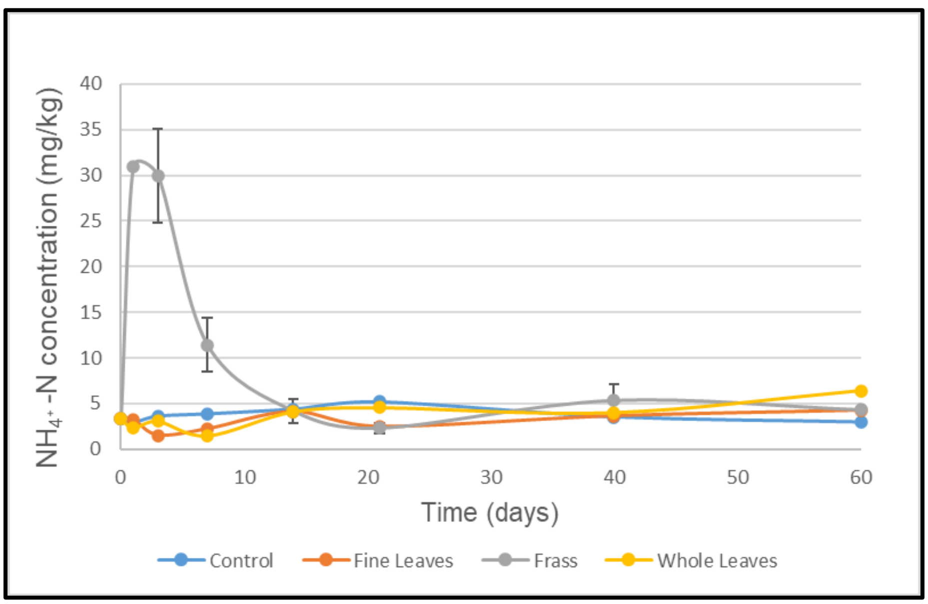


Figure 3. 8. Daily NH_4^+ concentration on selected incubation days for control, whole leaves, fine leaves and frass

3.3.3. Nitrate

NO_3^- -N concentration varied within treatment and across time (Fig. 3.9; Table 3.4). The control was notably high and increasing, contrasting to the results we expected. Control values of day 21, 40 and 60 were possibly contaminated (unclean/broken samples), and therefore discarded. However, it has been noted that this could have been to microbes active in the soil, thus high NO_3^- -N concentration values, however this cannot be verified as samples analysis were outsourced. Frass had significantly lower NO_3^- values compared to the control on days 1 & 3 (Table 3.4), whole leaves on day 1 and 3 (Table 3.4) and fine leaves on day 1 and 3 (Table 3.4). There was no significant differences between whole leaves, fine leaves or control during treatments days 7, 40 and 60 (Table 3.4). Frass and fine leaves treatments experienced a reduction in daily NO_3^- production directly after treatment application (Fig. 3.9; Table 3.4). The whole leaves had a slightly high response but also had a reduced NO_3^- concentration after day 3 (Fig. 3.9). While the concentrations in all the treatments varied, it remained relatively low. Towards the end of the trial, between Day 40 and 60, an increase in NO_3^- values were observed in all treatments, especially in the frass treatment, although not significant (Table 3.4). Unfortunately, the experiment ended on Day 60, and it cannot be established if this was the start of a new trend, or just normal variation.

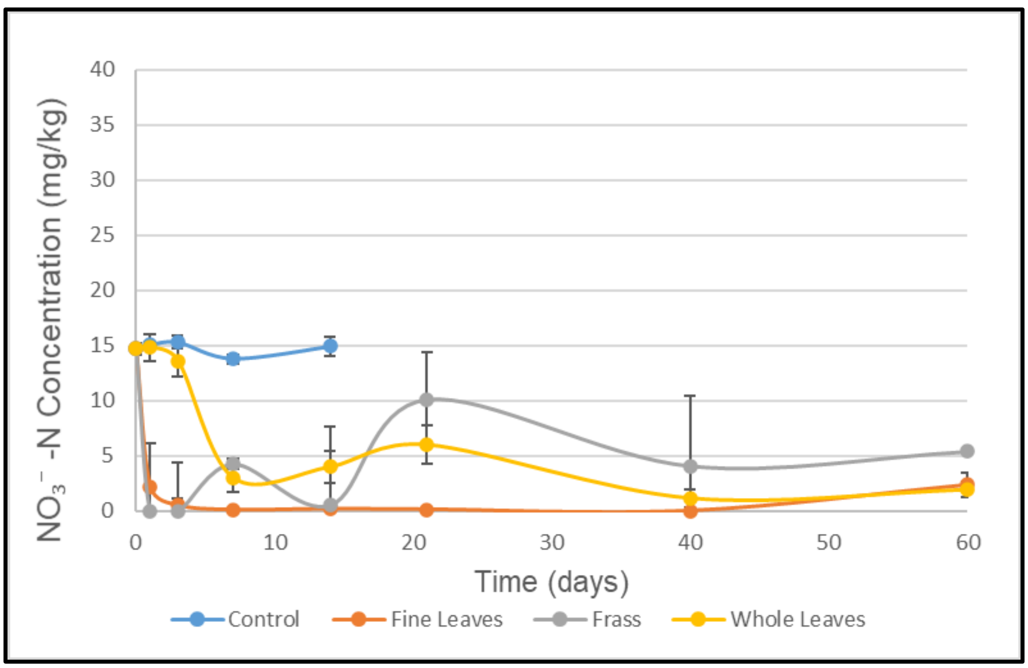


Figure 3. 9. Daily NO_3^- concentration on selected incubation days for control, whole leaves, fine leaves and frass

3.3.4. Total inorganic N release

Total inorganic N release over time was considerably higher in control than the three treatments, from day 7 onwards (Fig. 3.10). Inorganic N release was higher in frass during the first seven days, which is driven by the high NH_4^+ release in the first week but decreased below control. Standard deviation in frass treatment was higher than leaves treatments and control. There was a considerable decrease in inorganic N release in frass treatment in day 14 to below control and whole leaves and approximately equal to fine leaves. The total N released from the treatments with exception of control was higher in the first ten days but was lower than control for the rest of trial days (Fig. 3.10 below).

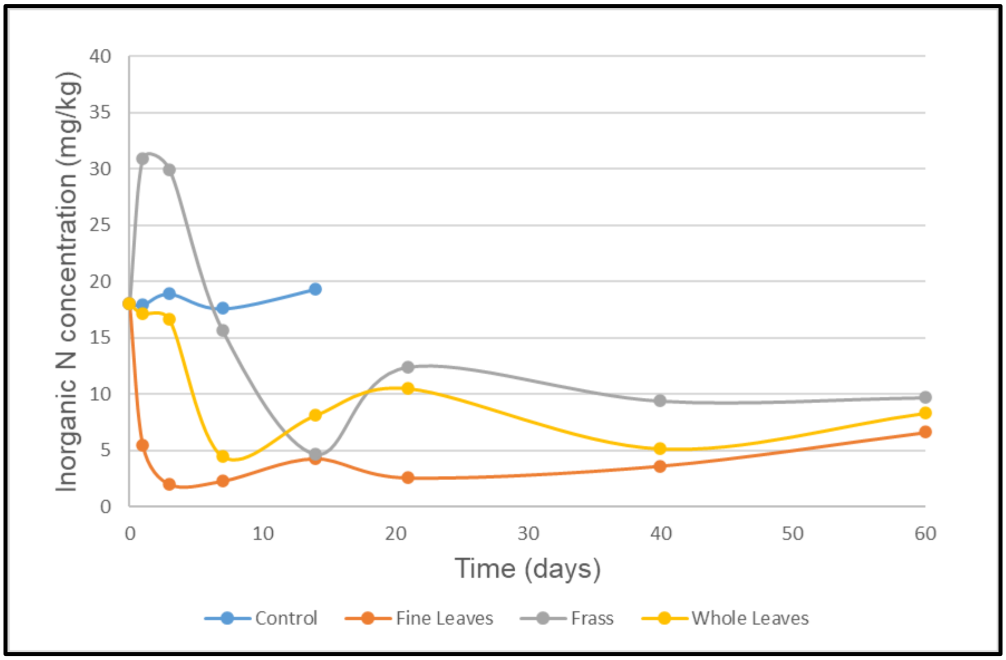


Figure 3. 10. Total daily inorganic N (NH_4^+ + NO_3^-) production

3.3.5. Cumulative inorganic N release

For the cumulative inorganic N release, we first expressed the cumulative release of NH_4^+ (Fig. 3.10), then that of NO_3^- (Fig. 3.11) and lastly the combined inorganic N release (NH_4^+ fraction + NO_3^- fraction) (Fig. 3.12). For these graphs, we removed the control sample, as the control produce relatively high inorganic values, which is not supposed to accumulate, since there was no N source added to this soil. The original soil also had very low C and N content, and thus the increase in inorganic N in the control is possibly due to contamination or laboratory error. We therefore only focus on the treatments that received organic materials to compare how the inorganic N content changed over time.

The NH_4^+ production in the frass treatment was higher compared to the other treatments. This was due to the high priming response during the first few days after application, however a corresponding increase in NO_3^- increase was not observed. Fine leaves and whole leaves had a gradual increase in NH_4^+ content, indicating a slow and steady release or breakdown of organic matter substrate. NO_3^- content unexpectedly resulted in high concentrations of NO_3^- concentration in the whole leave treatment, followed by frass and fine leaves. Overall, frass produced the most inorganic N (112.8 mg/kg), followed by whole leaves (70.5 mg/kg) and then fine leaves (26.9 mg/kg).

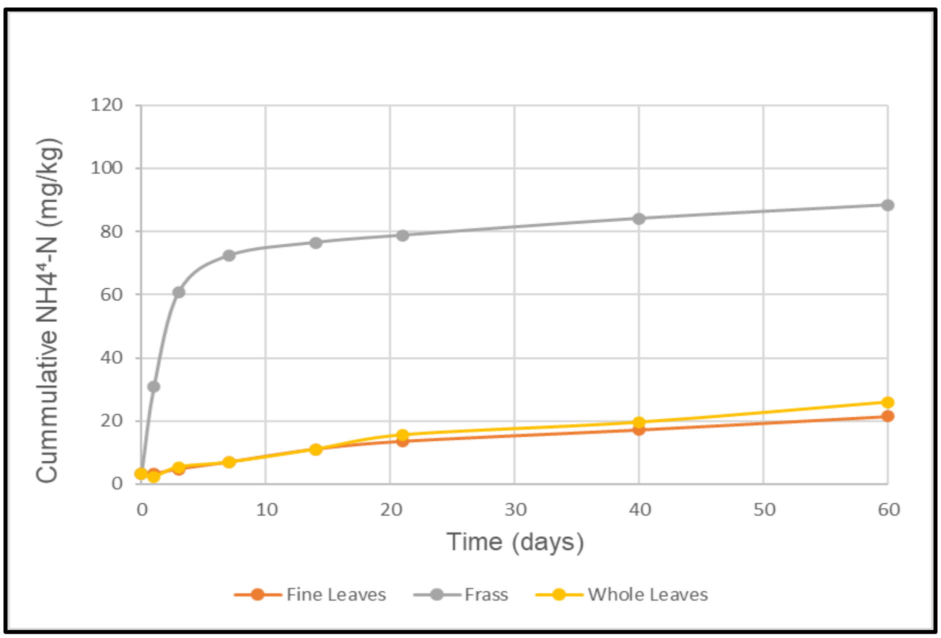


Figure 3. 11. Cumulative NH_4^+ production

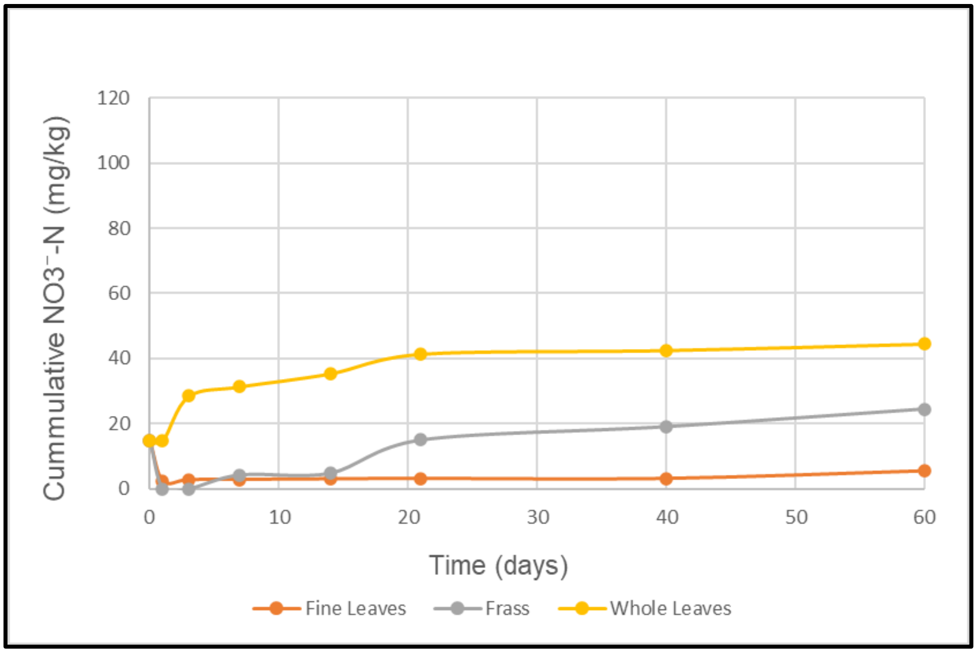


Figure 3. 12. Cumulative NO_3^- production

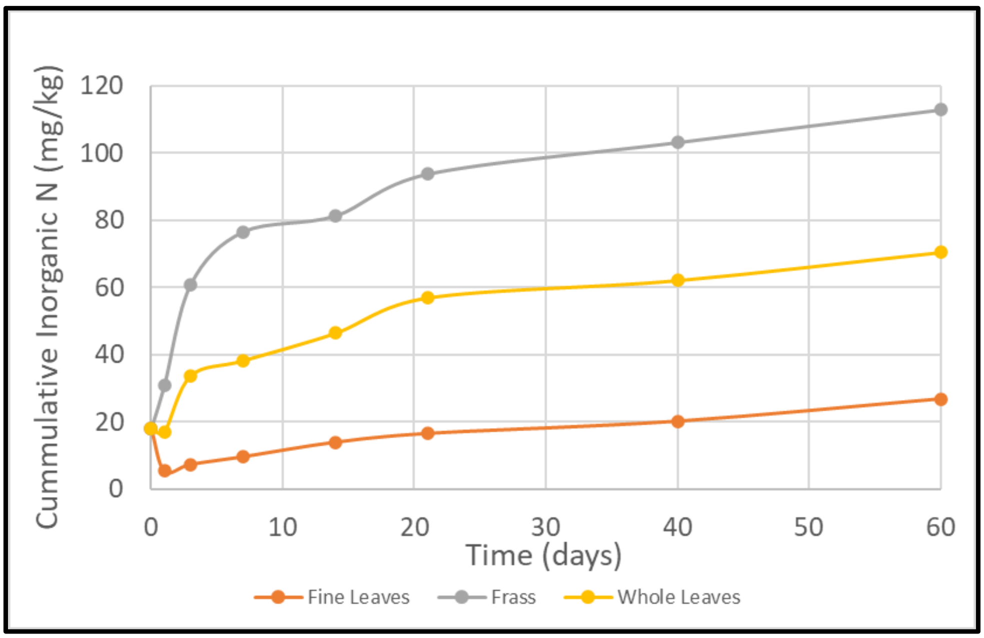


Figure 3. 13. Total cumulative inorganic N (NH_4^+ + NO_3^-) production

3.4. Discussion

3.4.1. Ammonium

Organic components added to the soil are subject to microbial processes that can convert nitrogen from one form to another (Mattson et al., 2009). One of the first nitrogen products resulting from the mineralization process is NH_4^+-N. In our experiment, the NH_4^+ concentration in the frass treatment showed an immediate increase after application. Organic components with easily mineralizable N can release a flush of NH_4^+ immediately after application to the soil (Stevenson, 1986; Drinkwater & Snapp, 2007). According to Lovett and Ruesink (1995) the N in frass is readily extractable; thus the high NH_4^+ release from the frass treatment occurs immediately after application. However, after three days, the daily production decreased. This decrease could have been due to N losses through nitrification or volatilization, which are both possible pathways for NH_4^+ in the soil. As the soil in the incubation trial was kept at field capacity, it is expected that there was enough oxygen to convert NH_4^+ into nitrate. However, microsites in the soil can be anaerobic and gaseous losses through denitrification can also occur (Coyne, 1999; Poffenbarger et al., 2018). There was a significant increase in NH_4^+-N concentration on day 60 in the whole leaves treatment. This could indicate a slow breakdown of organic materials and subsequent release of the first mineralization production, NH_4^+, after 60 days. In a natural system, this could possibly be accounted for by the addition of new organic matter into the system. Poffenbarger et al., (2018) noted that biologically- assimilated N may become plant available after some time when microbes die and release the absorbed nutrients. However, the experiment stopped at day 60, and thus we cannot explain this new activity.

The C:N ratio of the frass was 39.6 and that of the leaves 29.9; both are relatively high and could result in slower mineralization due to the relatively high C to N content (Coyne, 1999). Around days 14-33, NH_4^+-N concentrations in the frass treatment decreased, while it showed an increase in the control and whole leaves treatment. Lovett and Ruesink (1995) also found that frass has extremely labile C, stimulating microbial activity such as fungi, resulting in immobilization of N fractions within the first 10 days of the incubation (Lovett & Ruesink, 1995). Thus, this could have resulted in immobilization after day 14 in this present study. In the latter days of the experiment, NH_4^+-N concentrations for all treatments remained relatively low as most of the easily mineralizable N fractions in the frass and leaves were already consumed (Van Niekerk et al 2004).

3.4.2. Nitrate

NO_3^--N concentration varied within treatments and across time. The NO_3^--N from all the treatments decreased after application. Due to the relatively high C:N ratio in the leaves and frass, N immobilization is expected after application. The high C concentration compared to N in the organic material stimulates microbial activity but cannot provide enough N for microbial needs, thus extracting NO_3^- from the surrounding substrate or soil (Coyne, 1999), resulting in a decrease of NO_3^- in the soil. The NO_3^- concentrations will increase later as microbes go through their life cycle. From day 14, NO_3^--N concentration for frass increased and surpassed whole leaves treatment. NO_3^--N concentration in fine leaves treatment remained low throughout the experiment, taking a slight increase from day 40. NO_3^--N concentration was notably high in control, which was not expected as the control had no N source. After day 14, NO_3^--N concentration in frass and whole leaves treatment increased, corresponding with a decline observed in NH_4^+-N, which could mean nitrification occurred. From day 60, NO_3^--N concentration increased in all treatments, which unfortunately cannot be clearly explained as the experiment ended on day 60. However, this could be corresponding with remineralization (Poffenbarger et al., 2018), which was explained for NH_4^+-N concentration above. In a natural setting, this may be because of added organic matter resulting in a new cycle. Because this experiment was carried at field capacity, it could be that the soil was not enough or there was excess water leading to denitrification, as noted in Poffenbarger et al. (2018) in anaerobic conditions, denitrification could occur, resulting in gaseous N losses through reduction that can reduce NO_3^--N reduction, N_2O or N_2, thereby reducing the NO_3 concentration in the soil.

3.4.3. Total Inorganic N release

Total inorganic N was considerably higher in frass in the first seven days and was mainly driven by the high NH_4^+-N production in the first three days. However, from day 7 all three treatments had less total N than the control. This could be due to the N immobilization due to the high C:N ratio of the organic substrates. When the easily mineralizable N components are consumed by microbes, the total production will reduce (Van Niekerk et al. 2005). Our results followed the same trend with a high initial N release that levels off after the first few weeks to a stable, gradual release.

3.4.4. Cumulative Inorganic N release

The cumulative inorganic N release indicates the total N release from organic treatments over time. The cumulative inorganic NH_4^+- N release was higher in frass than in the leaf treatments, whereas NO_3^- -N cumulative concentration was higher in whole leaves treatment followed by frass and fine leaves. Frost and Hunter (2004) observed that frass deposition from red oak (*Quercus rubra*) saplings led to a relative increase in NH_4^+-N but not NO_3^--N. We could have explained the low NO_3^- -N and NH_4^+-N concentrations to leachate losses in an open system, as NO_3^- can dissolve in water (Frost & Hunter, 2004). Interestingly, whole leaves treatment had considerably lower cumulative NH_4^+-N concentration and a much higher cumulative NO_3^- -N concentration.

Overall, the frass treatment produced the highest inorganic N with 112.8 mg/kg of inorganic N released over 60 days, while the whole leave treatment released 70.5 mg/kg and fine leaves 26.9 mg/kg in the same period. In addition, De Swardt et al. (2018) found that frass of mopane caterpillars contributes to the nutrient cycling of N, K and P.

3.4.5. Surface area

Fine leaves treatment yielded the lowest concentrations for both NH_4^+-N and NO_3^- -N. Considering that the surface area of the fine leaves is closely related to that of frass, we expected that the fine leaves would display faster mineralization than whole leaves. However, fine leaves treatment had overall low N concentrations compared to whole leaves. This can possibly mean that the fraction size of the leaves and frass may not affect decomposition activities but rather the contents of the organic matter. In this study, size did not affect decomposition. Factors that is known to be mainly responsible include climate and microbes, such as bacteria and fungi (Aerts, 1997). However, this could not have affected the experiment as all treatments were under the same climatic conditions. It appeared that the surface area does not influence decomposition, as grinding the leaves seemed to have decreased its reactivity to decomposition. However, the larger surface area of whole leaves could mean more bacterial activity, and immobilization of inorganic N in these soil microbes, which will eventually be available for mineralization when the soil microbes die and thus the subsequent release of inorganic N observed in the later stages of the trial from whole leaves (Brust, 2019).

3.4.6. Mineralisation rate

It appears that frass has a high mineralisation rate as seen by the NH_4^+-N concentration that increased immediately after frass application but remained so low in the leaves treatment. The overall N release was high in frass treatment compared to whole and fine leaves. This supports that frass has easily mineralizable contents (Lovett & Ruesink, 1995; Reynolds & Hunter, 2004), making them important to nutrient cycling in the mopane ecosystem as they seem to have high nutrient release potential.

3.5. Conclusion

From the results of the incubation experiment, it is clear that frass produced by mopane caterpillars has a higher nitrogen turnover than whole leaves and fine leaves. We included a fine leaf treatment to compare the effect of surface area on the mineralization rate. Nevertheless, regardless of the finer fractions of mopane leaves, frass still decomposed faster and released much more N than leaves, indicating the role of mopane caterpillars in nutrient cycling. Not only does mopane caterpillars convert the leaves into small sizes, in the digestion process, the leaves are converted into an organic product that can decompose faster and release nutrients back into the environment at a faster rate and more effectively. This suggests that mopane caterpillars form an integral part of the nutrient cycling process. In a system where mopane caterpillars are absent, leaves would fall to the ground and dry out; mineralization in this system would be very slow. As the mineralization of leaves is very slow, nutrients can be removed from these systems if leaves are removed through burning or are blown away. Nutrient removal in these semi-arid regions is especially problematic, as sparse vegetation due to the low rainfall and high temperatures cannot be replaced easily. However, from day 60 NH_4^+-N concentration in whole leaves treatment started to rise again; thus it could be that although whole leaves released NH_4^+-N slower as compared to frass, they have the potential to release nutrients over a longer time period, thus sustaining nutrient availability in these systems after the complete decomposition of frass. Unfortunately, the NH_4^+-N and NO_3^--N concentrations in control samples were much higher, which was not expected for a sample with no N source. This could have been due to sample contamination.

3.6. References

Aerts, R. 1997. Climate, Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial Ecosystems: A Triangular Relationship. *Oikos,* 79 (3): 439-449. http://www.jstor.com/stable/3546886.

Bell, R. H. V. 1982. The effect of soil nutrient availability on community structure in African ecosystems. In B. J. Huntley, and B. H. Walker (Eds.). *Ecology of Tropical Savannas*, pp. 193–216. Springer, Berlin, Germany.

Belovsky, G.E. & Slade, J.B. 2000. Insect herbivory accelerates nutrient cycling and increases plant production. *PNAS*, 97 (26):14412–14417. www.pnas.org.

Bierman, P.M. & Rosen, C.J. 2005. Nutrient Management for Fruit & Vegetable Crop Production.

Brust, G. E. 2019. Management Strategies for Organic Vegetable Fertility. *Safety and Practice for Organic Food*. https://doi.org/1 0.101 6/ 8978-0-1 2-81 2060-6.00009-x 2019.

Chapman, S. K., Hart, S.C., Cobb, N.S., Whitham, T.G., & Koch. G.W. 2003. Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. *Ecology,* 84: 2867–2876.

Chen, B., Liu, E., Tian, Q., Yan, C. & Zhang, Y. 2014. Soil nitrogen dynamics and crop residues. A review. *Agron. Sustain. Dev*, 34:429–442. DOI 10.1007/s13593-014-0207-8.

Coyne, M. 1999. Soil Microbiology. An exploratory approach. USA: Delmar Publishers.

Crohn, D. 2004. Nitrogen Mineralization and its Importance in Organic Waste Recycling. In Proceedings, National Alfalfa Symposium. San Diego: University of California.

Dangerfield, J.M., McCarthy, T.S. & Ellery, W.N. 1998. The Mound-Building Termites *Macrotermes michaelseni* as an Ecosystem Engineer. *Journal of Tropical Ecology*, 14(4): 507-520.

De Swardt, D. B., Wigley-Coetsee, C. & O'Connor, T.G. 2018. Insect Outbreaks Alter Nutrient Dynamics in a Southern African Savanna: Patchy Defoliation of *Colophospermum Mopane* Savanna by Imbrasia belina Larvae. *Biotropica*, 50 (5): 789–796. 10.1111/btp.12565.

Ditlhogo, M., Allotey, J., Mpuchane, S., Teferra, G., Gashe, B.A. and Siame, B.A. 1996, November. Interactions between the mopane caterpillar, Imbrasia belina, and its host, Colophospermum mopane in Botswana. *Management of Mopane in Southern Africa*. Proceedings of a conference held at Ogongo Agricultural College, Namibia, 26-29.

Drinkwater LE & Snapp SS. 2007. Nutrients in agroecosystems. *Advances in Agronomy*, 92: 163-186.

Lavelle, P., Dugdale, R., et al. 2005. Chapter 12: Nutrient Cycling. In Hassan, R., Scholes, R. & Ash, N (Eds), *Millennium Ecosystem Assessment, Ecosystems and Human Well-being: Current State and Trends*, 1(331-353). Island Press: Washington. https://www.milleniumassessment.org/documents/document.281.aspx.pdf.

Fogal, W. H., and F. Slansky, Jr. 1985. Contribution of feeding by European pine sawfly larvae to litter production and element flux in Scots pine plantations. *Canadian Journal of Forest Research* 15:484–487.

Frost, C.J. & Hunter, M.D. 2004. Insect Canopy Herbivory and Frass Deposition Affect Soil Nutrient Dynamics and Export in Oak Mesocosms. *Ecology*, 85 (12): 3335–3347.

Grace, J. R. 1986. The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. Forest Science 32:855–870.

Grzyb, A., Wolna-Maruwka, A. & Niewiadomska, A. 2020. Environmental Factors Affecting the Mineralization of Crop Residues, Review. *Agronomy*, 10, 1951; 1-18. doi:10.3390/agronomy10121951. www.mdpi.com/journal/agronomy.

Hrabar, H., Hattas, D. & Du Toit, J.T. 2009. Differential Effects of Defoliation by Mopane Caterpillars and Pruning by African Elephants on the Regrowth of *Colophospermum mopane* Foliage. *Journal of Tropical Ecology*, 25:301–309. doi:10.1017/S0266467409005872.

Hrabar, H., & Du Toit. J.T. 2014. Interactions between Megaherbivores and Microherbivores: Elephant Browsing Reduces Host Plant Quality for Caterpillars. *Ecosphere* 5 (1):7. http://dx.doi.org/10.1890/ES13-00173.1

Hollinger, D. Y. 1986. Herbivory and the cycling of nitrogen and phosphorous in isolated California oak trees. *Oecologia*, 70:291–297.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous Inference in General Parametric Models. *Biometrical Journal*, 50(3), 346–363.

Jankielsohn, A. 2018. Insects: the answer to sustainable agriculture. In Coleman, A, Crops: Insects and Biodiversity. *Farmers Weekly*, 44-46.

Johnson, C., Albrecht, G., Ketterings, Q., Beckman, J. & Stockin, K. 2005. Nitrogen Basics – The Nitrogen Cycle. *Agronomy Fact Sheet Series,* Nutrient Management Spear Program. http://nmsp.css.cornell.edu.

Kerley, G.I.H., Landman, M., Kruger L. & Owen-Smith, N. 2008. Effects of Elephants on Ecosystems and Biodiversity. DOI: 10.18772/22008034792.

Knapp, J.S. & Bromley-Challoner, K.C.A. 2003. Recalcitrant organic compounds, In *Handbook of Water and Wastewater Microbiology.*

Kusumah, A. n.d. Soil Nutrient Cycling, Chapter 2.1. https://www1.agric.gov.ab.za.

Lacher, T.E. Jr., et al. 2019. The functional roles of mammals in ecosystems. *Journal of Mammalogy*, 100(3): 942–964. DOI:10.1093/jmammal/gyy183.

Lovett, G.M & Ruesink, A.E. 1995. Carbon and Nitrogen Mineralization from Decomposing Gypsy Moth Frass. *Oecologia*. 104:133- 138

Makhado, R., Potgieter, M., Timberlake, J. & Gumbo, D.A. 2014. Review of the Significance of Mopane Products to Rural People's Livelihoods in Southern Africa. *Transactions of the Royal Society of South Africa*, 69 (2): 117–122. DOI: 10.1080/0035919X.2014.922512. http://dx.doi.org/10.1080/0035919X.2014.922512.

Mapaure, I. 1994. The distribution of mopane. *Kirkia* 15: 1–5.

Mattson N, Leatherwood, R, Peters, C. 2009. Nitrogen: All Forms Are Not Equal. Cornell University.

McNeill, A. & Unkovich, M. 2007. The Nitrogen Cycle in the terrestrial ecosystems. DOI:10.1007/978-3-540-68027-7_2.

Mutakela, P.A. 2009. Biomass Prediction Models for *Colophospermum mopane* (mopane) in Botswana. MSc Thesis, University of Stellenbosch: South Africa, p25.

Poffenbarger et al., 2018, H., Coyne, M.S. & Frye, W.W. 2018. Nitrogen in Soils/ Cycle. Department of Plant and Soil Sciences, University of Kentucky, Lexington.

Pule-Meuelenberg, F. & Dakora, F.D. 2007. Assessing the biological potential of N_2-fixing Leguminosae in Botswana for increased crop yields and commercial exploitation. Review *African Journal of Biotechnology*. 6 (4): 325-334. http://www.academicjournals.org/AJB.

Reynolds, B.C. & Hunter, M. D. 2004. Nutrient Cycling. Chapter 19, 387-396.

Seastedt, T.R. & Crossley, D.A. Jr. 1984. The influence of arthropods on ecosystems. *Bioscience*, 34, 157-161.

Scholtz, C. H. 1976. Biology and ecological energetics of Lepidoptera larvae associated with woody vegetation in a savanna ecosystem. MSc thesis, University of Pretoria, Pretoria.

Schowalter, T.D. & Crossley, D.A. Jr. 1983. Forest canopy arthropods as sodium, potassium, magnesium, and calcium pools in forests. *Forest Ecology and Management*, 7, 143-148.

Schowalter, T.D., Hargrove, W.W. & Crossley, D.A. Jr .1986. Herbivory in forested ecosystems. *Annual Review of Entomology*, 31, 177-196.

Stevenson, F. J. 1986. Cycles of Soil, Carbon, Nitrogen, Phosphorus, Sulphur, Micronutrients. New York, USA: John Wiley and Sons, 106- 164.

Sinclair, A. R. E. 1975. The resource limitation of trophic levels in tropical grassland ecosystems. *J. Anim. Ecol*. 44: 497–520.

Sinclair, A.R.E. 2003. The Role of Mammals as Ecosystem landscapers.

Swank, W. T., J. B. Waide, D. A. Crossley, Jr., and R. L. Todd. 1981. Insect defoliation enhances nitrate export from forest ecosystems. *Oecologia*, 51:297–299.

Styles, C. V. & Skinner, J. D. 1996. Possible Factors Contributing to the Exclusion of Saturniid Caterpillars (mopane worms) from a Protected Area in Botswana. *Afr. J. Ecol*, 34: 276-283.

R Development Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://cran.r-project.org/bin/windows/base/old/3.6.0/

USDA NRCS. 2011.Carbon to Nitrogen Ratios in Cropping Systems. http://www.soils.usda.gov/sqi.

Van Niekerk, C.M., Claassens, A. & Snyman, H. 2005. N transformation in Incubated Sewage Sludge and Commercial Fertilizer Enriched Soil. *Communications in Soil Science and Plant Analysis* 36 (4): 743-757.

Van Voorthuizen, E. 1976. The Mopane tree. Botswana. Notes & Records. 8: 223–230.

Wood, T.G. & Sands, W.A. 1978. The Role of Termites in Ecosystems. *Production Ecology of Ants and Termites*, 245-292.

Wolf, A., Doughty, C.E. & Malhi, Y. 2013. Lateral Diffusion of Nutrients by Mammalian Herbivores in Terrestrial Ecosystems. *PLOS ONE* 8(8):1-10. doi: 10.1371/journal.pone.0071352.

Zimmer, M. & Topp, W. 2002. The Role of Coprophagy in Nutrient Release from Feces of Phytophagous Insects. *Soil Biology & Biochemistry*, 32: 1093-1099.

CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

Mopane caterpillars are a food source for both humans and animals such as mongooses, birds, fish and livestock. They are also a source of income to those involved in their harvesting and trade. They are of considerable social, economic and cultural significance. Mopane caterpillars research has focused more on their importance as food and as an income source, with less concern for their ecological importance, particularly their role in nutrient cycling in the African Savanna (mopane woodland). Mopane caterpillars are one of the main defoliators of mopane trees, thus there is a need to study how they influence the mopane woodland through defoliation and frass release to reveal whether they have an influence on nutrient cycling in these systems.

There was a lack of studies in countries that have been reported to have distribution of mopane caterpillars, thus studies are needed in such countries to bridge the gap in knowledge found. Mopane caterpillars are being harvested unsustainably, and rules governing harvesting are not so effective because they are often overridden. Climate change, land use change, lack of effective laws threatens the sustainability of this resource.

We recommend more research on gap areas found on the study such as ecosystem service in terms of nutrient cycling and on sustainable use. There is need for actual implementation of stricter laws to govern the harvesting process and quantity of caterpillars harvested, and stricter fines for those who override the laws to ensure the sustainability of mopane caterpillars, especially at the local scale where there is excessive harvesting and overriding of laws set by local Chiefs.

Compared to mopane leaves (fine and whole leaves), mopane caterpillars' frass had higher mineralisation rates, because the mineralisation of frass released inorganic N faster than mopane leaves, thus increasing amount of inorganic N released into the ecosystem at a faster rate than in a system where frass would be absent. Overall, the frass treatment produced the highest inorganic N as compared to the other treatments. However, it was worth noting that mopane leaves showed an increase in NH_4^+-N concentration during the end of the trial, whilst it remained low for frass treatment, thus it could be that although leaves release inorganic N at a slower rate, they can sustain the system for a longer time by releasing inorganic N when frass has completely decomposed, thus the co-existence of frass and leaves in the mopane woodland is essential for nutrient availability.

Additionally, the release of inorganic N from whole leaves during the end of the trial could possibly be due to immobilization of the N, which mineralizes when soil microbes eventually die. The high NH_4^+-N and NO_3^--N concentrations in control samples are of interest as it was not expected considering there was no added N source. This consequently led to a questions of why the absence of N source led to high inorganic N and the addition of N source led to lower inorganic N concentrations.

The trial also revealed that the assumption that the fast mineralisation of frass as opposed to leaves could be due to their fraction size could not possibly hold, as fine leaves had notably the lowest inorganic N released, lower than for whole leaves. This could mean that frass have some easily mineralizable content or an added advantage due to digestion in the caterpillars gut.

Mopane caterpillars have shown to have potential in terms of nutrient cycling through high mineralising frass. This study however led to more questions such as how much caterpillars consumes on average in a day and throughout their life span, how much leaf biomass is returned to soil as frass and how much of this is nitrogen. Thus the average leaves consumed by caterpillars, frass released, biomass returned to the ecosystem by mopane caterpillars would be important topics for future research.

Appendix 1: Publications used in systematic review

Adelabu, S., Mutanga, O. & Cho, M.A. 2012. A Review of Remote Sensing of Insect Defoliation and its Implications for the Detection and Mapping of Imbrasia belina Defoliation of Mopane Woodland. *The African Journal of Plant Science and Biotechnology*, 6(1): 1-13.

Akpalu, W., Muchapondwa, E. & Zikhali, P. 2007. Can the Restrictive Harvest Period Policy Conserve Mopane Worms in Southern Africa? A bio-economic modelling approach. *Working papers*, no 65.

Amadi, E.N. & Kiin-Kabari, D.B. 2016. Nutritional Composition and Microbiology of Some Edible Insects Commonly Eaten in Africa, Hurdles and Future Prospects: A critical review. *Journal of Food: Microbiology, Safety & Hygiene*, 1(1):1-7. doi:10.4172/2476-2059.1000107.

Baiyegunhi, L.J.S. & Oppong, B.B. 2016. Commercialisation of Mopane Worm (*Imbrasia belina*) in Rural Households in Limpopo Province, South Africa. Forest Policy and Economics, 62:141-148. http://dx.doi.org/10.1016/j.forpol.2015.08.012.

Baiyegunhi, L.J.S., Oppong, B.B. & Senyolo, G.M. 2015. Socio-Economic Factors Influencing Mopane Worm (*Imbrasia belina*) Harvesting in Limpopo Province, South Africa. J. For. Res. DOI 10.1007/s11676-015-0168-z.

Baiyegunhi, L.J.S., Oppong., B.B. & Senyolo, G.M. 2016. Mopane Worm (*Imbrasia belina*) and Rural Household Food Security in Limpopo province, South Africa. Food Security, 8:153–165. DOI:10.1007/s12571-015-0536-8.

Chagwena, D.T., Matanhire, G.T., Jombo, T.Z. & Maponga, C.C. 2019. Protein Quality of Commonly Consumed Edible Insects in Zimbabwe. *African Journal of Food, Agriculture, Nutrition and Development*, 19(3): 14674-14689. DOI: 10.18697/ajfand.85.17645.

Chiripasi, S.C., Moreki, J.C., Nsoso, S.J. & Letso, M. 2013. Effect of Feeding Mopane Worm Meal on Mineral Intake, Retention and Utilization in Guinea Fowl under Intensive System. *International Journal of Poultry Science*, 12 (1): 19-28.

Cmelik, S.H.W. 1969. Carotenoids from the Haemolymph of the Mopane Moth Caterpillars *Gonimbrasia belina*. *Experientia*, 25(11).

Cmelik, S.H.W. 1970. Sterols in the Diet and Various Organs of the Caterpillars of the Mopane Moth *Gonimbrasia belina*.351: 360-372.

Dangwa. N., Mwenje, E., Dhlamini, Z. & Siwela. A.H. 2014. Molecular Characterization of Aflatoxigenic *Aspergillus* species in dried Traditional foods in Zimbabwe. Advances in Bioresearch, 5 (1): 29-36. http://www.soeagra.com/abr.html.

De Swardt, D. B., Wigley-Coetsee, C. & O'Connor, T.G. 2018. Insect Outbreaks Alter Nutrient Dynamics in a Southern African Savanna: Patchy Defoliation of Colophospermum Mopane Savanna by *Imbrasia belin*a Larvae. *Biotropica*, 50(5): 789–796.

Ditlhogo, M., Allotey, J., Mpuchane, S., Teferra, G., Gashe, B.A. and Siame, B.A. 1996, November. Interactions between the mopane caterpillar, *Imbrasia belina*, and its host, Colophospermum mopane in Botswana. Management of Mopane in Southern Africa. Proceedings of a conference held at Ogongo Agricultural College, Namibia, 26-29.

Douglas, R.M. 1991. Mopane Worms in Bloemfontein? *Culna*, 41:20.

Dube, S., Dlamini, N.R., Mafunga, A., Mukai, M. & Dhlamini, Z. 2013. A Survey on Entomophagy Prevalence in Zimbabwe. *African Journal of Food, Agriculture, Nutrition and Development*, 13(1): 7242-7253.

Dube, S & Dube, C. 2010. Towards improved utilization of macimbi *Imbrasia belina* Linnaeus, 1758 as food and financial resource for people in the Gwanda district of Zimbabwe. *ZJST*, 5: 28-36.

Duffy, K.J., O'Connor, T.G. & Collins, O.C. 2018. A Lepidopteran (*Imbrasia belina*) might Influence Tree-Grass Balance of Colophospermum mopane Savanna. Theoretical Ecology. https://doi.org/10.1007/s12080-018-0379-y.

Ekosse, G.E., Van den Heever, D.J., De Jager, L. & Totolo, O. 2005. Heavy Metals Concentrations in *Imbrasia belina* (Phane Caterpillar) around the Selebi Phikwe Ni-Cu Mine and Smelter/Concentrator Plant, Botswana. *Global Journal of Pure and Applied Sciences*, 11(4): 553-559.

Ekpo, K.E., Azeke, M.A. & Ugbenyen, A.M. 2008. Functional Properties of Dried Imbrasia Belina Larvae Flour as Affected by Mesh Size and pH. *Nigerian Annals of Natural Sciences*, 8(1): 6-9. www.nansjournal.com.

Frears, S.L., Chown, S.L. & Webb, P.I. 1997. Behavioural Thermoregulation in the Mopane Worm (Lepidoptera). *Journal of Thermal Biology*, 22(4/5): 325-330.

Frears, S.L., Chown, S.L. & Webb, P.I. 1999. Temperature Dependence of Feeding Behaviour in the Mopane Worm (Lepidoptera). *Journal of Thermal Biology*, 24:241-244.

Gabaza, M., Shumoy, H., Muchuweti, M., Vandamme, P. & Raes, K. 2018. Baobab Fruit Pulp and Mopane Worm as Potential Functional Ingredients to Improve the Iron and Zinc Content and Bioaccessibility of Fermented Cereals. *Innovative Food Science and Emerging Technologies*, doi: 10.1016/j.ifset.2018.04.005.

Gashe, B.A., Mpuchane, S.F., Siame, B.A., Allotey, J. & Teferra, G. 1997. The Microbiology of Phane, an Edible Caterpillar of the Emperor Moth, Imbrasia belina. *Journal of Food Protection* (11):1376-1380.

Gaston, K.J., Chown, S.L. & Styles, C.V. 1997. Changing Size and Changing Enemies: The case of the mopane worm. *Acta Ogcologica*, 18 (1):21-26.

Glew, R.H., Jackson, D., Sena, L., VanderJagt, D.J., Pastuszyn, A. & Millson, M. 1999. *Gonimbrasia belina* (Lepidoptera: Saturniidae): A nutritional food source rich in protein, fatty acids, and minerals. *American Entomologist*, 45(4): 250- 253.

Gondo, T., Frost, P., Kozanayi, W., Stack, J. & Mushongahande, M. 2010. Linking Knowledge and Practice: Assessing Options for Sustainable Use of Mopane Worms (*Imbrasia Belina*) In Southern Zimbabwe. *Journal of Sustainable Development in Africa*, 12(4): 127-145.

Greenfield, R., Akala, N. & Van der Bank, F.H. 2014. Heavy Metal Concentrations in Two Populations of Mopane Worms (*Imbrasia belina*) in the Kruger National Park Pose a Potential Human Health Risk. *Bull. Environ. Contam. Toxicol*., 93:316–321. DOI:10.1007/s00128-014-1324-4.

Greyling, M., van der Bank, F.H., Grobler, J.P. & Wessels, D.C.J. 2001. Allozyme Variation in Two Populations of the Mopane Worm, *Imbrasia belina* (Saturniidae), and the Effect of

Developmental Stage and Staggered Generations. *South African Journal of Animal Science*, 31(1): 15-24. http://www.sasas.co.za/Sajas.html.

Hope, R.A., Frost, P.G.H., Gardiner, A. & Ghazoul, J. 2009. Experimental Analysis of Adoption of Domestic Mopane Worm Farming Technology in Zimbabwe. *Development Southern Africa*, 26(1):29-46, DOI: 10.1080/03768350802640065.

Hrabar, H., Hattas, D. & Du Toit, J.T. 2009 (a). Differential Effects of Defoliation by Mopane Caterpillars and Pruning by African elephants on the regrowth of Colophospermum mopane Foliage. *Journal of Tropical Ecology*, 25:301–309. doi:10.1017/S0266467409005872.

Hrabar, H., Hattas, D. & Du Toit, J.T. 2009 (b). Intraspecific Host Preferences of Mopane Moths (*Imbrasia belina*) in Mopane (*Colophospermum mopane*) Woodland. *African Zoology*, 44(2):131–140.

Hrabar, H. & Du Toit, J.T. 2014. Interactions between Megaherbivores and Microherbivores: Elephant Browsing Reduces Host Plant Quality for Caterpillars. *Ecosphere*, 5(1): 1-6. www.esajournals.org.

Illgner, P & Nel, E. 2000. The Geography of Edible Insects in Sub-Saharan Africa: A Study of the Mopane Caterpillar. *The Geographical Journal*, 166(4):336-351. The Royal Geographical Society: Grahamstown. https://www.jstor.org/stable/823035.

Jennings, G., Mc Geoch, M.A., Rebe, M., Jackson, S., Roberts, J., Styles, C. & Scholtz, C.H. 2001. The Nutritive Value of *Imbrasia belina* Caterpillars and their Role in the Diet of Human Consumers in South Africa. *Entomological Society of Southern Africa*.

Kachapulula, P.W., Akello, J., Bandyopadhyay, Y. R. & Cotty, P.J.2018. Aflatoxin Contamination of Dried Insects and Fish in Zambia. *Journal of Food Protection*, 81(9):1508-1518. doi: 10.4315/0362-028X.JFP-17-527.

Kamwi, J.M., Endjala, J. & Siyambango, N. 2020. Dependency of Rural Communities on Non-timber Forest Products in the Dry Lands of Southern Africa: A case of Mukwe Constituency, Kavango East Region, Namibia. *Trees, Forests and People*, 2. https://doi.org/10.1016/j.tfp.2020.100022.

Kozanayi, W. & Frost, P. 2002. Marketing of Mopane Worm in Southern Zimbabwe. Mopane Worm Market Survey: Southern Zimbabwe.

Kung, S., Fenemore, B. & Potter, P.C. 2011. Anaphylaxis to Mopane Worm (*Imbrasia Belina*). *Annals of Allergy, Asthma & Immunology*, 106: 538-540.

Kung, S., Mazhani, L. & Steenhoff, A.P. 2013. Allergy in Botswana. *Current Allergy & Clinical Immunology*, 26(4): 202-209.

Kwiri, R., Winini, C., Muredzi, P., Tongonya, J., Gwala, W., Mujuru, F. & Gwala, S.T. 2014. Mopane Worm (*Gonimbrasia belina*) Utilisation, a Potential Source of Protein in Fortified Blended Foods in Zimbabwe: A Review. *Global Journal of Science Frontier Research: D Agriculture and Veterinary*,14(10): 55-67.

Langley, J., Van der Westhuizen, S., Morland, G., & van Asch, B. 2019. Mitochondrial Genomes and Polymorphic Regions of Gonimbrasia belina and Gynanisa maja (Lepidoptera: Saturniidae), Two Important Edible caterpillars of Southern Africa. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.12.055.

Madibela, O.R., Mokwena, K.K., Nsoso, S.J. & Thema, T.F. 2009. Chemical Composition of Mopane Worm Sampled at Three Sites in Botswana and Subjected to Different Processing. *Trop Anim Health Prod*, 41:935–942. DOI 10.1007/s11250-008-9282-7.

Madibela, O.R., Seitiso, T K., Thema, T.F. & Letso, M. 2007. Effects of Traditional Processing Methods on Chemical Composition and In Vitro True Dry Matter Digestibility of Mophane Worm (*Imbrasia belina*). *Journal of Arid Environments*, 68:492-500. Doi: 10.1016/j.jaridenv.2006.06.002.

Makhado, R.A, Potgieter, M.J, Wessels, D.C.J, Saidi, A.T, & Masehela, K.K. 2012. Use of mopane woodland resources and associated woodland management challenges in rural areas of South Africa. *Ethnobotany Research & Applications*, 10:369-379. www.ethnobotanyjournal.org/vol10/i1547-3465-10-369.

Makhado, R., Potgieter, M., Timberlake, J. & Gumbo, D.A. 2014. Review of the Significance of Mopane Products to Rural People's Livelihoods in Southern Africa. *Transactions of the*

Royal Society of South Africa, 69(2): 117–122. DOI: 10.1080/0035919X.2014.922512. http://dx.doi.org/10.1080/0035919X.2014.922512.

Manyeula, F., Tsopito, C. M., Kamau, J. M., Mogotsi, K. K., Nsoso, S. J. & Moreki, J. C. 2018. Effect of *Imbrasia belina* (Westwood) or *Vigna subterranea* (L) Verde or *Tylosema esculentum* (Burchell) Schreiber as Protein Sources in Diets Fed to Tswana Hens on Egg Quality. *Journal of Animal Science and Veterinary Medicine*, 3(6):190-196. https://doi.org/10.31248/JASVM2018.118.

Manyeula, F., Tsopito, C.M., Mogotsi, K., Nsoso S.J., Kamau, J.M. & Moreki, J.C. 2019. Chemical Composition and Sensory Evaluation of Meat of Tswana Hens Fed Different Protein Sources: Mopane Worm, Bambara Groundnut and Morama Bean. *Online Journal of Animal and Feed Research*, 9(2): 92-102. www.ojafr.ir.

Marareni, M. & Mnisi, C.M. 2020. Growth Performance, Serum Biochemistry and Meat Quality Traits of Jumbo Quails Fed with Mopane Worm (*Imbrasia belina*) Meal-Containing Diets. *Veterinary and Animal Science*, 10:1-6. https://doi.org/10.1016/j.vas.2020.100141.

Mataboge, M.S, Mulaudzi, F.M., Wenhold, F.A.M., Nkunika, P.O.Y., Dlamini, N.R. & Alders, R.G. 2016. Effects of Climate Variability on the Harvesting and Preservation of Mopane Worms. *Indilinga - African Journal of Indigenous Knowledge Systems*, 15 (2): 34-48.

Mbata, K.J. Chidumayo, K.J. 2003. Traditional Values of Caterpillars (Insecta: Lepidoptera) among the Bisa People of Zambia. Insect Sci. Applic., 23 (4): 341-354.

Mbhenyane, X.G., Venter, C.S., Vorster, H.H. & Steyn, H.S. 2005. Nutrient Intake and Consumption of Indigenous Foods Among College Students in Limpopo Province. *SAJCN*, 18(1):32-38.

Mogwase, S.S., Moreki, J.C. & Nsoso, S.J. 2018. Analysis of Drumsticks and Offal of Guinea Fowl (*Numida Meleagris*) fed Mophane Worm (*Imbrasia Belina*) as Protein Source and Raised Up to 13 Weeks Under Intensive Management System. *International Journal of Innovative Science and Research Technology*, 3(4): 741-746. www.ijisrt.com.

Motshegwe, S.M., Holmbackb, J. & Yeboah, S.O. 1998. General Properties and the Fatty Acid Composition of the Oil from the Mophane Caterpillar, *Imbrasia belina*. *JAOCS*, 75(6): 725-728.

Moyo, S., Masika, P.J. &. Muchenje, V. 2019. The potential of *Imbrasia belina* worm as a poultry and fish feed. A review. *Journal of Animal and Feed Sciences*, 28: 209–219 https://doi.org/10.22358/jafs/112156/2019.

Mpuchane, S., Gashe, B.A., Allotey, J., Siame, B., Teferra, G. & Ditlhogo, M. 2000. Quality deterioration of phane, the edible caterpillar of an emperor moth *Imbrasia belina*. *Food Control*, 11:453-458.

Mpuchane, S., Taligoola, H.K. & Gashe, B.A. 1996. Fungi Associated with *Imbrasia belina*, an Edible Caterpillar. *Botswana Notes and Records*, 28: 193-197. http://www.jstor.com/stable/40980141.

Mufandaedza, E., Moyo, D.Z. & Makoni, P. 2014. Management of Non-timber Forest Products Harvesting: Rules and regulations governing (*Imbrasia belina*) access in South-Eastern in Lowveld Zimbabwe. *African Journal of Agricultural Research*, 10(12):1521-1530. Doi:10.5897/AJAR2013.7720. http://www.academicjournals.org/AJAR.

Mufandaedza, E., Moyo, D.Z. & Makoni, P. 2015. Models of Valuation of Non-Timber Forest Products: Study of *Imbrasia belina* dynamics in the South-East Lowveld, Zimbabwe. *Agricultural Journal*, 9(2): 132-147.

Mufandaedza, E., Moyo, D.Z., Makoni, P. 2018. Effect of Soil Type and Foliar Factors on the Distribution of *Imbrasia belina* in the Southeastern Lowveld of Zimbabwe. Scientifica, 1-8. https://doi.org/10.1155/2018/9273184.

Mughogho, D.E.C. & Munthali, S.M. 1993. Ecological Interaction between *Gonimbrasia belina* (L.) and *Gynanisa maia* (L.), and the Impact of Fire on Forage Preferences in a Savanna Ecosystem. *Afr. J. Ecol*., 33: 84-87.

Mujuru, F. M., Kwiri, R., Nyambi, C., Winini, C. & Moyo, N.D. 2014. Microbiological quality of Gonimbrasia belina processed under different traditional practices in Gwanda, Zimbabwe.

International Journal of Current Microbiology and Applied Sciences, 3(9): 1085-1094. https://www.ijcmas.com.

Murefu, T.R., Macheka, L., Musundire, R. & Manditsera, F.A. 2019. Safety of Wild Harvested and Reared Edible insects: A review. *Food Control*,101: 209-224. https://doi.org/10.1016/j.foodcont.2019.03.003.

Mushaphi, L.F., Dannhauser, A., Walsh, C.M., Mbhenyane, X.G. & van Rooyen, F.C. 2017. The Impact of a Nutrition Education Programme on Feeding Practices of Caregivers with Children Aged 3 to 5 Years in Rural Limpopo Province, South Africa. *South African Journal of Clinical Nutrition*, 30(4):101-108. DOI: 10.1080/16070658.2017.1322823.

Musundire, R., Zvidzai, C.J., Chidewe, C., Samende, B.K. & Chemura, A. 2016. Habitats and Nutritional Composition of Selected Edible Insects in Zimbabwe. *Journal of Insects as Food and Feed*, 2(3): 189-198. DOI: 10.3920/JIFF2015.0083.

Mwimanzi, L. & Musuka, C.G. 2014. The Potential of Mopane Worm (*Gonimbrasia belina*) as an Alternative Protein Source in Fish Feed. *International Journal of Aquaculture*, 4(12):73-78. http://ija.sophiapublisher.com.

Nantanga, K.K.M. & Amakali, T. 2020. Diversification of Mopane Caterpillars (*Gonimbrasia belina*) Edible Forms for Improved Livelihoods and Food Security. *Journal of Arid Environments*, 177:1-5.

Nawases, B., Uzabakiriho, J. & Chimwamurombe, P. 2018. Identification of Fungi Associated with Processed-Food Contamination at Open Markets of Windhoek, Namibia. *Journal of Pure and Applied Microbiology*, 12(3): 1489-1494. http://dx.doi.org/10.22207/JPaM.12.3.53.

Ndeinoma, A. & Wiersum, K.F. 2017. Diversity of governance arrangements for indigenous natural products in communal areas of Namibia. *Forests, Trees and Livelihoods*, 26(2):124-141, DOI: 10.1080/14728028.2016.1268545.

Ndlovu, I., Nunu, W.N., Mudonhi, N., Dube, O. & Maviza, A. 2019. Land Use−Land Cover Changes and Mopane Worm Harvest in Mangwe District in Plumtree, Zimbabwe. Environmental Systems Research, 8(11): 1-9. https://doi.org/10.1186/s40068-019-0141-5.

Nobo, G., Moreki, J.C. & Nsoso, S.J. 2012(a). Growth and Carcass Characteristics of Helmeted Guinea Fowl (Numida meleagris) fed Varying Levels of Phane Meal (*Imbrasia belina*) as Replacement of Fishmeal Under Intensive System. *IJAVMS*, 6(4): 296-306. doi:10.5455/ijavms.164.

Nobo, G., Moreki, J.C. & Nsoso, S.J. 2012(b). Feed Intake, Body Weight, Average Daily Gain, Feed Conversion Ratio and Carcass Characteristics of Helmeted Guinea Fowl Fed Varying Levels of Phane Meal (*Imbrasia belina*) as Replacement of Fishmeal under Intensive System. *International Journal of Poultry Science*, 11 (6): 378-384.

Nunu, N.W., Ncube, B., Dube, O., Mpofu, C., Ndlovu, B., Dzinomva, T. & Khumalo, N. 2019. Nutritional Factors Associated with Distribution of Mopane Worms in Mopane Woodlands in Tsholotsho and Gwanda Districts, Zimbabwe: A comparative survey. *Scientific Reports*, 9(17320):1-7. https://doi.org/10.1038/s41598-019-53923-7.

Obopile, M. & Seeletso, T.G. 2013. Eat or Not Eat: An analysis of the status of entomophagy in Botswana. *Food Sec.*, 5:817–824. DOI 10.1007/s12571-013-0310-8.

Okezie, O. A., Kgomotso, K.K. & Letswiti, M.M. 2010. Mopane Worm Allergy in a 36-Year-Old Woman: A case report. *Journal of Medical Case Reports*, 4(42): 1-4. http://www.jmedicalcasereports.com/content/4/1/42.

Payne, C.L.R., Umemura, M., Dube, S., Azuma, A., Takenaka, C. & Nonaka, K. 2015. The Mineral Composition of Five Insects as Sold for Human Consumption in Southern Africa. *African Journal of Biotechnology*, 14(31): 2443-2448. DOI: 10.5897/AJB2015.14807.

Pharithi, M.T., Suping, S.M. & Yeboah, S.O. 2004. Variations of the Fatty Acid Composition in the Oil from the Larval Stages of the Emperor Moth Caterpillar, *Imbrasia belina*. *Bull. Chem. Soc. Ethiop*., 18(1):67-72.

Potgieter, M.J. & Ramalivhana, N. 2020. Data on Mopane Worm (*Imbrasia belina*) Microorganisms from Limpopo Province, South Africa. Data in Brief. https://doi.org/10.1016/j.dib.2020.105695.

Potter, P. 2013. An Overview of the Indigenous and Alien Allergens of Southern Africa. *Current Allergy & Clinical Immunology*, 26(4).

Quin, P.J. 1964. Foods and Feeding Habits of the Pedi. *S.A. Medical Journal*, 969-971.

Ramashia, S.E., Tangulani, T., Mashau, M.E. & Nethathe, B. 2020. Microbiological Quality of Different Dried Insects Sold at Thohoyandou Open Market, South Africa. Food Research, 1-9. http://www.myfoodresearch.com.

Rapatsa, M.M. & Moyo, N.A.G. 2017. Evaluation of Imbrasia belina meal as a fishmeal substitute in *Oreochromis mossambicus* diets: Growth performance, histological analysis and enzyme activity. *Aquaculture Reports*, 5: 18–26. http://dx.doi.org/10.1016/j.aqrep.2016.11.004.

Rapatsa¸ M.M. & Moyo, N.A.G. 2019. Enzyme activity and histological analysis of Clarias gariepinus fed on *Imbrasia belina* meal used for partial replacement of fishmeal. *Fish Physiol. Biochem*, 45:1309–1320. https://doi.org/10.1007/s10695-019-00652-3.

Rasengwatshe, A. & Madibela, O.R. 2005. Rumen Degradability of *Imbrasia belina* (mophane worms) and Carcass Meal. Journal of Animal and Veterinary Advances, 4(12): 1021-1024.

Ribeiro, J.C., Cunha, L.M., Sousa-Pinto, B. & Fonseca, J. 2017. Allergic Risks of Consuming Edible Insects: A systematic review. *Molecular Nutrition & Food Research*, 1-31. www.mnfjournal.com.

Roberts, C. 1998. Long-term Costs of the Mopane Worm Harvest. *Oryx*, 32(1).

Sekonya, J.G, McClure, N.J. & Wynberg, R.P. 2020. New Pressures, Old Foodways: Governance and Access to Edible Mopane Caterpillars, *Imbrasia (Gonimbrasia) belina*, in the Context of Commercialization and Environmental Change in South Africa. *International Journal of the Commons*, 14(1): 139–153. DOI: https://doi.org/10.5334/ ijc.978.

Simpanya, M.F., Allotey, J. & Mpuchane, S.F. 2000. A Mycological Investigation of Phane, an Edible Caterpillar of an Emperor Moth, *Imbrasia belina*. *Journal of Food Protection*, 63(1):137–140.

Siulapwa, N., Mwambungu, A., Lungu, E. & Sichilima, W. 2014. Nutritional Value of Four Common Edible Insects in Zambia. *International Journal of Science and Research (IJSR)*, 3(6): 876-884. www.ijsr.net.

Stack, J., Dorward, A., Gondo, T., Frost, P., Taylor, F. & Kurebgaseka, N. 2003. Mopane worm utilisation and rural livelihoods in Southern Africa. Presented at The International Conference on Rural Livelihoods, *Forests and Biodiversity*, 19-23 May 2003, Bonn, Germany.

Styles, C. V. & Skinner, J. D. 1996. Possible Factors Contributing to the Exclusion of Saturniid Caterpillars (mopane worms) from a Protected Area in Botswana. *Afr. J. Ecol*, 34: 276-283.

Swemmer, L., Grant, R., Annecke, W. & Freitag-Ronaldson, S. 2015. Toward More Effective Benefit Sharing in South African National Parks. *Society & Natural Resources: An International Journal*, 28(1): 4-20. DOI: 10.1080/08941920.2014.945055.

Swemmer, L., Landela, R., Mdungasi, P., Midzi, S., Mmatho, W., Mmethi, H. Shibambu, D. Symonds, A., Themba, S. & Twine, W. 2020. It's Not Just About the Worm: Social and Economic Impacts of Harvesting Imbrasia Belina larvae (Kruger National Park, South Africa). *Conservation and Society*, 18(2): 183-199. www.conservationandsociety.org.

Teferra, G. 2003. Assimilation Efficiency in Two Herbivores, *Oreochromis Niloticus* and Larvae of *Imbrasia Belina*: A comparison. *Sinet: Ethiop. J. Sci.*, 26(1):73–76.

Teferra, G., Allotey, J., Mpuchane, S., Siame, B.A. & Gashe, B.A. 2000. The nutritional status of three species of plant leaves as food for the larvae of Imbrasia belina, an edible caterpillar. *Botswana Notes and Records*, 32:143-150.

Thomas, B. 2013. Sustainable Harvesting and Trading of Mopane Worms (*Imbrasia belina*) in Northern Namibia: An experience from the Uukwaluudhi area. *International Journal of Environmental Studies*, 70(4): 494-502. https://doi.org/10.1080/00207233.2013.829324.

Togarepi, C., Nashidengo, E. & Siyambango, N. 2020. Effects of Climatic Variability and Non-Climatic Factors on Mopane Worms' (*Gonimbrasia Belina*) Distribution and Livelihood Options in North Central Namibia. Environment and Natural Resources Research, 10(2):14-25. https://doi.org/10.5539/enrr.v10n2p14.

Van Den Berg, M. A., Catling, H. D. & Vermeulen, J. B. 1973. The Distribution and Seasonal Occurrence of Saturniidae (Lepidoptera) in Transvaal. *Phytophylactica*, 5:111-114

Van Huis, A. 2003. Insects as Food in Sub-Saharan Africa. *Insect Sci. Applic*., 23(3):163–185.

Wiggins, D.A. 1997 (a). Clutch size and oviposition site characteristics of a nocturnal African silk moth *Imbrasia belina* (Lepidoptera: Saturniidae). *Annales Zoologici Fennici*, 34 (4): 235-240. http://www.jstor.com/stable/23735486.

Wiggins, D.A. 1997 (b). Fluctuating Asymmetry in *Colophospermum Mopane* Leaves and Oviposition Preference in an African Silk Moth *Imbrasia belina*. *Oikos*, 79(3): 484-488. http://www.jstor.com/stable/3546891.

Yeboah, S.O. & Mitei, Y.C. 2009. Further Lipid Profiling of the Oil from the Mophane Caterpillar, *Imbrasia belina*. *J. Am. Oil. Chem. Soc.*, 86:1047–1055. DOI 10.1007/s11746-009-1443-y.

Zinzombe, I.M. & George, S. 1994. Larval Lipid Quality of Lepidoptera: *Gonimbrasia belina*. *Botswana Notes and Records*, 26: 167-173. http://www.jstor.org/stable/40959191.