

Influence of Soil Properties on Pre-pupal Burrowing, Pupation and Nutritional Quality of *Gonimbrasia belina* Westwood

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Abstract

Mopane worms (*Gonimbrasia belina*) are important sources of nutrition among many people in southern Africa. Threats of climate change and over-harvesting have provided compelling evidence to conserve and domesticate these edible insects through a better understanding of their natural habitat. A study was conducted in Zimbabwe, with the objectives of determining the influence of soil properties on mopane larvae soil burrowing, pupal metamorphosis and its nutritional quality. Field sampling was conducted by identifying *Colophospermum mopane* trees that had mopane larvae and then characterizing soil properties under each identified tree. An adjacent area with similar vegetation but with no known mopane occurrence was the control site. Soil and leaf samples were taken from each site and analysed for selected physical and chemical properties. Data were analysed using detrended correspondence analysis and Student t-test. Results show that *G. belina* larval abundance was highest on trees rooted in soils with 80–97% sand. Pupae population was positively linked to soil organic matter content, litter content, and exchangeable Ca, Mg and K concentrations but negatively linked to soil bulk density. The concentrations of Mg and K in the mopane tree leaves and mopane worm were at least seven times that of soil. Protein (55.6% DM) and fat (18.5% DM) were relatively higher in mopane worm than foliar samples. We conclude that mopane worms are more likely to burrow and metamorphose to pupae into less compacted sandy soils with relatively high content of organic matter and litter content.

1. Introduction

Mopane worms (*Gonimbrasia belina* Westwood) are large edible caterpillars of the emperor moth species (Anomalous emperor moth) that are important sources of nutrition among many people in southern Africa. These edible insects are mainly found in specific regions of Botswana, South Africa and Zimbabwe that are dominated by their preferred host tree *Colophospermum mopane* (mopane) (Kirk ex Benth.) Kirk ex J.Léonard). Known locally in Zimbabwe as macimbi (isiNdebele) and madora (Shona), mopane worms occur in the mopane woodland belt of the country, covering the drier regions of Matabeleland North Province (Tsholotsho District) and Matabeleland South Province (Gwanda, Plumtree, Mangwe, Matobo and Beitbridge districts) (Kwiri et al., 2014). Mopane worm distribution follows closely the distribution of *C. mopane* but is not restricted to it as the larvae can also feed on *Trema bracteolate* Hochst Blum, *Terminalia sericea* Burch. ex DC., *Searsia lancea* (L.f.) F.A. Barkley, *Ficus* spp., *Carissa grandiflora* (E.Mey.) A.DC. (Pinhey, 1972). However, not all areas with mopane trees are host to mopane worms suggesting that other environmental factors are important in the distribution of the *G. belina*. Skinner and Styles (1992) postulated that the extinction of mopane worms in some areas could have been assisted by edaphic factors and browsing quality.

In Zimbabwe the mopane worms are bivoltine (producing two generations per rainy season) with the moths emerging during the summer months when the mopane trees are in leaf, raining and typically high temperatures (Frears et al., 1999). The first generation occurs around October-November, at the beginning of the rainy season and a second one in February-March (Gondo et al., 2010). However, this seem to be a shift/change in emergence season as observed in the past three years. The adults lay eggs

in cluster which hatch in about 10 days into black larvae, which subsequently grow and moult 4 times, with each instar lasting about 5–7 days (Ditlhogo et al., 1996). The larval period generally lasts 4–6 weeks, during which time, the voraciously feeding larvae increase their body mass 4,000 times (Gondo et al., 2010) with the fifth instar caterpillars usually weighing about 12–13 g and measuring approximately 80 mm in length (Frears et al., 1999, Hrabar, 2007). The late instar caterpillars burrow 10–15 cm into the soil near the base of mopane trees to pupate (Hrabar, 2007, Gondo et al., 2010). According to Gondo et al. (2010), the first and second generation pupae take on average about one-and-a-half months and 6–7 months, respectively to complete pupation.

The period spent in the soil may be critical as it could determine the survival of the larvae and pupae and eventual emergence as moths. More so, mopane worms are threatened with extinction, which is partly attributed to climate change, unregulated harvesting, population pressure, land use change and overexploitation (Mataboge et al., 2016, Ndlovu et al., 2019). Therefore, it is imperative that appropriate measures, such as legislation and domestication of the mopane worms, that promote sustainable management and exploitation of mopane worms be implemented. However, such measures require a better understanding of the mopane worm habitat and life cycle. Although some stages of the mopane worm life cycle are well known (Mataboge et al., 2016, Bara et al., 2022), there are limited studies on the soil habitat. In addition, the nexus between the soil habitat, especially soil properties and nutrients, and the nutritional content of the plant habitat and the mopane worm is not yet understood. As such, a study was conducted in Matabeleland Province, Gwanda District, Zimbabwe, with the aim to better understand the influence of soil properties on mopane pre-pupal soil burrowing and pupation and the nexus between soil, host plant and mopane worm nutrients. Findings from this study will provide in-depth insights that are important for the successful rearing and sustainable management of mopane worms.

2. Materials and Methods

2.1 Study location

This study was conducted in Gwanda District, Matabeleland South Province, Zimbabwe (Fig. 2) with soil samples being collected in December 2021. The vegetation of the study area was dominantly *Colophospermum mopane* trees and some scattered tree species of *Grewia*, *Combretum*, and *Acacia*. Trees with mopane worms were first identified and soil samples taken underneath them.

2.2 Soil sampling

Three random points under each identified mopane tree were selected for soil sampling. The soil sampling depths at each point were 0–12 cm and 12–25 cm. Soil samples for bulk density analysis were taken using cylindrical core samplers and those for chemical analyses were taken from a vertical section of a soil pit using a knife. Soil samples were put in clean polythene bags and transported to the laboratory for analysis. Soil under *Colophospermum mopane* trees was also carefully excavated using

knives to locate the mopane worm pre-pupae and pupae. The depths to the pupae and pre-pupae in the soil were measured using a measuring tape.

2.3 Sampling of mopane worm and mopane leaves and sample preparation

Mopane worm (also called larvae in this paper) and mopane leaves were sampled from the trees in which the soil were sampled. Approximately 0.25–0.5 kg of mopane worm were obtained from each mopane tree in the larvae site. The mopane worms were collected according to local harvesting practices (a combination of handpicking from the mopane trees and shaking the host trees followed by degutting). Larvae were degutted manually by pressing its gut out followed by washing with distilled water. Naturally degutted larvae were collected on the ground when they were burrowing into the ground. Mopane leaves were sampled from each of the selected sites. The leaves were air-dried and then crushed in an electrical blender (AE Electrical, Zimbabwe) for 10 seconds.

Samples were packed in zipping polyethylene bags before freezing. The samples were transported to laboratory on flaked ice in cooler boxes and stored at – 18°C until further analyses.

Prior to analysis, approximately 100 g of frozen mopane worm samples were allowed to thaw. Before analysis, samples were allowed to defrost, followed by crushing in an electrical blender (AE Electrical, Zimbabwe) at high speed for 10 seconds.

2.4 Laboratory analysis

2.4.1 Nutrient composition determination

Proximate composition

Moisture content was assessed by drying in a forced-air oven (Fischer Scientific, South Africa) at 105°C for three hours (Nielsen, 2010). The Kjeldahl method, which was modified slightly from Mihaljev et al. (2015), was used for the determination of crude protein and a nitrogen to protein conversion factor of 5.6 was used (Boulos et al., 2020). Crude fat content was determined using the Soxhlet extraction method. Briefly, the sample (5 g) was refluxed with technical grade petroleum ether (Merck (Pvt) Ltd, Johannesburg, South Africa) for 8 hours. The extraction solvent was then vacuum-evaporated to recover the fat in a rotary evaporator (Büchi R-124, Büchi Labortechnik, Flawil, Switzerland). Ash content was determined by heating sample in porcelain crucibles in a muffle furnace (Scientific, Johannesburg, South Africa) at 600°C for six hours. Crude fibre was determined using the procedure previously explained by Anaduaka et al. (2021) with minor adjustments. Each of the fresh (ground) mopane worm samples (2.0 g) (F1) was placed into a 300 ml conical flask, to which 200 ml of 0.128 M H₂SO₄ was then added and boiled for 30 min. Thereafter the material was filtered through Whatman filter paper 42 (Merck, Johannesburg, South Africa) followed by washing three times with hot water (80°C) and the residue was placed back into the conical flask. Following the addition of 200 ml of 0.223 M NaOH, the sample was heated for 30 min before being filtered through Whatman filter paper 42 (Merck, Johannesburg, South

Africa). The residue was then washed with 25 ml of 0.128 M H₂SO₄, three 50 ml portions of water and 25 ml of ethanol. Thereafter, the residue was transferred into a pre-weighed crucible (F2), dried at 130°C for 2 h, cooled in a desiccator and weighed (F3). The samples were then ignited for 30 min at 600°C, cooled in a desiccator and reweighed (F4). The percentage crude fibre was calculated as shown in Eq. 1:

$$\text{Crude fibre (\%)} = \frac{m_i}{m_{\text{tot}}} \times 100 \text{ Eq. (1)}$$

Where m_i – mass (g) of residue (F₃-F₂) minus mass of ash (F₄-F₂) and m_{tot} – is the total mass (g) of sample (F₁)

Mineral profiling

Mineral profiling was according to the procedure previously explained by Paul et al. (2014) with minor modifications. Wet ashing with 65% nitric acid and 70% perchloric acid (both from Sigma-Aldrich, St. Louis, MO, USA) was used to determine the mineral (Ca, Mg, K, Na, Fe, Zn, Mn, and Cu). Briefly, 0.5 g of ground fresh mopane worm, 5 ml of 65% HNO₃ and 10 ml of HClO₄ were added and heated until the solution was clear. After cooling, the solution was transferred to a 100 ml volumetric flask. The results were read on an atomic absorption spectrophotometer (AAS) (PinAAcle 900F, Perkin Elmer, Massachusetts, USA). Extraction, detection, identification, and quantification was in duplicate for each sample.

Phosphate content

Phosphate content was measured using colorimetric method according to Pulliainen and Wallin (1996) on an Ultraviolet-visible spectrophotometer (Lampda 365- UVSN410020, Perkin Elmer Massachusetts, USA). The samples were dry-ashed in the presence of zinc oxide, and total phosphorus content was measured colorimetrically as molybdenum blue at 430 nm. All determinations were in duplicate.

2.4.2 Soil analysis

Soil samples for chemical analysis were first air-dried and passed through a 2-mm sieve. Particle-size distribution was analysed using the sedimentation method (Gee and Bauder, 1986) and soil pH was determined in 0.01M CaCl₂ (Thomas, 1996). Cation exchange capacity and exchangeable bases (Ca, Mg, K, Na) were determined following the method described by Sumner and Miller (1996). Soil organic matter was determined using wet oxidation methods (Nelson and Sommers, 1996). Soil bulk density was determined using the core method (Black and Hartge, 1986). Data was analysed using detrended correspondence analysis.

2.5 Statistical analysis

Statistical analysis was done using Sigma plot for windows (Version 12, IBM Corporation, Armonk, New York) software. Variation on nutritional composition of mopane leaves from control site and larvae site

and mopane worm and leaves from the larvae site was assessed using a Student's t-test. Shapiro-Wilk test for normality was used. Soil data was analysed using detrended correspondence analysis.

3. Results and Discussion

3.1 Soil properties and mopane worm pupae

Soil properties showed an increasing trend in bulk density from 0–12 cm to 12–25 cm in both the larvae/mopane worm and control sites (Table 1). As would be expected, plant litter content was only found on the soil surface due to leaf-fall and surface grass cover. The texture of soil was sand to loamy sand on the surface layers but varied from loamy sand to sandy loam in the sub-horizons. According to Gardiner and Taylor (2003), sandy soils are suitable pupation sites for *G. belina* because of the low water holding capacity inherent in sandy loams. Subsequently, sandy soils do not offer a conducive environment for the proliferation of bacterial, fungal and viral disease pathogens. It is also easier for *G. belina* to burrow and emerge from sandy soils that have relatively low bulk density. The association of mopane trees with sandy soils could also explain the subsequent soil habitat for the mopane pupae.

Soil pH was slightly acidic (pH 6.0-6.1 range) and was attributed to the high percentage of sand. Although the soil has low cation exchange capacity, the concentration of Na was generally high.

Table 1
Summary of selected soil properties of the study area (mean $\pm$ standard deviation), $n = 60$.

Property	Larvae sites		Control sites	
	(with mopane larvae)		(without mopane larvae)	
	Soil depth (cm)			
	0–12	12–25	0–12	12–25
Bulk density (g/cm ³)	1.25 ± 0.18	1.51 ± 0.13	1.52 ± 0.05	1.55 ± 0.30
Plant litter (%)	13.2 ± 22.5	0	2.0 ± 3.22	0
Organic C (%)	1.12 ± 0.71	0.21 ± 0.38	0.52 ± 0.23	0.12 ± 0.05
Gravel (%)	54.8 ± 25.1	69.6 ± 16.38	54.9 ± 10.08	77.8 ± 10.9
Sand (%)	91.2 ± 5.72	85.0 ± 8.01	87.8 ± 4.5	81.5 ± 6.13
Silt (%)	5.3 ± 3.96	7.7 ± 4.16	7.75 ± 3.1	9.5 ± 4.51
Clay (%)	2.9 ± 1.24	7.3 ± 4.09	4.5 ± 2.1	9.0 ± 7.52
pH	6.1 ± 0.6	6.0 ± 0.6	6.1 ± 0.6	6.1 ± 0.58
Exchangeable Ca (cmol _c /kg)	4.68 ± 2.34	4.51 ± 2.26	4.4 ± 0.77	4.38 ± 0.78
Exchangeable Mg (cmol _c /kg)	3.05 ± 0.74	3.5 ± 1.34	3.1 ± 0.8	3.48 ± 1.15
Exchangeable K (cmol _c /kg)	0.14 ± 0.04	0.57 ± 0.37	0.16 ± 0.06	0.64 ± 0.54
Exchangeable Na (cmol _c /kg)	0.70 ± 0.47	0.49 ± 0.61	0.62 ± 0.53	0.68 ± 0.62
Cation exchange capacity (cmol _c /kg)	9.51 ± 3.31	10.16 ± 3.86	10.4 ± 2.6	10.63 ± 2.51

Detrended correspondence analysis showed that pupae population is positively linked to soil organic matter, plant litter content, exchangeable Ca, K and Mg but negatively linked to soil bulk density (Fig. 3). Interactive-forward test indicated that bulk density significantly ($p < 0.05$) affected pupae and larvae population of *G. belina* in the soil (Table 2). During its life cycle, the mopane worm burrows into the soil and undergoes metamorphosis into pupa. The negative relationship between soil bulk density and pupal population is attributed to the difficulty of the *G. belina* larvae encounter when trying to burrow into compacted soil. Relatively high amounts of soil organic matter reduce soil bulk density, regulate soil

temperature and moisture (Senesi and Loffredo 1999). Therefore, the positive relationship of the abundance and depth (6-10cm) of pupae location in the top layers of the soil suggests a conducive soil environment for burrowing and pupation than the underlying soil layer (Table 1). This finding is somewhat in contrast to earlier studies which suggested that the late instar caterpillars burrow 10–15 cm into the soil (Hrabar, 2007, Gondo et al., 2010).

The dominance of mopane trees (on which mopane worms feed) in light-textured soils in this study area could explain the link between *G. belina* abundance and the percentage of sand (Table 2). Exchangeable Ca and litter percentage were the second most influential attributes controlling pupal population in the soil (Table 2). High percentage of litter on the soil surface moderates soil temperature and reduces soil moisture loss through evaporation and could provide an ideal hiding habitat from soil burrowing predators.

Table 2
Detailed results of forward selection test analysis

External variables	Explains %	Contribution %	pseudo-F	P
Bulk density	43.1	48.9	10.6	0.004
% Plant litter	9.6	10.8	4.5	0.038
% Organic matter	0.5	0.6	0.2	0.688
%Sand	0.3	0.4	< 0.1	0.74
% Silt	2.1	2.4	1.0	0.308
% Clay	1.8	2.1	0.8	0.322
Ca	22.0	25.0	8.2	0.016
Mg	3.8	4.3	1.9	0.196
Na	0.2	0.3	0.1	0.772

3.2. Mopane worm

Soil-leaf-mopane worm mineral transfer

Similar to the general trend in most natural soils, the concentrations of available basic cations in the soil followed the order Ca > Mg > Na > K (Table 1). However, the concentrations of these elements in the leaves of *C. mopane* showed the preferential uptake and accumulation of K and Mg and in the order K > Mg > Ca (Table 3). Since *C. mopane* is adaptive to the drier regions (Makhado et al., 2014), it is postulated that such plants would absorb relatively higher concentrations of K from the soil for osmo-regulation (Mengel and Kirkby, 2012). On the other hand, Mg, Fe and Mn are vital in photosynthesis (Mengel and Kirkby, 2012). Previous studies of *C. mopane* foliage in the Kruger National Park (Ferwerda

et al, 2006), revealed that leaf composition was related to geomorphology/soil, vegetation structure, and fire history.

Mineral concentrations in the mopane worms were several times higher than that in the soil (Table 3). These trends show the absorption, transfer and bio-concentration of nutrients from the soil to the plant. Although the concentration of Ca in the leaves was similar to that in the mopane worms, it was about 5 times lower than that in the soil (Tables 2.) The concentration of Mg in the leaves and in mopane worms was about 7 times higher than that in soil. Mineral nutrient accumulation was highest for K that showed more than 200 times in the leaves and in mopane worms. These trends are attributed to the high demand for K in the leaves of *C. mopane* as this nutrient is required for osmo-regulation and starch formation in plants (Mengel and Kirkby, 2012). It is likely that K performs similar functions in the mopane worm, hence its higher bio-concentration. Although Na concentration was relatively high in the soil, only less than half of the concentration of this mineral accumulated in the leaves. In plant mineral nutrition, Na is not regarded as a nutrient hence its low concentrations in plants.

However, significant differences ($p < 0.05$) were observed in mineral composition between the leaves from the study sites. The control site had the highest Ca, Mg, Na, Fe, Zn, Mn and Cu (Table 3) whilst mopane leaves from larvae site had higher K and P. From the results, it can be hypothesised that the nutritional composition of the leaves had no link in habituating mopane worms.

Table 3
Mineral composition of mopane leaves from the control and larvae sites

Parameter (mg/kg)	Control site (without mopane larvae)	Larvae site (with mopane larvae)
Ca	$274^y \pm 3.2$	$156^x \pm 2.0$
Mg	$2285^y \pm 3.3$	$2256^x \pm 2.2$
K	$7291^x \pm 2.0$	$8890^y \pm 1.3$
Na	$23^x \pm 0.2$	$22^x \pm 0.2$
P	$1726^y \pm 5.3$	$1792^y \pm 3.1$
Fe	$187^y \pm 2.0$	$158^x \pm 2.2$
Zn	$63^y \pm 2.0$	$49^x \pm 2.4$
Mn	$66^y \pm 2.1$	$51^x \pm 2.1$
Cu	$3.5^y \pm 0.2$	$1^x \pm 0.1$

Values are mean $\pm$ SD (n = 4). Different superscript letters x and y represent significant differences ($p < 0.05$) between the control site and the mopane larvae site.

Results in Fig. 4 showed no significant difference in protein content of the mopane leaves (approx. 13% DM) from control and larvae sites. The values agree with Makhado et al. (2014) who reported a range of 9.2–13.9%. In addition, there were no significant differences in fat, ash and crude fibre of foliar samples from the control and larvae sites ($p > 0.05$).

Sites with mopane larvae showed significant differences ($p < 0.05$) between the proximate composition of foliar and mopane larvae. Both protein (55.6% DM) and fat (18.5% DM) were higher in mopane worm than foliar samples. However, ash (5.0% DM) and crude fibre (25.6% DM) were higher in foliar. As for the mineral composition, mopane worm had the highest Ca (860), K (14068), Na (425), P (4364) and Zn (143) mg/kg DM. There was no significant difference in Cu between mopane leaves and mopane worms.

4. Conclusion

Soil physical and chemical properties influence mopane worm habitat and the nutritional composition of the host trees. Mopane larvae are more likely to burrow less compacted sandy soil with relatively high organic matter and litter contents. These soil properties suggest a conducive environment for pupation. Although the concentrations of basic cations (Ca, Mg, K, Na) in the soil between the two sites were similar, significant differences in the concentrations of these elements were observed in the leaves and mopane larvae. The nutritional quality of mopane larvae reflected on the foliar composition of its host trees.

Declarations

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Statements and Declarations

Competing Interests and Funding

The authors declare no competing interests. Moreover, the authors have no relevant financial or non-financial interests to disclose.

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Figures

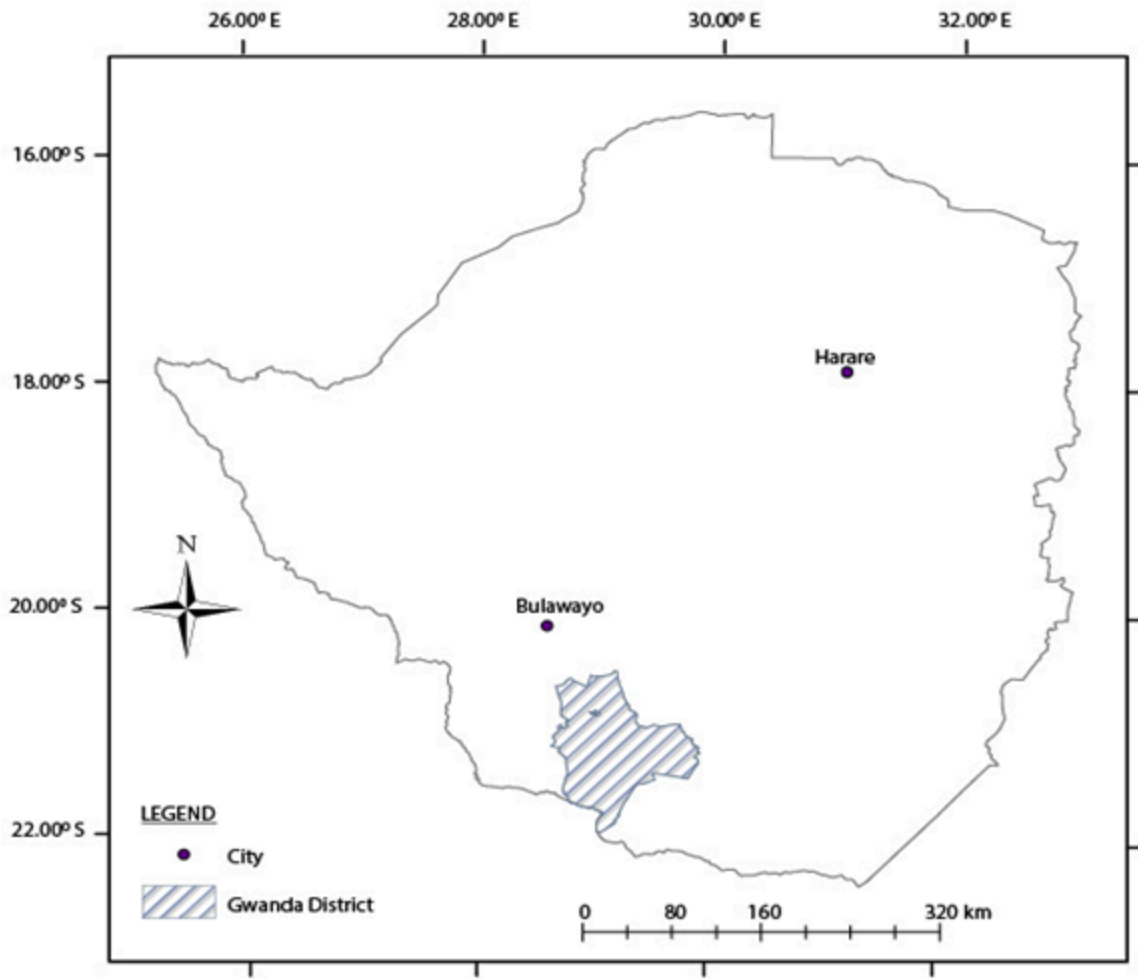


Figure 1

Location of the study site in Zimbabwe

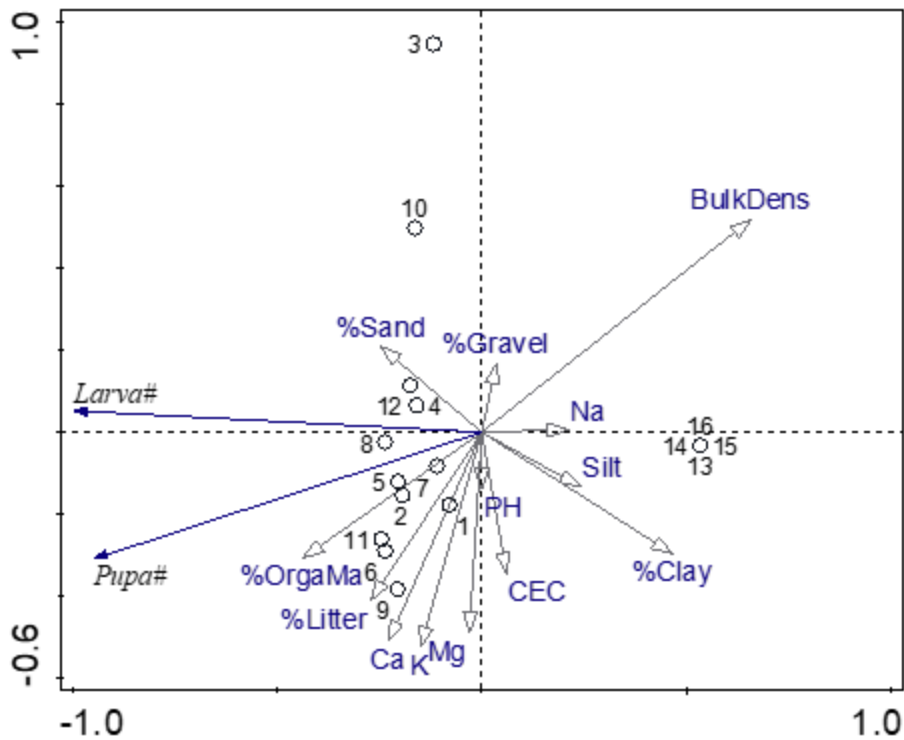


Figure 2

Detrended correspondence analysis (DCA) diagrams of species and environmental variable. The direction of the vectors indicates the direction of maximum change of that variable, whereas the length of the vector represents how well the parameter explains the distribution of the data. External variable (% Orga Ma = Soil organic carbon, % Litter= Litter ground cover, Ca = soil exchangeable calcium, K = soil exchangeable Potassium, Mg = soil exchangeable Magnesium, Na = soil exchangeable Sodium, CEC= cation exchange capacity, pH = soil pH, Ca=Calcium, Mg=Magnesium, K=Potassium, Na= Sodium).

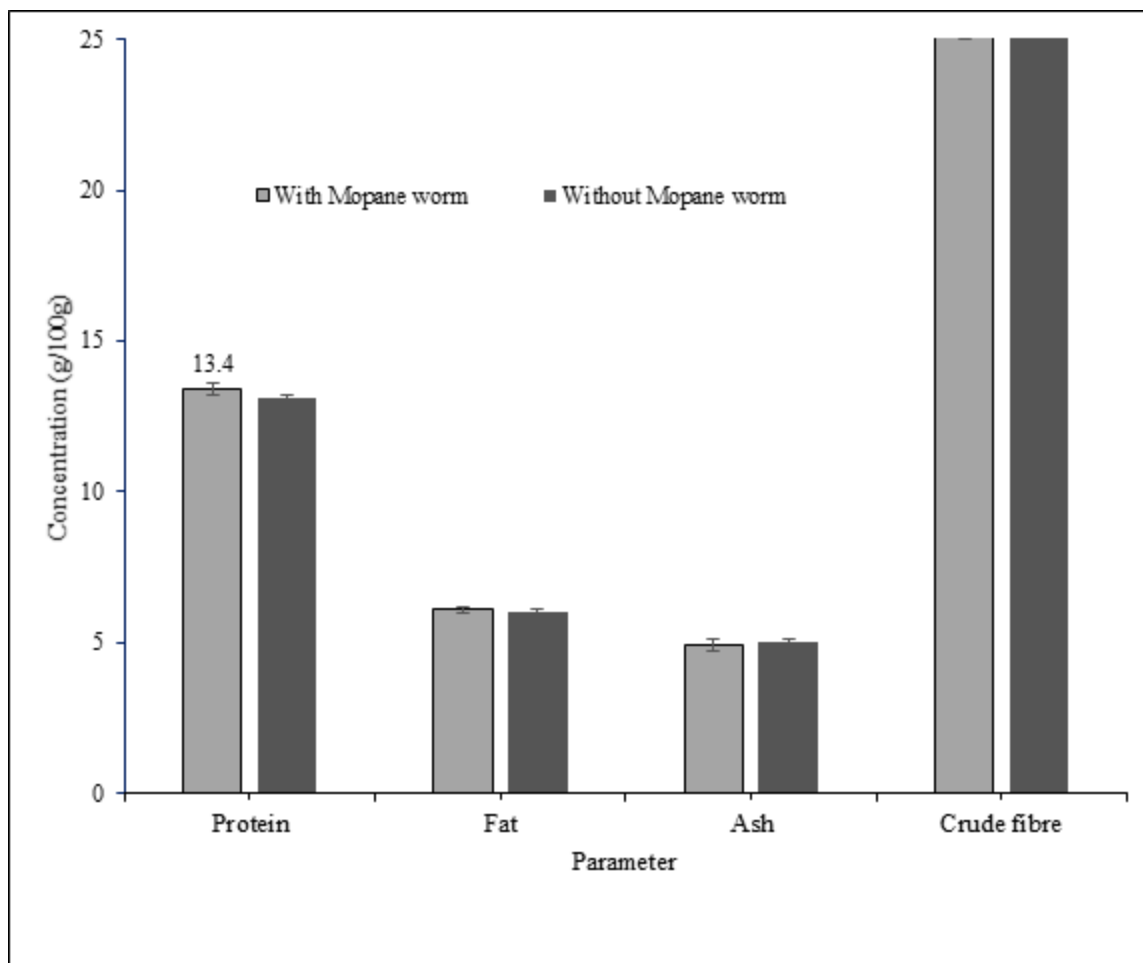


Figure 3

Proximate analysis of C mopane leaves from the study sites with mopane worm/larvae and without (control).