SUPPORTING INFORMATION

Quantifying the role of termite decomposition in a mesic savanna

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Appendix S1.

Methods

Chemical treatments

Non-target invertebrate effects

To assess the effect of the poisoning on non-target soil invertebrates we removed 15 soil monoliths (0.2 x 0.2 x 0.2 m) randomly from each plot. The soil monoliths were placed in large white trays, and the soil was searched carefully and exhaustively for all invertebrates. All invertebrates that were found were placed in a vial. In the laboratory, invertebrates were identified to order and counted.

Poison residue analyses

We also sampled soil, leaves from the most dominant tree (*Terminalia sericea*) and grass (mixed) on our termite suppression plots to test for environmental contamination associated with our selected chemical treatments, imidacloprid and fipronil. Five 5 cm^3 soil samples, taken from the topsoil layer (0-5 cm) were collected from each plot and pooled. Five handfuls of *Terminalia sericea* leaves from different trees and five handfuls of mixed grass from different locations were also collected from each plot and combined. These samples were then frozen and transferred to the laboratory for analysis. The soil was then homogenised and the grass and leaves were finely ground.

In preparation for chemical extraction, sub-samples of the homogenised soil, finely ground grass and finely ground leaves were extracted using 20 ml Acetonitrile:MeOH (1:1) (ultra-

purity lc methanol/ acetonitrile (Romil-UpS™, Microsep, South Africa)) and sonicated for 15 minutes. The samples were filtered using 0.2µm Nylon filters (Agilent, Captiva) and placed in sample vials for analyses with UPLC. Validation soil samples, spiked with imidacloprid and fipronil, were prepared to show that the Gas chromatography–mass spectrometry (GC-MS) analysis was able to detect these pesticides at doses used in the field. The GC-MS analyses were performed on a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system hyphenated to a quadrupole-time-of-flight (QTOF) instrument. The system was operated with MassLynx (version 4.1) software (Waters Inc., Milford, Massachusetts, USA) for data acquisition and processing. Further details can be found in Walker *et al.* (2022).

Non-target invertebrate and termite effects

We used a an ANOSIM to test for any effect of termite suppression on both termite community composition and non-target invertebrate abundance within soil samples.

Decomposition rate

To determine the decomposition rate we calculated the decomposition rate of each substrate. Following Olson (1963), we calculated the decomposition constant (k) and half-life (T1/2) for each substrate (wood: *Pinus*: 1 year and *Terminalia:* 2 years; dung 56 days and grass 112 days) We calculated k using the equation below:

(1)

$$k = - \frac{\text{natural log } (X/X0)}{t}$$

Half-life was calculated as below:

(2)

$$T1/2 = \frac{\text{natural log } (2)}{k}$$

where t is the time in years since the decomposition bag was placed on the plot, X is the substrate mass remaining at time point t, and X0 is the original mass at t = 0 years. This method assumes that k is constant.

It is likely that k will change across the different collection time points due to seasonal effects. However, it is useful for estimating an average decomposition rate and as a means of comparing between substrates.

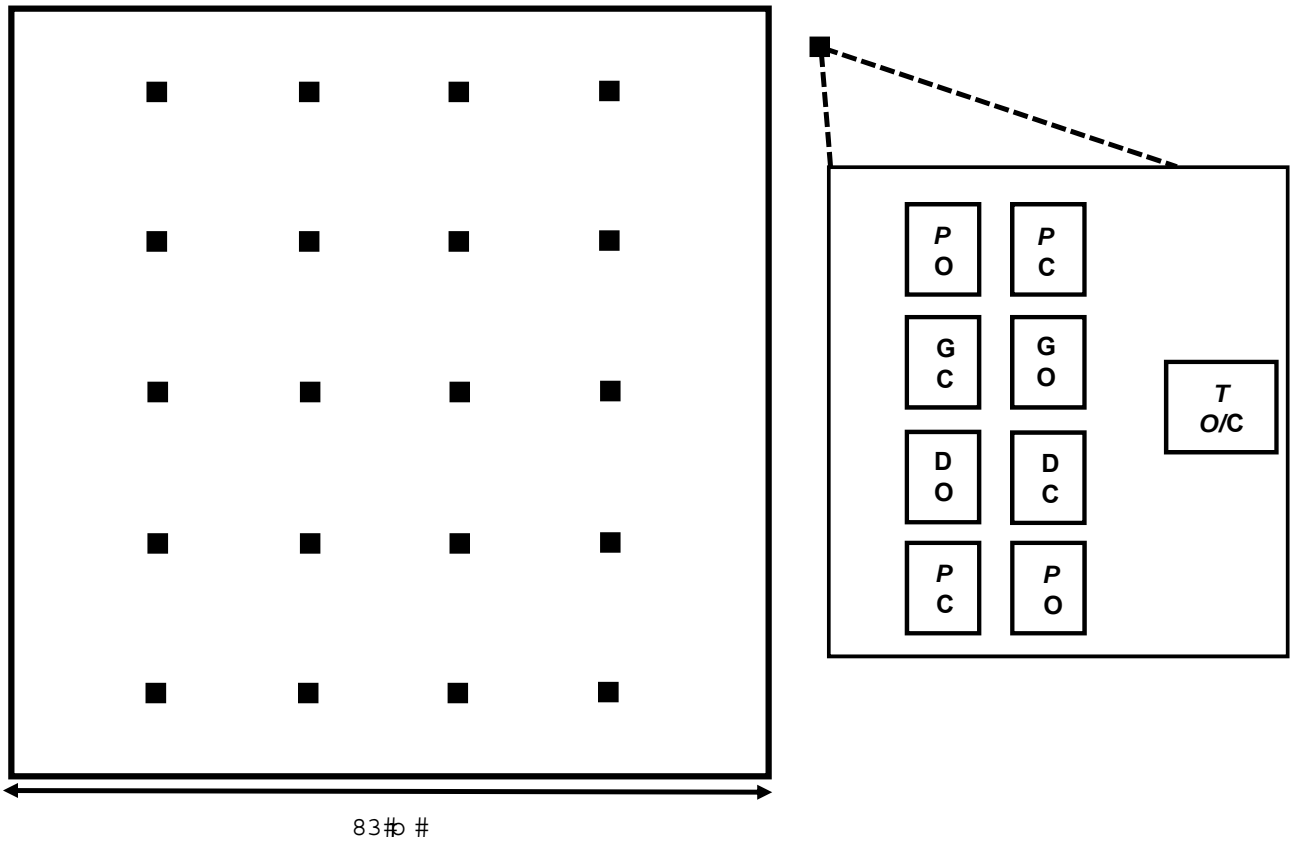

Figure S1: Layout of decomposition bags across the 50 m x 50 m (0.25 ha) plots. At each of the 20 positions (indicated by black squares), in the 4 x 5 grid, there were 9 decomposition bags. Five wood (*P: Pinus* x 4 & *T*: *Terminalia* x 1) – *Pinus*: two open (O) and two closed (C) and *Terminalia*: one open (O)/closed (C); two grass (G) – one open (O) and one closed (C) and two dung (D) - one open (O) and one closed (C).

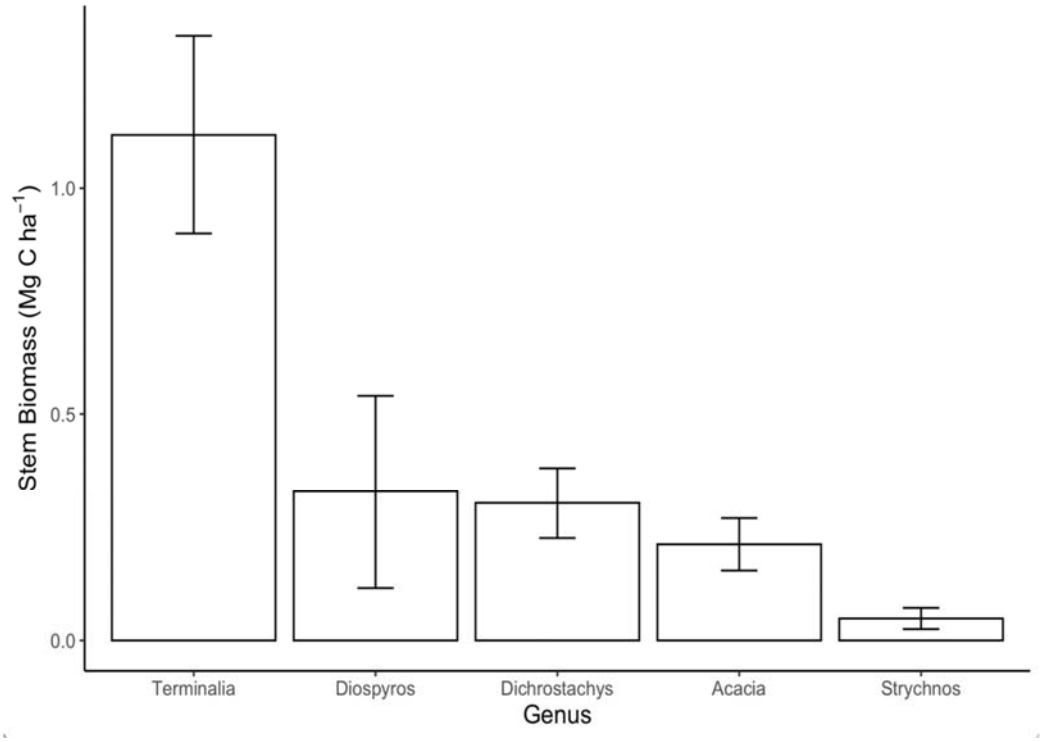

Figure S2. Biomass of top 5 tree genera across our study site (Wits Rural Facility). Sampling of trees (>=5 cm) was done within six GEM (Global Environmental Monitoring) plots (40 m x 40 m) placed within a subset of our existing plots.

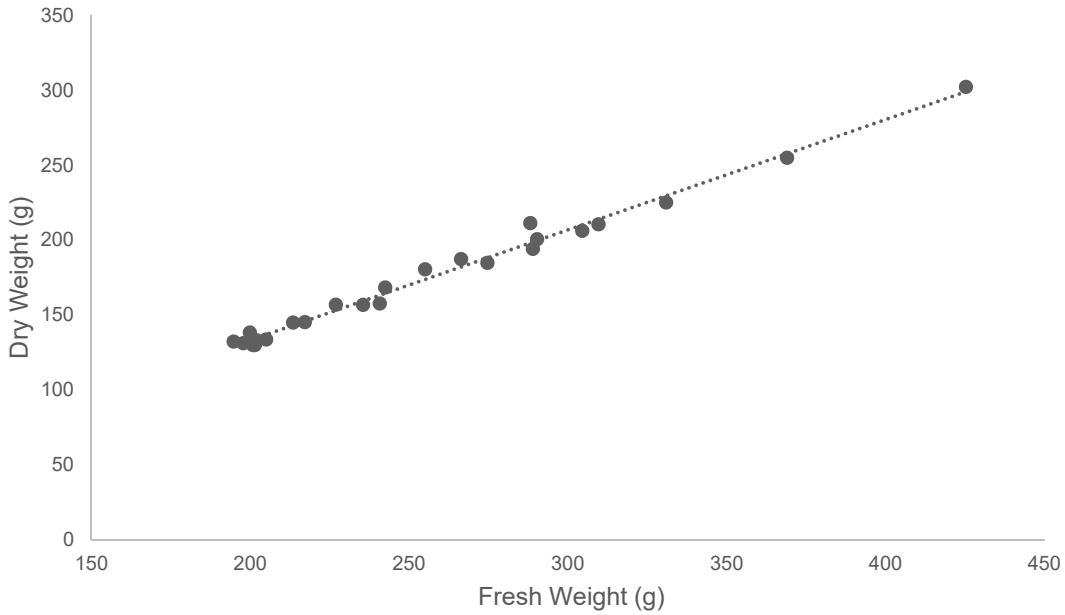

Figure S3. Regression between *Terminalia* fresh and dry weights (n = 24, y = 0.7357x - 13.887; R^2 = 0.989)

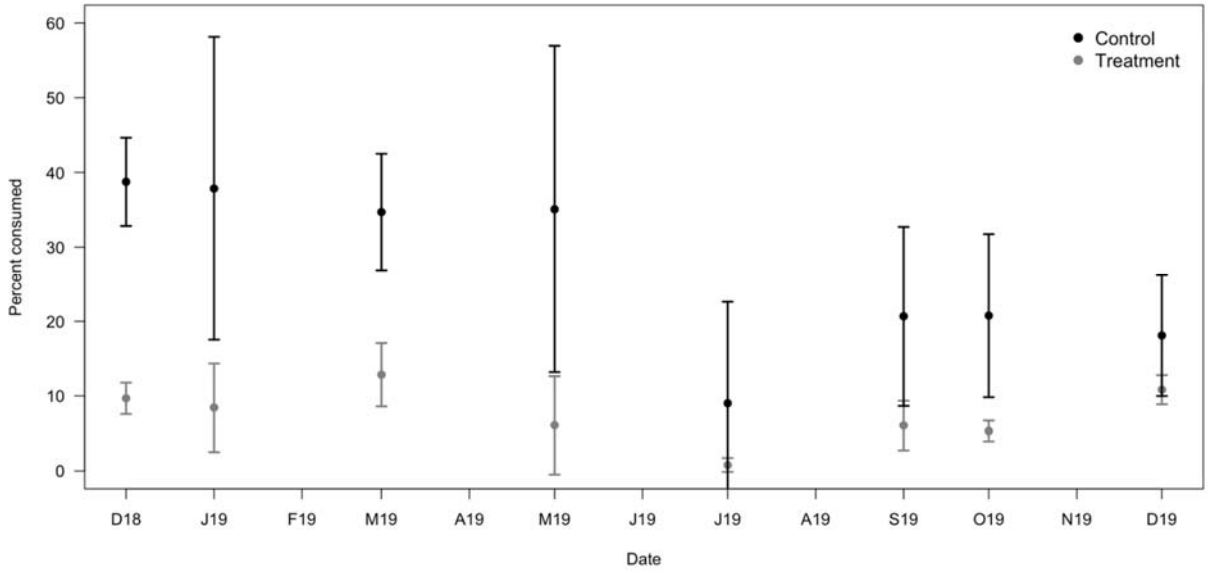

Figure S4. Consumption of bait toilet paper rolls (TPR) by termites in control versus termite suppression plots between December 2018 and December 2019. Error bars are standard errors for replicate plots in each treatment group. The average percent consumption across the plots: control 26.87% & suppression: 7.5% which equates to 72% suppression effect across the year.

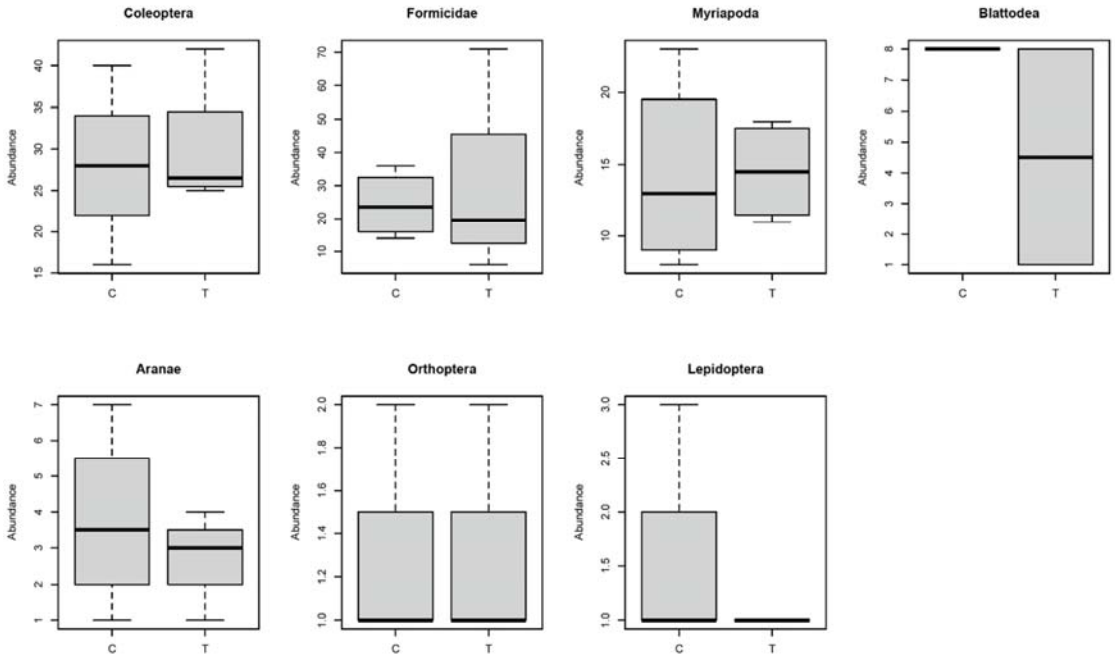

Figure S5. Median abundance plus abundances plus interquartile and range of the seven most common non-target invertebrate groups sampled from soil pits across control (C) and termite

suppressed (T) plots. The scale of abundance (y-axis) differs by taxon. Blattodea represent non-termite cockroaches.

Table S1. The seven dominant non-termite soil groups (with average abundance of >1 individuals per soil monolith). Blattodea represent non-termite cockroaches.

Group	N	Mean	SE
Coleoptera	232	29	2.77
Formicidae	213	26.63	6.64
Myriapoda	115	14.38	1.65
Blattodea	25	3.13	1.34
Araneae	23	2.88	0.74
Orthoptera	9	1.13	0.21
Lepidoptera	8	1	0.31

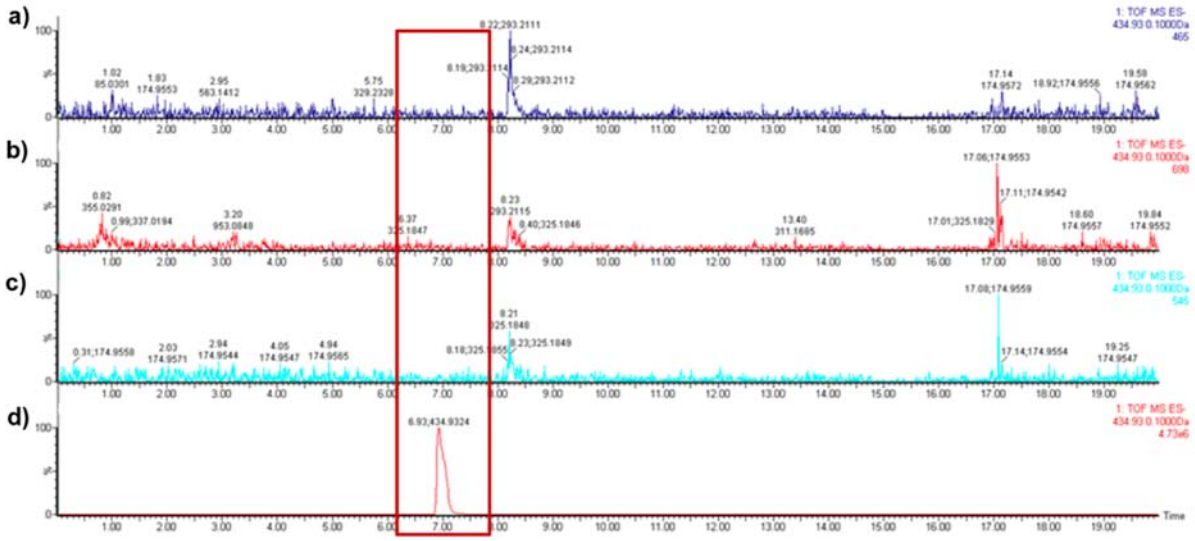

Figure S6. Extracted ion chromatograms of a) grass b) leaves c) soil above d) soil spiked with fipronil (peak: 6.93).

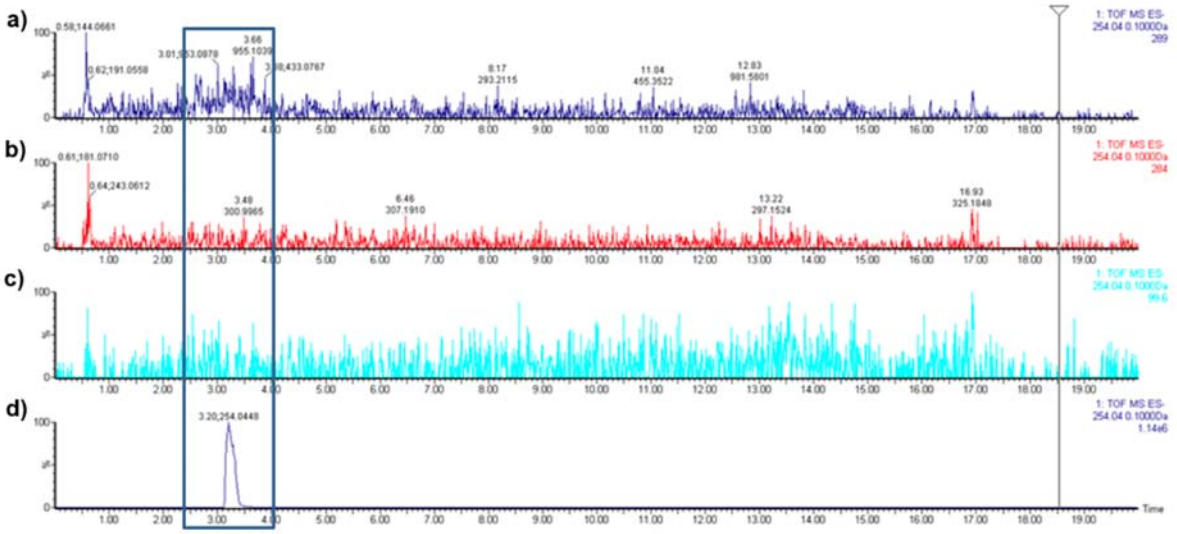

Figure S7. Extracted ion chromatograms of a) leaves b) grass c) soil above d) soil spiked with imidacloprid (peak: 3.20).

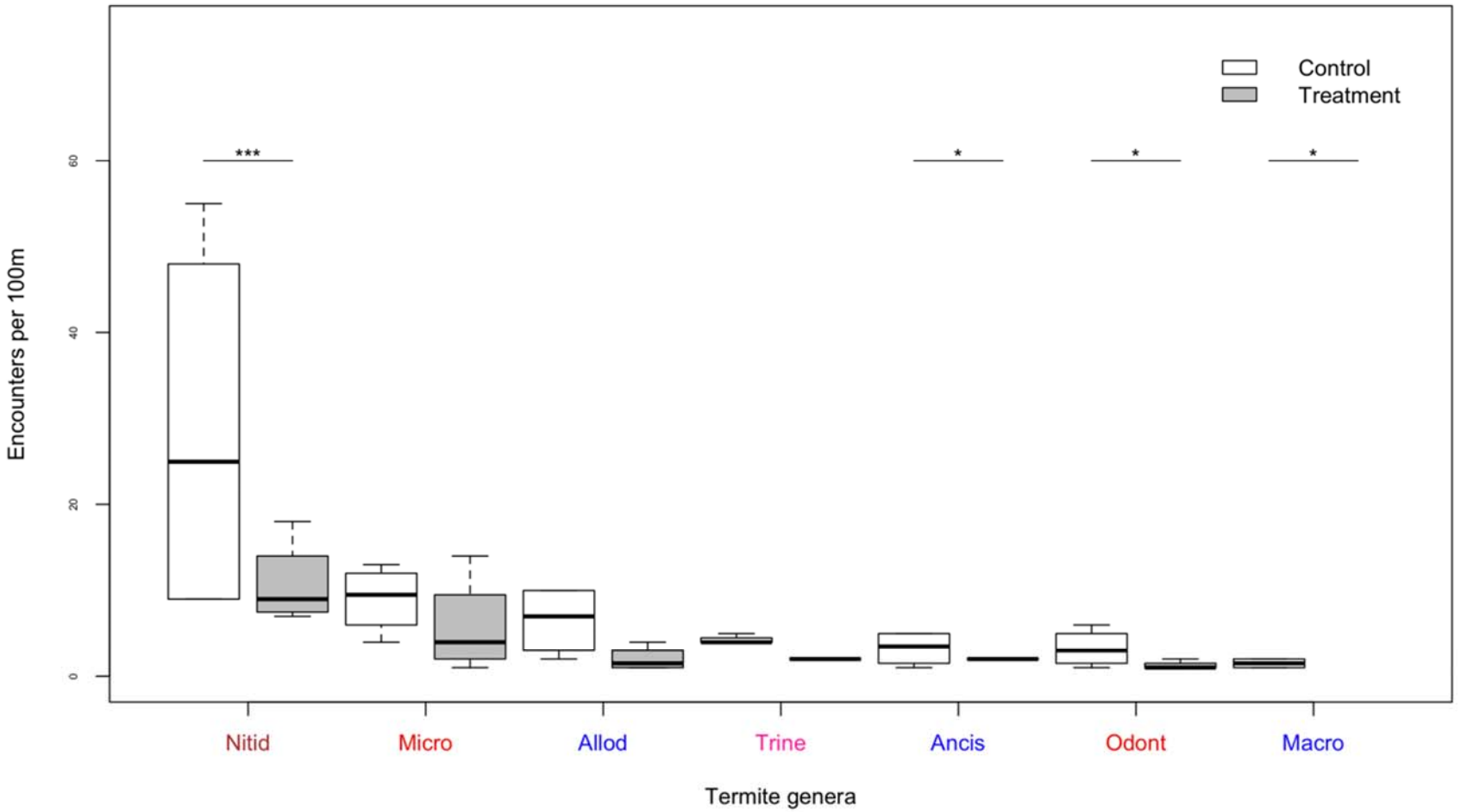

Figure S8. Median termite genera encounter rate plus interquartile range on control and termite suppression plots. Genera are colour-coded by feeding group: soil feeders (brown), wood-feeders (red); mixed feeders (blue) and grass feeders (pink). Nitid = *Nititidermes*, Micro = *Microtermes*, Allod = *Allodontermes*, Trine = *Trinervitermes*, Ancis = *Ancistrotermes*, Odonto = *Odontotermes*, Macro = *Macrotermes*. Level of significance (Tukey test results (z-values): '***' – 0.001; '**' – 0.01; '*' – 0.05) displayed above solid lines where applicable.

Table S2. Termite genera across feeding groups based on Uys (1993)

Termite Genera	Feeding Group
Allodontermes	mixed
Amitermes	wood
Ancistrotermes	mixed
Anguilitermes	dung
Nititermes (formerly Cubitermes)	soil
Macrotermes	mixed
Microcerotermes	wood
Microtermes	wood
Odontotermes	wood
Rhadinotermes	wood
Schedarhinotermes	wood
Trinervitermes	grass

Table S3. Mean consumption of wood (*Pinus* and *Terminalia)*, dung and grass attributed to microbes, termites, and other invertebrates in a mesic savanna (Wits Rural Facility).

*Calculation: Microbes = mean of control closed & treatment closed; Termites = control open – treatment open; Other invertebrates = treatment open – treatment closed.

Treatment and bag type	Consumption by	Wood *Pinus* (mean)	Wood *Terminalia* (mean)	Dung 56 days (mean)	Grass 112 days (mean)
control – closed	microbes	6.7	12.3	25.9	23.9
control – open	microbes + termites + other invertebrates	18.9	19.8	44.6	37.4
treatment – closed	microbes	7.0	12.9	21.5	22.8
treatment – open	microbes + other invertebrates	6.1	20.2	25.0	22.3

Table S4. Decomposition: k-values and half-life values for wood (*Pinus* and *Terminalia*), dung and grass. Values taken from open bags on the control plots.

Decomposition measures	Wood Pinus (mean)	Wood Terminalia (mean)	Dung 56 days (mean)	Grass 112 days (mean)
k-value	0.31	0.24	1.27	0.71
half-life (years)	3.29	5.12	0.24	1.14

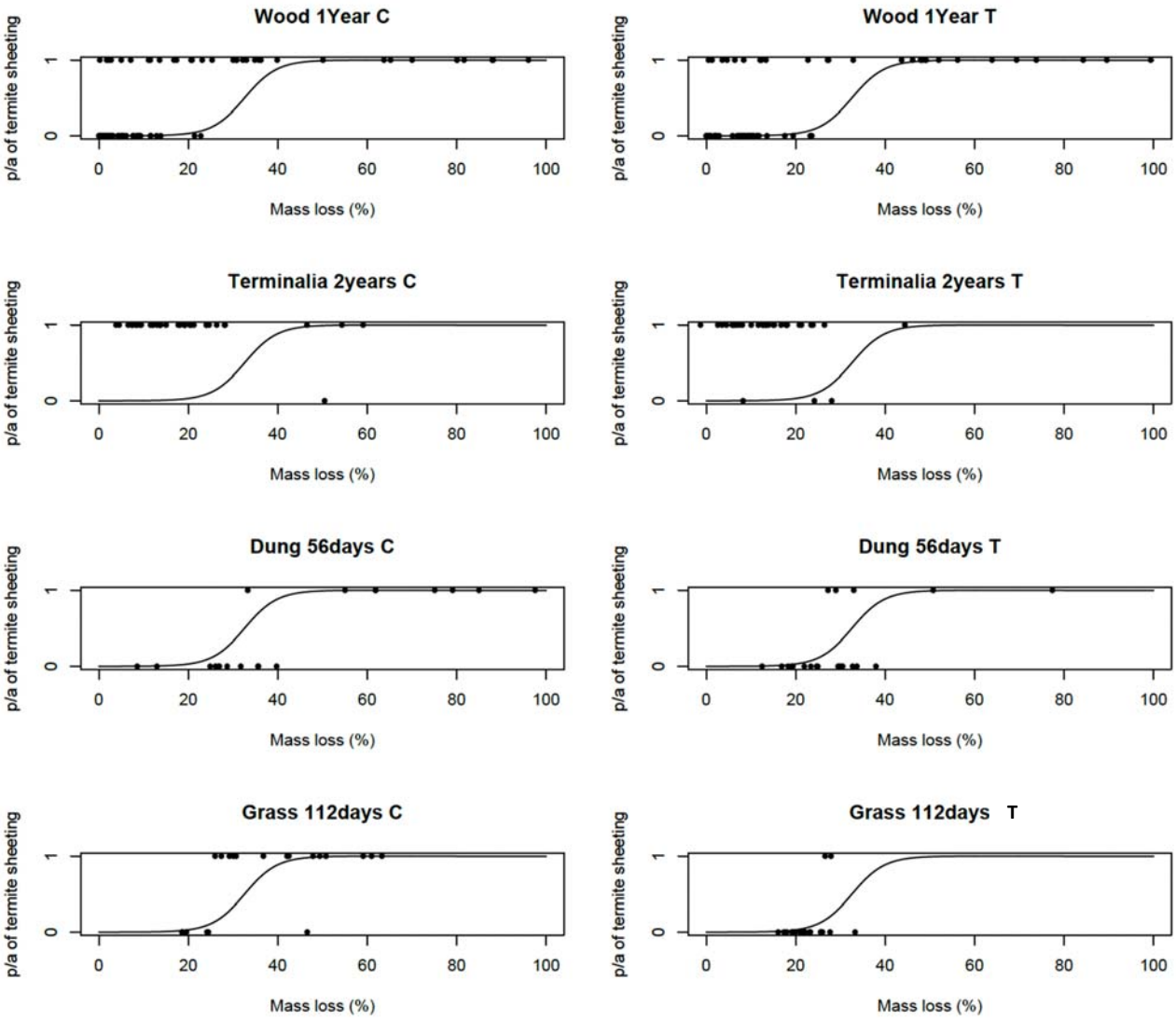

Figure S9. The relationship between the presence of sheeting in open bags and mass loss (%) within open decomposition bags containing wood 1 year, *Terminalia* 2 years, dung 56 days and grass 112 days across control (C) and suppression (T) plots, where 0 represents the absence of sheeting and 1 represents the presence. Logistics curves fitted for each regression.