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RESEARCH ARTICLE

Secondary compounds increase litter removal by termites across 23 savanna grass species

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Abstract

1. Termites—one of the most abundant animal groups in tropical ecosystems—are vital in nutrient recycling, contributing significantly to maintaining ecosystem functioning. However, how selective they are in their litter food choice, and whether they prefer nutritious or less nutritious litter substrates, are still important unresolved questions.
2. Here, we test the effect of litter traits on the removal of litter by fungus-growing termites, a dominant group of macrodetritivores in the Serengeti-Mara ecosystem. We used metal-mesh litterbags to measure the mass loss of stem and leaf litter from 23 grass species after an incubation period of 61 days.
3. Sheeting—soil deposits indicating termite presence—strongly increased with tannin and phenolic compounds, especially in leaf litter ($R^2 = 0.54$), where highly aromatic species like *Cymbopogon caesius* and *Bothriochloa insculpta* exhibited the highest sheeting rates. Litterbags with sheeting displayed 66% higher mass loss on average compared to those without. Once termites had selected the substrate, mass loss increased consistently irrespective of litter traits.
4. Quantifying the combined effects of sheeting frequency and differential mass loss, we found that fungus-growing termites predominantly increased the mass loss of high-nutrient litter. This was mainly due to additional mass loss in leaf litter, not stem litter, with tannin concentration being the primary predictor of enhanced overall decomposition by termites. This suggests termites ameliorate the otherwise adverse influence of plant secondary compounds on litter decomposition.
5. *Synthesis*: Our results support the idea that fungus-growing termites are generalists, as termites collected organic substrates with varying quality. The strong effect of phenolic compounds on sheeting rate implies that secondary compounds play an important role in termite substrate selection. The selective removal of substrates high in secondary compounds suggests fungus-growing termites lift important constraints to microbial breakdown, potentially enhancing ecosystem-level carbon and nutrient recycling rates. Termites play crucial roles in recycling organic matter across tropical biomes and our study highlights that the role of

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secondary compounds in their selection and detection in decomposition studies needs more attention

KEYWORDS
African savannas, decomposition, litter quality, litter traits, nutrient recycling, plant traits, plant–soil interactions, Serengeti, termites, Termitomyces

1 | INTRODUCTION

Termites are ecologically significant organisms in tropical ecosystems, playing a crucial role in nutrient recycling and maintaining ecosystem functioning and productivity (Govorushko, 2019; Griffiths et al., 2019; Jouquet et al., 2006; Veldhuis et al., 2017). The subfamily Macrotermitinae, within the family Termitidae, comprises fungus-growing termites which have emerged as dominant decomposers of plant material throughout the savannas of the Old World (Aanen et al., 2002; Jones & Eggleton, 2011). These termites can harvest up to 30% of annual litter production in savanna ecosystems (Wood & Sands, 1978), paralleling the organic matter turnover by mammalian herbivores and fires that contribute roughly 20% of carbon mineralization in these ecosystems (Bignell & Eggleton, 2000). Besides their important roles in nutrient recycling and redistribution, fungus growers are considered soil physical engineers that improve soil fertility and aeration through bioturbation (Jouquet et al., 2011). However, human disturbance and land use changes are now affecting termite mound distribution, which can reduce the ecosystem services provided by fungus-growing termites in human-dominated areas (Davies et al., 2020). These activities, by intensifying herbivory and changing fire frequencies, also drive shifts in plant species composition, subsequently altering the availability and characteristics of litter that termites rely on.

Fungus-growing termites are commonly considered generalists, capable of exploiting various food resources, from wood to grass (da Costa et al., 2019; Krishna et al., 2013), potentially rendering them less sensitive to shifts in litter properties. However, this generalist view may oversimplify the complex feeding strategies and preferences underlying their success. The efficiency of fungus-growing termites in consuming low-quality and stoichiometrically imbalanced food sources stems from a specialized three-way symbiosis with their gut microbiome and a basidiomycete fungus (Brune, 2014; da Costa et al., 2019). Nitrogen-fixing bacteria in the gut enable these termites to utilize nitrogen efficiently, enhancing the nutritional quality of their diet (Sapountzis et al., 2016). Additionally, mechanisms such as selective respiration by the fungal comb help regulate the C:N ratio of collected substrates, demonstrating their specialized adaptations to thrive on poor-quality substrates (Higashi et al., 1992; Nobre et al., 2011). These adaptations suggest that selecting high-quality substrates (with low C:N ratios) might not be as critical for fungus-growing termites. Moreover, consuming substrates rich in sugars may lead to rapid fermentation in their gut, resulting in by-products that could harm their digestive system (Abe et al., 2000).

Contrary to the generalist label, experiments have shown that fungus-growing termites demonstrate a degree of selectivity (Smith et al., 2019) and indeed select low-nutrient substrates over high-nutrient substrates (Ouédraogo et al., 2004; Shanbhag et al., 2019; Sundsdal et al., 2020). Most studies on termite foraging patterns typically examined only two or three substrate types, for instance, contrasting elephant or cattle dung with straw. As a result, it is challenging to determine whether termites indeed favour lower-quality substrates or whether other chemical traits are important as well, and whether there is selectivity within the same type of organic substrate (e.g. among leaf litter differing in quality among species). Mammalian dung may not be the preferred substrate for fungus-growing termites due to the higher probability of encountering entomopathogenic fungi or bacteria in dung than in dry plant litter (Freymann et al., 2008). Termite nests are warm and humid, which should make them prone to infectious diseases. Interestingly, termite nests are exceptionally sterile environments (Otani et al., 2019), effectively protecting the *Termitomyces* monoculture termites rely on. This sterility is likely maintained through a range of behaviours aimed at reducing pathogen transmissions, such as grooming, nest cleaning, secretion of antibiotics, avoidance of substrates exposed to mycopathogens and colony relocation (Bodawatta et al., 2019; Roy et al., 2006; Schmidt et al., 2022). Consequently, it is plausible to suggest that termite substrate preferences may be influenced by factors beyond simple nutrient content, including the necessity to maintain nest sterility.

While the significance of variation in litter traits across species in decomposability is well-established (Canessa et al., 2021; Cornwell et al., 2008; Freschet et al., 2012; Makkonen et al., 2012), a current imperative lies in understanding how dominant groups of macrodetritivores, such as fungus-growing termites, contribute to the mass loss of different litter substrates. Macrodetritivore effects generally increase with aridity (Sagi & Hawlena, 2023). For example, fungus-growing termites can accelerate recycling rates by as much as 123% under water-limited conditions (Veldhuis et al., 2017), highlighting the need to investigate how specific litter traits influence their foraging activities.

Here, we performed a litter decomposition experiment in an African savanna that included 46 different substrate types (23 grass species, both leaf and stem litter), in litter bags, to understand how litter traits predict litter turnover, both through non-termite-assisted decomposition and through substrate removal by fungus-growing termites. We focus on two measures: relative mass loss of litter substrates and the phenomenon of 'sheeting'. Sheeting, a behaviour

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commonly associated with fungus-growing termites in African savannas, involves creating a covering over litter substrates made from a mix of soil particles, saliva, and organic materials (Sileshi et al., 2010). This covering serves dual purposes: it acts as a protective barrier against UV radiation and modifies the microclimatic conditions around the litter, facilitating termite activity (Sileshi et al., 2010). It is crucial to note that while the presence of sheeting is a reliable indicator of termite presence, it does not directly equate to active termite foraging. Active foraging and litter removal is inferred from the observed changes in the mass loss of litter substrates. Therefore, in cases where sheeting is observed, any significant difference in mass loss between litter bags accessible to termites and those that are not can be confidently attributed to termite foraging activity. This distinction is important for understanding the termites' ecological role, particularly in how they select and process different litter substrates based on their traits.

Termite activity in litter decomposition may thus operate in two distinct phases: initial selection of the litter based on its traits, evident from the rates of sheeting, followed by the impact of these traits on the extent of mass loss post-colonization. Our research questions are therefore as follows: (1) What is the effect of litter traits on the percentage of sheeted litter bags (a measure of termite presence)? (2) How do litter traits affect mass loss of leaf and stem litter, both with and without the presence of fungus-growing termites? (3) How do litter traits influence the overall contribution of fungus-growing termites to litter mass loss, considering the combined effects of sheeting rates and differential mass loss between sheeted and unsheeted litter bags?

Based on our conceptual model (Figure 1), we propose three alternative hypotheses concerning the selectivity of fungus-growing termites in terms of interspecific variation in litter quality: (A) Fungus-growing termites do not preferentially select litter with high- or low-litter quality (with quality representing both nutrient content and plant secondary compounds). The added effect of termites on the mass loss of plant litter (on top of the effect of other soil-based decomposers on mass loss) is, therefore, equal across litter trait spectra. (B) Fungus-growing termites select litter with low quality, thereby ameliorating the well-established constraint of litter quality on decomposition by free-living soil microorganisms. (C) Fungus-growing termites select high-quality litter and thereby amplify the positive effects of nutrient-rich litter on mass loss.

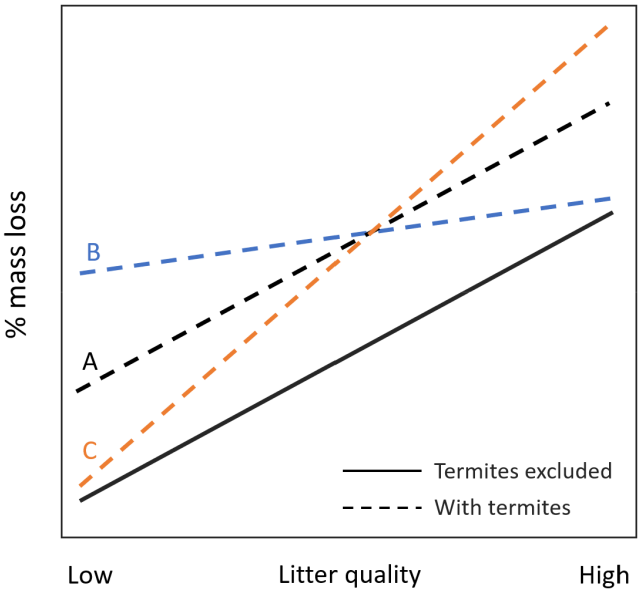


FIGURE 1 Conceptual model showing how fungus-growing termites may contribute to mass loss of litter with varying quality or other litter characteristics. A: Termites speed up the mass loss of all litter types equally (black dashed line) on top of the effect of the mass loss through the decomposing activities of the free-living soil microbiome (black solid line). B: Termites select low-quality litter, thereby lifting the constraint that poor-quality litter has on mass loss (blue dashed line). C: Termites select high-quality litter and thereby amplify the positive effects of high-quality litter on mass loss (orange dashed line).

2 | MATERIALS AND METHODS

2.1 | Study area and design

The study was conducted near the Serengeti Wildlife Research Centre in Seronera, situated in the centre of the Serengeti National Park (SNP), North-western Tanzania (Latitude 2°26′28.856 S, Longitude 34°51′7.865 E). Fieldwork was completed under research permit 2020-307-NA-2020-188, issued by the Tanzania Commission for Science and Technology (COSTECH). The Greater Serengeti-Mara ecosystem, which includes the SNP, is an expansive and well-preserved tropical savanna ecosystem renowned for its abundant and diverse wildlife, including termites (Sinclair et al., 2008). The area is inhabited by different genera of fungus-growing termites, such as *Macrotermes* spp. and *Odontotermes* spp., which contribute significantly to litter removal, underscoring their ecological impact on this ecosystem (Freymann et al., 2010). Mean annual rainfall is around 800 mm and peaks during the short rainy season in November–December and during the long rainy season in March–May (Mahony et al., 2021). The study area is characterized by undulating pediplains on granitic rocks (Jager, 1982) and a continuous C4-grass-dominated herbaceous layer with scattered trees and shrubs.

We set up a litter bed experiment during the rain-to-dry season transition from May to June 2021. This transition period is marked by litter production and, therefore, an appropriate time to study litter removal by termites. Metal-mesh litterbags were used to estimate leaf and vegetative stem litter decomposition for 23 grass species (see Table S1 for species list). These species were selected based on known differences in the quality of the fresh leaves (unpublished dataset by the authors), ensuring a wide range of litter trait values. For example, the leaf litter nitrogen content, a key litter trait, ranged from 0.46% in *Loudetia arundinacea* to 1.24% in *Digitaria abyssinica* while stem litter nitrogen content ranged from 0.20% in *Hyperthelia dissoluta* to 0.73% in *Chloris pycnothrix* (Table S1). The

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litter of each species was collected in February 2021 (the short dry season), as much as possible from the Seronera region to avoid any influence of soil properties or disturbance regimes on trait values. Undecomposed leaves and stems in an advanced state of senescence were collected for this study by carefully assessing the presence of residual green or yellow hues at the base of the leaves. This was done to avoid collecting samples that had been significantly influenced by photodegradation as such processes can strongly alter litter characteristics (McCalley & Sparks, 2009). The litter was air-dried in paper bags at ambient temperature until it reached a constant mass. This method was chosen over oven drying to avoid altering the litter's properties, thereby ensuring that its characteristics remained similar to field conditions until the start of the experiment. A subsample of each litter type was kept separate for the litter trait and moisture content measurements. We oven-dried subsamples at 70°C for 48 h for each litter type (species × organ) to correct for the differences in moisture content in the pre-incubation air-dried litter substrates. The metal-mesh litterbags (15 × 15 cm, mesh size 1 mm) were filled with 0.5 g (±0.001 g) of each litter type at the start of the experiment. The 1 mm mesh size of the litterbags was specifically chosen to exclude larger macroinvertebrates, particularly fungus-growing termites, while allowing smaller soil-based decomposers to access the litter, thereby facilitating a comparative analysis of litter turnover with and without the influence of these larger termite species. A double mesh layer was used to prevent litter from falling out of the litterbags and as extra protection against fungus-growing termite intrusion. Half of the litterbags had four 1 cm^2 openings at the bottom of the litterbags to allow termite access while having a likely negligible effect on the microclimate inside the mesh bags. The litterbags were installed in three sites—at least 200 m apart—in close proximity to active termite mounds. While the specific termite species were not identified, the observed mounds were low, flattened and featured open ventilation systems, characteristics typical of *Odontotermes* sp. mounds (Uys, 2002). Although no other mounds were visible in the immediate vicinity, contributions from other fungus-growing termites in the area cannot be ruled out. For each species × organ (leaf or stem) combination, we installed two litterbags that were accessible to termites and two that were not. The litterbags were placed at random in a circle around the termite mound at an approximate distance of 15 m from the edge of the termite mound. In total, 552 litterbags (23 species × 2 organs × 2 treatments × 3 sites × 2 replicates) were incubated for 2 months.

2.2 | Sheeting and mass loss measurements

After collection, litterbags were first visually examined for 'sheeting' on the remaining litter or the litterbag itself, which indicates termite presence (see Section 1). Litterbags were also inspected for integrity. Fungus-growing termites have a remarkable capacity to break down a variety of materials, including some forms of metal mesh (Smith et al., 2019). In our experiment, the metal mesh was not damaged. However, 44 of the 'inaccessible' litter bags had sheeting on the litter inside, a significant number despite the lower sheeting rate than for the 'accessible' litterbags, where 117 litterbags were sheeted. This indicates that our treatment designed to restrict termite access was not fully effective. Consequently, we adjusted our analysis to focus solely on comparing mass loss between sheeted and unsheeted bags, directly assessing the impact of fungus-growing termites. For future studies, we recommend using a finer mesh size (0.5–0.8 mm) to more effectively control access of fungus-growing termites. The remaining litter was extracted from the litterbags, oven-dried at 70°C for 48 h and weighed. In addition, to account for the weight of soil particles (whether in sheeting or loose) mixed in with the remaining litter, the mix was ashed for 6 h at 550°C. The difference in weight before and after ashing represents the undecomposed organic component of the litter. To correct for the undecomposed minerals, we ashed subsamples to obtain the ash fraction for each litter type. The weight before (moisture-corrected) and weight after (ash-corrected) incubation was used to calculate litter mass loss and was expressed as a percentage of initial dry mass. Unfortunately, the first batch of 78 samples that were ashed were lost due to incorrect settings of the furnace leading to only 474 samples for the analysis. These samples were distributed across the different substrates in a randomized manner, meaning no specific species-organ combination was completely lost. Specifically, for the final analysis we had 130 unsheeted leaf litter, 91 sheeted leaf litter, 183 unsheeted stem litter and 70 sheeted stem litter.

2.3 | Litter traits

Of each of the 23 grass species, we measured the following litter traits: mass-based carbon content (% C), nitrogen content (% N), phosphorus content (% P), phenolic compounds (%), tannins (%), magnesium (Mg, ppm), calcium content (Ca, ppm) and pH. In the same locations where we collected leaf and stem litter, we additionally collected leaf and stems of green plants to measure dry matter content of leaves (LDMC) and stems (SDMC) in order to have an indicator of the physical toughness of the litter substrates. The nutrients and structure were measured because they have been widely recognized in previous studies as important predictors of litter decomposition rates through both microbial decay and the activities of (macro)detritivores (Aerts, 1997; Freschet et al., 2012; Liu et al., 2015). Meanwhile, pH serves as a proxy for basic cation content and antimicrobial organic acids (including secondary compounds involved in plant defences), influencing decomposition processes across different decomposer groups (Cornelissen et al., 2006). For each species, leaf litter and stem litter were individually ground into fine powder and then each type was thoroughly mixed separately to ensure a representative and homogenous sample for the respective leaf and stem analyses. The content of C and N was determined by dry combustion using an elemental analyser (Flash EA 1112, Thermo Scientific, Rodana, Italy). For P content, a 50 mg subsample was digested in 1 mL of a 1:4 mixture of 37% (v/v) HCl and 65% (v/v) HNO_3, in a closed Teflon cylinder for 6 h at 140 C. Samples were

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then diluted with 4 mL demineralized water and total P content was quantified by spectrophotometry, using the ammonium molybdate method (Murphy & Riley, 1962). Total phenolic content was measured with the Folin Ciocalteau method after extraction in 50% methanol, using tannic acid (Merck, Darmstadt, Germany) as a standard. Tannin content was calculated after removing the tannins from the extract with PVPP and subtracting the remaining non-tannin phenolics from the total phenolics (Makkar, 2003). The pH-H2O was measured according to Cornelissen et al. (2006). Briefly, a 0.15 mL sample was added to 1.2 mL demi-water in an Eppendorf tube and shaken for 1 h at 250 rotations per minute. Then the tubes were centrifuged for 5 min at 13,000 rpm, and the supernatant was used to measure pH-H_2O value using a narrow (5 mm diameter) SenTix Mic electrode connected to an Inolab Level 2 pH meter (both: Wissenschaftlich-Technische Werkstätten GmbH & Co. KG, Weilheim, Germany). Ca and Mg, extracted using acid digestion with a 1:4 mixture of HCl and HNO3, were measured with atomic absorption spectroscopy (1100B Spectrometer, PerkinElmer Inc., Waltham, MA, USA). For the dry matter content measurements, we followed the protocol for measuring functional traits by Cornelissen et al. (2003), and collected five mature, sun-exposed leaves and stems of five individuals as much as possible in one area. We weighed the fresh leaves and stems immediately after collection in the field and then oven-dried them at 70°C for 48 h to obtain the dry weight. LDMC and SDMC were calculated as the ratio of dry weight to fresh weight.

2.4 | Data analysis

Some of the litter traits data (P, Mg, Ca, phenolic compounds, tannins, and phenolic compound to N ratio) were log-transformed to correct for deviations from normality. We used a Principal Component Analysis (PCA) to identify patterns and relationships among the 11 litter traits. All variables were centred and scaled to ensure standardization. The first two principal components (PC1 and PC2), which explained a high proportion of variance, were used as proxies for overall litter quality in subsequent analyses. This approach aligns with established methodologies (see Freschet et al., 2012) for summarizing trait data. The specific interpretation of PC1 and PC2 in terms of litter traits is detailed in the results section. Initially, linear mixed models (LMM) were fitted using the *lme4* package in the R coding environment (Bates et al., 2007). Litter quality (PC1, PC2), termite presence (sheeted, unsheeted), and their interaction were used as fixed terms, and site was used as a random term. The effect of the different sites was similar to the extent that between-site variability was insufficient (estimated variance = 0), leading to a singular fit. Therefore, we averaged the mass loss for each species × organ combination across the sites and conducted an analysis of covariance (ANCOVA), with termite presence (sheeted/unsheeted) as a factor and the PCs as covariates. Due to some substrate types being more frequently sheeted than others, resulting in uneven sample sizes for 'termite presence', we applied weights in the ANCOVA to balance the influence of each group and ensure a fair comparison of mass loss between sheeted and unsheeted conditions. All candidate models were evaluated based on AIC scores (Burnham & Anderson, 2004). The best model was used to construct partial residual plots using the *Visreg* package to visualize the results (Breheny & Burchett, 2017). Model fit was assessed using R^2; the proportion of explained variance by each model. To assess how litter traits explain termite presence, we calculated the percentage of litterbags with sheeting for each litter type. Some of the closed litterbags had sheeting on the outside (rather than on the litter inside of the litterbag), which could mean that the termites may have been attracted to the substrate but could not access it. Therefore, we used all 12 litter bags (accessible + inaccessible) in the calculation of percentage sheeted bags. This approach also takes into account the possibility that termites breached 'inaccessible' bags in response to particularly attractive substrates. We acknowledge that termites might have entered both the 'inaccessible' and 'accessible' bags, inspecting the substrate without necessarily finding it suitable for collection, as indicated by the absence of sheeting, which fungus-growing termites typically produce only when actively harvesting a substrate. The observed percentage of sheeting, therefore, does not necessarily indicate 'detection' of the substrate by fungus-growing termites. Instead, it serves as an indicator of their active effort to harvest the material, whether they were successful in this attempt or not. Because the percentage data comes from discrete counts, we analysed the percentage sheeted litterbags with beta regression using the *betareg* package (Zeileis et al., 2016). The model with the lowest AIC score was selected (Burnham & Anderson, 2004) and model fit was assessed using pseudo R^2. Finally, to assess the overall impact of termites on litter decomposition, we calculated the additional mass loss attributable to termites by multiplying the sheeting rate by the difference in mass loss between sheeted and unsheeted litterbags. To identify litter traits that best predict termite-induced mass loss, we used linear regression to analyse both principal components (PC1 and PC2) and individual traits against the calculated additional mass loss. All analyses were performed in the statistical environment R, version (4.2.3) (R Core Team, 2023).

3 | RESULTS

3.1 | Variation in litter traits

The first two principal components accounted for 65% of the total variation in litter traits among species and organs. PC1 (42%) was primarily associated with nutrient-related traits (N, Mg, Ca) and the structure-related trait DMC, in the opposite direction (Figure 2). Total phenols and tannins were also associated with this axis, in the same direction as the nutrients (Figure 2). PC2 (23%) was associated with the phenolic compounds to N ratio and pH, indicating a possible relationship with plant defence traits. Phenols, tannins and P were also associated with PC2, in opposing directions (Figure 2). In summary, both PC1 and PC2 are related to defence and nutrient-related traits; however, PC1 predominantly represents nutrient-related

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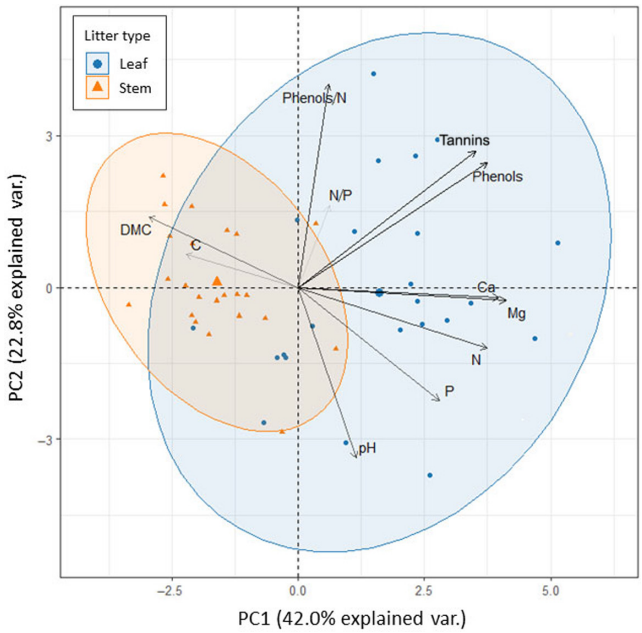


FIGURE 2 Principal Components Analysis (PCA; first vs. second axes) of stems (blue circles) and leaves (orange triangles) of 23 grass species characterized by 11 litter traits. The ellipses indicate the 95% confidence intervals of trait variation of leafs (blue) and stems (orange). The length of the arrows corresponds to their significance, and the angle between two arrows represents the strength of the correlation between variables. For a list of used traits and abbreviations, see Table S1.

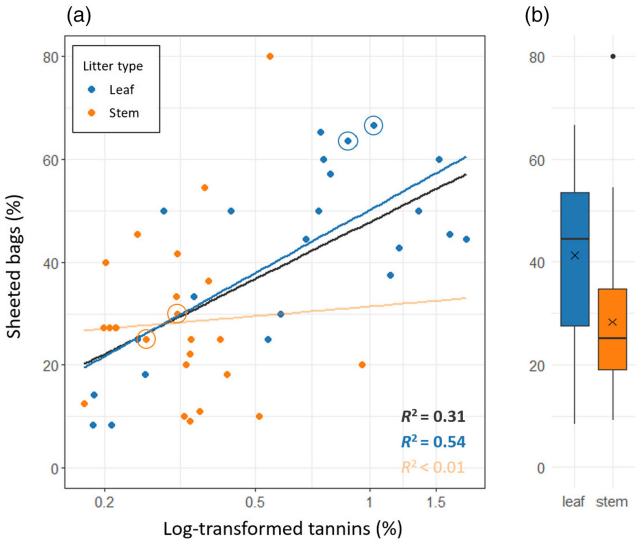


FIGURE 3 Relationship between the percentage tannins (log-transformed) in leaf (blue) and stem (orange) litter and the percentage of sheeted litterbags (a). Solid lines represent regression lines for leaf litter (blue line; beta regression, $Z = 4.670$, $p < 0.001$, pseudo $R^2 = 0.54$), stem litter (orange line) and both litter types combined (black line: Beta regression, $Z = 4.447$, $p < 0.001$, pseudo $R^2 = 0.31$). The two leaf litter substrates with high aromatic compounds (leaf litters of *Cymbopogon caesius* and *Bothriochloa insculpta*) are highlighted with a blue circle. The stem litter of both aromatic species are highlighted with an orange circle. Boxplots indicate the interquartile range of sheeting percentages for leaf (blue) and stem (orange) litters, with medians and means denoted by lines and 'x' symbols, respectively (b).

traits as all nutrient indicators load on this axis, whereas PC2 is associated with defence traits, with only phosphorus showing a significant loading on this axis. As expected, leaf litter had higher nutrient content than stem litter, as indicated by the higher positive values along PC1 (Figure 2). Leaf litter traits were also more variable across species than stem litter.

3.2 | Termite presence

Of the 474 litterbags that were included in the final analysis, 161 had sheeting (34%). On average, leaf litter substrates had a higher incidence of sheeting at 41.3%, across species compared with 28.3% for stem litter substrates (Figure 3). The best predictor of sheeting on litterbags was litter tannin concentration and this relationship was strongly driven by variation in leaf litter tannins (Figure 3). Total phenolic compounds also predicted the percentage of sheeting on leaf litter reasonably well (beta regression, $Z = 3.992$, $p < 0.001$, pseudo $R^2 = 0.45$) and overall better than PC1 ($Z = -2.222$, $p = 0.026$, pseudo $R^2 = 0.19$) and PC2 ($Z = -2.618$, $p = 0.009$, Pseudo $R^2 = 0.23$). Two of the substrates that had the highest sheeting percentages (>60%) were leaf litters of *Cymbopogon caesius* and *Bothriochloa insculpta*, species that are known to be aromatic (Figure 3, blue circles). When only considering stem litter, tannin concentration did not correlate with sheeting incidence (Figure 3, orange line), nor did any other traits.

3.3 | Leaf and stem litter mass loss

Percentage mass loss of litter increased by 66% in litterbags with sheeting (mean = 33.5%, SD = 13.1) compared to litterbags without sheeting (mean = 20.2%, SD = 10.1). Mass loss of stem litter nearly doubled (+95.5%) in litterbags that were accessed by fungus-growing termites (mean = 24.4%, SD = 10.4) compared with those that were not accessed (mean = 12.5%, SD = 5.0). Although leaf litter displayed a slightly greater absolute difference in mass loss due to sheeting than stem litter, the relative increase was less pronounced at 53.1% for sheeted versus unsheeted litterbags (mean = 42.6%, SD = 8.3% and mean = 27.8%, SD = 7.7%, respectively). This implies that fungus-growing termites may be particularly effective at accelerating mass loss in stem litter. Nutrient-rich litter (high PC1 values; $p < 0.001$) and litter with lower levels of chemical defences (low PC2 values; $p < 0.001$) experienced the highest percentage of mass loss (Figure 4, Table S2). Termite access increased mass loss consistently irrespective of litter quality, with no significant interactions between the different trait spectra and termite intrusion (Figure 4, Table S2).

The additional mass loss of litter attributed to fungus-growing termites—determined by multiplying the difference in average mass loss between sheeted and unsheeted litterbags by the sheeting rate—was best predicted by PC1, with the greatest effect of termites on high-nutrient litter (Figure 5a). This is not surprising, given the much higher

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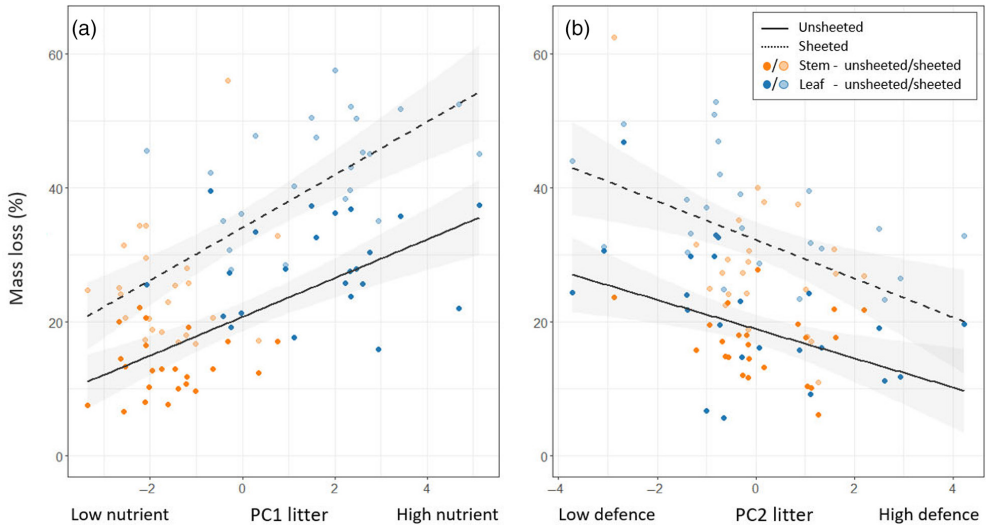


FIGURE 4 Partial residual plots showing the effect of the two main axes of trait variation PC1 (related mostly to nutrient content, a) and PC2 (related to chemical defences, b) on leaf and stem litter mass loss (%) for litterbags with signs of fungus-growing termite presence (sheeted; dashed lines) and without signs of their presence (unsheeted; solid lines; $F_{3,88}=61.79$, adj. $R^2=0.67$, $p<0.001$). The partial residual plots are based on the best model and allow for the evaluation of the effect of each predictor on the response variable, given the effect of the other predictor in the model.

sheeting rate on high-nutrient leaf litter compared to (generally low-nutrient) stem litter (Figure 3). For leaf litter, tannin concentration was the best predictor of additional mass loss (Figure 5d) while %N was not correlated with additional mass loss for either leaf or stem litter separately (Figure 5c). The higher contribution of fungus-growing termites to mass loss of high-nutrient litter is thus best explained by the increased sheeting on leaves (Figure 3), which in turn is driven by the higher tannin concentration in leaves (Figure 2).

4 | DISCUSSION

Our study investigated the role of leaf and stem litter traits on litter turnover focusing particularly on the impact of fungus-growing termites within the broader framework of soil-based decomposition processes. We found support for an amplified contribution of termites to the mass loss of high-nutrient litter (Figure 5). Therefore, we reject the hypothesis that fungus-growing termites select low-quality litter (Figure 1, line B), at least on the nutrient content dimension of litter quality (Figure 5). However, the apparent selection of high-nutrient litter may be indirectly influenced by higher tannin concentrations typically associated with nutrient-rich leaf litter compared with nutrient-poor stem litter (Figure 2). Tannin concentration was a key predictor for both termite sheeting (Figure 3), and the resultant additional mass loss caused by termites (Figure 5). This suggests that the increased sheeting on high-tannin substrates controlled the overall contribution of fungus-growing termites to litter decomposition. Therefore, our study does not falsify the hypothesis that fungus-growing termites select low-quality litter in relation to plant secondary compounds (Figure 6). These results raise new questions about the mechanistic role of tannins, and phenolic compounds in general, in regulating substrate removal by fungus-growing termites. Termites may be attracted to litter containing phenolic compounds and actively select for it (i.e. preference). Alternatively, but not mutually exclusive from preference, chemical cues associated with litter high in tannins and phenolics may aid termites in locating food sources within their environment (i.e. detection). Below, we will elaborate on these two mechanisms, aiming to shed light on the intricate interplay between termite behaviour, chemical signalling, and the ecological significance of litter traits in shaping the dynamics of substrate removal by fungus-growing termites.

While several prior studies have indicated that fungus-growing termites select low-quality litter substrates (Ouédraogo et al., 2004; Shanbhag et al., 2019; Sundsdal et al., 2020), we found the opposite result when including a large number of different substrates of less extreme qualities. A possibility is that the limited range of nutrient content in our study may have influenced the termites' selection behaviour, potentially masking preferences that could have emerged under conditions of wider nutrient variability. For instance, the green tea used in Sundsdal et al. (2020) has a C:N ratio of 12.23 (Keuskamp et al., 2013), which is much lower than the C:N ratio of the grass litter substrates in the current study. However, the grass species used in our experiment represent the full spectrum of litter quality available in the ecosystem, ensuring a realistic representation of the termites' natural foraging choices, at least for the grass litter-feeding fungus-growing termites. A second explanation for the positive effect of termites on the mass loss of high-nutrient litter could be that nutrient content and plant secondary compounds were linked in our study. Leaf litter not only had higher nutrient content, but also higher phenol and tannin content than stem litter (Figure 2). The large effect of termites on the additional mass loss of leaf litter, as compared to stem litter, may thus be mostly driven by the selection (or improved detection) of litter high in secondary compounds

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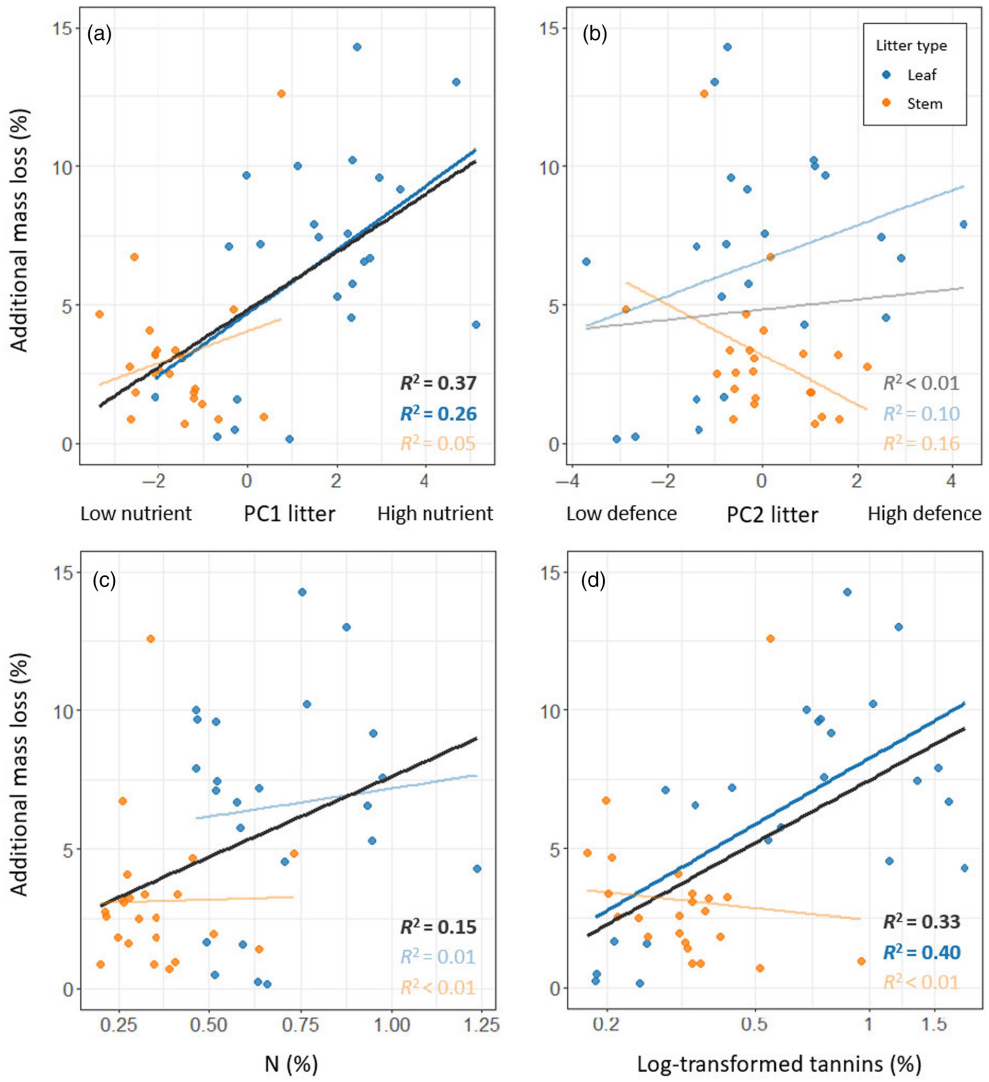


FIGURE 5 Effect of litter quality on the additional mass loss by fungus-growing termites, accounting for both the frequency of sheeting and the average mass loss across litter types. Shown are the regression lines for leaf litter (blue lines), stem litter (orange lines) and both litter types combined (black lines), where bold lines represent significant correlations. Additional mass loss was highest for high-nutrient litter (a, black line; $F_{1,44} = 25.65$, $R^2 = 0.37$, $p < 0.001$) while PC2 was not related to additional mass loss through termite activity (b, black line). Specifically for leaf litter, tannin concentration was the best predictor of additional mass loss ($F_{1,21} = 14.08$, $R^2 = 0.40$, $p = 0.001$; d, blue line), while %N (which just like tannins was related to PC1) did not predict additional mass loss within leaves or stems (c, blue and orange lines).

(Figures 3 and 5) with no role for nutrient content at all. This explains why the analyses revealed no significant relationships between stem litter traits with either sheeting (Figure 3) or termite-assisted mass loss (Figure 5). Given that all stem litter contains low levels of plant secondary compounds (Table S1), it is expected that the nutrient content (%N; %C) should predominantly influence stem litter mass loss over the effects of secondary compounds.

Termites could select for secondary compounds for several reasons. First, phenolic compounds—such as hydroquinone—have been identified as a key phagostimulating signal used by termites to mark their feeding sites (Reinhard et al., 2002). This signalling mechanism appears to be widespread among termite species globally (Reinhard et al., 2002). Substantial quantities of hydroquinone and its derivative—methyl hydroquinone—have been identified in extracts from soldiers of the fungus-growing termite species *Macrotermes bellicosus* (Hammoud Mahdi et al., 2020). Hydroquinone is a compound also commonly produced by plants, including grasses in the *Cymbopogon* genus (Gaba et al., 2020), as part of their secondary metabolites. Termites may be attracted to leaf litter containing hydroquinone, potentially because it marks the feeding sites of termite colonies. Other aromatic compounds or substances with similar chemical properties might also play a role in termite signalling. Termites are highly sensitive to chemical cues, and it is possible that compounds sharing structural similarities with hydroquinone (i.e. benzene rings) could also attract termites to potential feeding sites. This could explain why the highly aromatic leaf litters of *B. insculpta* and *C. caesius* (authors' field experience) had such high sheeting percentages in our experiment

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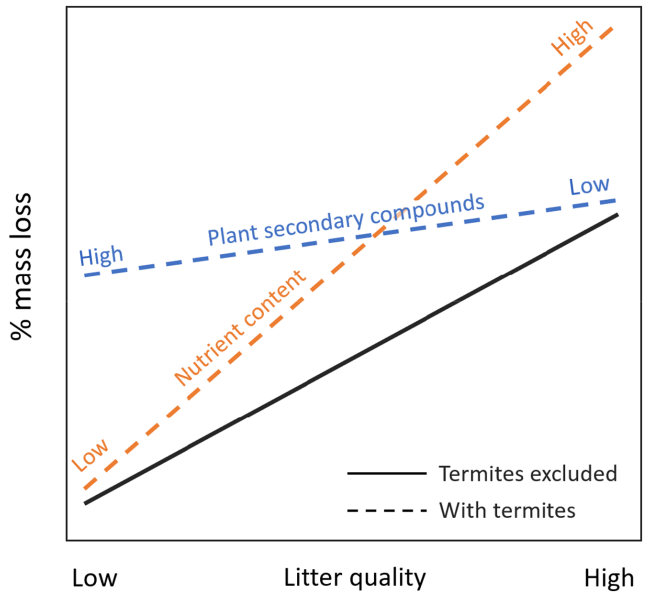


FIGURE 6 Contribution of fungus-growing termites to mass loss of litter with varying quality. Fungus-growing termites were found to speed up the mass loss of high-nutrient litter and thereby amplified the positive effects of high-nutrient litter on mass loss (orange dashed line). At the same time, termites selected litter high in tannins, thereby lifting the constraint that leaf litter rich in plant secondary compounds had on mass loss (blue dashed line).

(Figure 3). In a multispecies litterbed study in subtropical China, Guo et al. (2023) found that termites accelerated gymnosperm litter decomposition much faster compared with angiosperm litter. The authors linked these results to aromatic compounds (i.e. resins) as well (Guo et al., 2023).

In addition to making litter more detectable, termites may also favour litter rich in secondary compounds to prevent disease. Phenolic compounds have antimicrobial properties which could be beneficial against fungal competitors of the *Termitomyces* fungiculture inside the termite mounds. Fungus-growing termites promote the activity of their fungal symbiont by optimizing the humidity and temperature inside their mounds (Aanen & Eggleton, 2005). In combination with the constant addition of plant litter, the fungal garden should be prone to infections (Schmidt et al., 2022). Termites avoid disease by avoiding mycopathogen-exposed litter substrates that could harm their crop (Bodawatta et al., 2019). It is plausible that the phenolic compounds in grass litter keep it free from mycopathogens for extended periods while accumulating in the top layer of the soil; this could make these substrates more attractive to fungus-growing termites. The fact that *Termitomyces* spp. themselves produce a range of phenolic and tannic acids with strong antimicrobial activity against both bacteria and fungi (Borges et al., 2013; Puttaraju et al., 2006; Schmidt et al., 2022) underscores the importance of suppressing competitors. Two of the phenolic acids produced by *Termitomyces* spp., ferulic acid and caffeic acid, are also found in grass species (Lowry et al., 1993) and are likely still present in the litter of these species. The collection of litter rich in phenolic and tannic acids with high antimicrobial activity on top of the production of these compounds by *Termitomyces* spp. may provide a comprehensive strategy for termites to maintain the health and productivity of their fungal gardens in the face of potential infections and competitors. They would not be the only nest or mound-building insects using plant materials as 'antibiotics': Formica wood ants use resin to protect themselves against pathogens as well (Chapuisat et al., 2007).

Plant secondary compounds in senesced plant material slow litter decomposition by free-living soil microorganisms (Hättenschwiler & Vitousek, 2000; Kraus et al., 2003; Makkonen et al., 2012). The selection of litter substrates high in plant secondary compounds and consequent increased mass loss of these substrates compared to litterbags inaccessible to termites, confirms the idea that fungus-growing termites lift important substrate quality-related constraints to microbial activity and thereby increase ecosystem homeostasis of carbon and nutrient recycling rates (de Jonge et al., 2023). Because plant secondary compounds play important roles in mitigating abiotic stress in plants (Possell & Loreto, 2013), current and future changes in climate (e.g. droughts, rising temperatures and CO_2 levels) can alter plant secondary compounds production in plants (Holopainen et al., 2018; Peñuelas & Estiarte, 1998), with cascading effects on soil functioning and the decomposition process (Ehlers et al., 2020). Fungus-growing termites may therefore have an important buffering effect against increasing substrate limitations (de Jonge et al., 2023). Further research is needed to assess the roles (e.g. signalling, antibiotics) of specific compounds (e.g. hydroquinone, ferullic acid) that may be responsible for the selective removal of litter by fungus-growing termites. Moreover, experiments are needed to uncover the mechanism (detection, preference or both) behind the selection of litter high in phenols and tannins.

AUTHOR CONTRIBUTIONS

Inger K. de Jonge, J. Hans C. Cornelissen, and Michiel P. Veldhuis conceived the ideas and designed the methodology; Inger K. de Jonge collected the data; Inger K. de Jonge and Richard S. P. van Logtestijn analysed the data; Inger K. de Jonge led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

To the best of our knowledge, none of the above-mentioned persons has any conflict of interest, financial or otherwise. Hans Cornelissen

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is an Associate Editor for *Journal of Ecology*, but took no part in the peer review or decision-making process for this manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data are available from the Zenodo Digital Repository: https://zenodo.org/doi/10.5281/zenodo.12592492 (de Jonge, 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Table of measured litter traits of stems and leaves of 23 grass species: Dry matter content (DMC, ratio), mass-based carbon content (% C), nitrogen content (% N), phosphorus content (% P),

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phenolic compounds (%), tannins (%), magnesium (Mg, ppm), calcium content (Ca, ppm) and pH used in the PCA analysis.

Table S2. Model fits (AIC, the Akaike information criterion) for percentage mass loss, considering different predictors (PC1, PC2, and termite sheeting). Models are ordered based on AIC scores in ascending order. The best model (lowest AIC score) is indicated in bold.

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