

Litter type and termites regulate root decomposition across contrasting savanna land-uses

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ABSTRACT

Decomposition is a vital ecosystem process, increasingly modified by human activity. Theoretical frameworks and empirical studies that aim to understand the interplay between human land-use, macro-fauna and decomposition processes have primarily focused on leaf and wood litter. For a whole-plant understanding of how land-use and macro-fauna influence decomposition, investigating root litter is required. Using litterbags, we quantified rates of root decomposition across contrasting tropical savanna land-uses, namely wildlife and fire-dominated protected areas and livestock pastureland without fire. By scanning litterbags for termite intrusion, we differentiated termite and microbial driven decomposition. Root litter was buried underneath different tree canopies (leguminous and non-leguminous trees) and outside canopies to account for savanna landscape effects. Additionally, we established a termite cafeteria-style experiment and common garden to explore termite selectivity of root litter and root trait relationships, respectively. After one year, we found no significant differences in root litter mass loss between wildlife dominated areas and pastureland. Instead, we found consistent species differences in root litter mass loss across land-uses and additive and non-additive effects of termites on root decomposition across plant species. Termite selectivity for root litter species occurred for both root and leaf litter buried near termite mounds, but was not explained by root traits measured in the common garden. Termite foraging was greater under leguminous tree canopies than other canopies; however, this did not influence rates of root decomposition. Our study suggests that land-use has a weak direct effect on belowground processes in savannas. Instead, changes in herbaceous species composition and termite foraging have stronger impacts on belowground decomposition. Moreover, termites were not generalist decomposers of root litter, but their impact varies depending on plant species identity and likely associated root traits. This root litter selectivity by termites is likely to be an important contributor to spatial heterogeneity in savanna nutrient cycling.

Keywords: leguminous trees, plant–soil interaction, root traits

INTRODUCTION

Plant productivity is increasingly utilised and altered by humans globally. Importantly, this extends to the afterlife of plants – or plant litter (Dias et al. 2017). Across biomes, large-scale replacement of wild herbivores by livestock is changing the balance between processes that regulate litter production and removal (Bakker et al. 2004, Cornwell et al. 2008, Hempson et al. 2017). Typically, litter decomposition is regulated through microbes, burning and soil fauna (Cornwell et al. 2009). However, more intense livestock grazing can suppress fire frequency and intensity by consuming so much wet season plant biomass that there is insufficient litter fuel-load in the dry season. In turn, litter removal and decomposition becomes more dependent on soil fauna and microbes. Theoretical frameworks that aim to better understand this interplay between herbivory, fire and litter decomposition have mainly considered leaf and woody litter (Cornelissen et al. 2017, Grootemaat et al. 2015). However, root litter can potentially interact differently with herbivory and fire. For example, roots often escape elevated temperatures and consumption by fire as they are insulated from its effects by soil (Coetsee et al. 2010). At the same time, herbivory and fire can modify species composition and aboveground litter quality and similar changes may occur in root litter (Buitenwerf et al. 2011, Davies et al. 2013, Okullo and Moe 2012). Thus, consideration of the impact of herbivory and fire dominated land-uses on root decomposition is essential in order to improve the understanding of processes regulating litter.

Soil fauna consume, digest and/or stimulate microbial activities, accelerating litter decomposition in many tropical ecosystems. In savanna ecosystems, termites are the primary soil fauna decomposer shaping the spatial distribution of nutrients. Many termite species, but particularly fungus-growing termites (family *Macrotermitinae*), concentrate litter-derived carbon and nutrients within their nests (Jones 1990, Pringle et al. 2010, Sileshi et al. 2010). Humans can intervene in termite nutrient cycling by preventing nest formation or destroying and/or redistributing soil from nests (Jones 1990). However, more commonly humans intervene indirectly via changes in herbivory (Buitenwerf et al. 2011, Okullo and Moe 2012) or fire regimes (Davies et al. 2013, Moe et al. 2009), thus altering the plant species composition, litter quantity and quality and termite foraging patterns. That said, changes in species and litter quality may have little impact on termite foraging with a number of studies suggesting that termites have a weak preference for different species' leaf, wood and woody root litter (Collins 1981, Manlay et al. 2004, Parker et al. 1984, Veldhuis et al. 2017, Whitford et al. 1988). Yet, many of these studies consider only two to three plant species. Given potentially larger interspecific physiochemical and morphological variation in roots compared to aboveground plant pools (Freschet et al. 2013, Freschet et al. 2017), we would expect strong root litter selectivity by termites. Whether or not termites are selective

root litter foragers will have important implications for their role in redistributing nutrients in savanna ecosystems. Assuming a diverse plant community, weak root litter foraging selectivity would reinforce greater concentration of nutrients within the ‘fertility islands’ of termite nests (Sileshi et al. 2010), whereas strong selectivity, with plant species being avoided by termites, would alter the spatial distribution of belowground nutrient cycling with more litter decomposing *in situ* below plants.

Savanna ecosystems are characterized by the coexistence of grasses and trees (Scholes and Archer 1997). Due to their more extensive spread of roots as well as symbioses with nitrogen fixing microbes, mature savanna trees can alter sub-canopy water and nutrient availability and thus belowground processes (Belsky et al. 1989). Conditions underneath mature savanna trees are typically cooler, wetter and enriched in nitrogen, particularly underneath leguminous trees with nitrogen fixing symbionts (Belsky 1994, Blaser et al. 2013). Soil microorganism diversity can also be greater underneath savanna trees (van der Heijden et al. 2008). Therefore, presence of trees would be expected to accelerate root decomposition; however, in wetter regions trees have been shown to compete and reduce soil water and nutrient availability (Ludwig et al. 2004a,b), thus potentially inhibiting the decomposition process. Mutualistic ants, associated with many savanna tree species, prey upon foraging termites (Palmer 2003), thus, in turn, presumably reducing termite litter removal. Thus, trees potentially influence multiple drivers of belowground decomposition simultaneously, though it remains unclear which (if any) of these drivers have a prevailing influence on rates of root litter decomposition underneath trees.

In this study we attempted to unravel the complexity of interacting processes regulating root litter decomposition in savanna ecosystems. We aimed to: (1) quantify rates of annual root litter decomposition across contrasting land-uses (wildlife and fire-dominated protected areas versus livestock pastureland without fire) for four widespread grass species; (2) Contrast rates of microbial versus termite driven root decomposition; and (3) Investigate the relationship between root decomposition and tree canopy (underneath leguminous and non-leguminous trees and outside canopies). Overall this allowed us to test the influence of land-use, litter type, termites and tree canopy on root decomposition.

METHODS

Study area and experimental design

The root litter decomposition study was undertaken inside and outside wildlife management areas surrounding the Serengeti National Park, Tanzania (latitude 2° to 2°30'S and longitude 34° to 34°30'E). We focused our study in the savanna woodlands in the northern and central region of Serengeti to minimize spatial variation in rainfall, elevation, soil type and dominant canopy tree species (Table S1).

During the incubation period, rainfall for our study regions ranged from 760 mm in wetter west to 500 mm in drier east and central Serengeti (Huffman 2017), while average soil temperatures were 29°C in the upper 10 cm (McNally et al. 2017). This rainfall is seasonal with a distinct dry season from June to October with typically <50mm of rain, and a bimodal wet season between November and May including a short and long rainfall periods (Krebs 1999). Elevation of selected sites varied from 1360 to 1935 m a.s.l. Underlying soil type across the northern and central region are organic accumulating phaeozems intermixed with small pockets of solonchaks (Table S1) (ISRIC 2018).

Apart from the migrating wildebeest and zebra, densities of wild herbivores (including elephant, buffalo, impala and various species of gazelle) within wildlife protected areas were low in contrast to the high densities of livestock (cattle, sheep and goat) outside management areas. Outside the park, district-level cattle densities were higher in west with 135 cattle km⁻² and 40 cattle km⁻² in east (Figure 1) with similar patterns for other livestock: 58 and 45 goats km⁻² and 34 and 21 sheep km⁻² in the west and east, respectively (Tanzania National Bureau of Statistics 2012 census). Nevertheless, it should be noted livestock densities tend to be far greater closer to the borders of protected areas. Due to intense livestock grazing outside protected areas, there are insufficient fuel-loads for fires and burning is almost non-existent (Figure 1), while in protected areas wildlife management authorities undertake regular burning. All sites inside wildlife protected areas had fires within the last 3 years (MODIS MCD 45A burn product area years 2000 - 2016), whereas outside the last fire was over 6 years ago in the west and over 16 years ago in the east region.

Herbaceous root litter was incubated in savannas in five regions around the Serengeti. Inside protected areas in Ikorongo Game Reserve in the west, Seronera, central Serengeti, and Klein's gate in east Serengeti and outside protected areas on village pastureland in Park Nyigoti and Ololosokowan (Figure 1). Protected area sites were between 0.3 – 4 km from the park boundaries to ensure selected sites had no illegal livestock grazing and this was confirmed after consulting both villagers and rangers. Within each region, four replicate blocks spaced 500 m apart were selected based upon a co-dominance of leguminous tree *Vachellia gerrardii* (formerly *Acacia gerrardii*) and non-legume tree *Balanites aegyptiaca*. Due to limited cover of *V. gerrardii* in Seronera roots were buried under *Vachellia tortilis* (formerly *Acacia tortilis*) and at this site we only established three replica blocks. At each block, roots were incubated underneath a legume tree, non-legume tree and outside the tree canopy. Only mature trees were selected with average heights 5.0 m, 4.6 m and 6.5 m for *V. gerrardii*, *V. tortilis* and *B. aegyptiaca*, respectively (± 0.2 m standard error). Furthermore, there were two sets of burial locations per tree canopy for root litter either open to or excluding soil termites using metal mesh (details below). In total we had

114 burial locations inside and outside the Serengeti National Park. GPS coordinates for all burial locations were recorded.

Root litterbags for field experiments

Litterbags were used to estimate annual root decomposition for four common grass species: *Cynodon dactylon*, *Chloris pycnothrix*, *Panicum maximum* and *Themeda triandra*. The species were selected to represent those dominant inside wildlife protected areas (*Panicum* and *Themeda*) and outside in pastureland (*Chloris* and *Cynodon*) (Figure S1). In March 2016, root material was collected during the short dry season between the bimodal rainy seasons as it is presumed to be the period of peak root senescence (McNaughton et al. 1998). As much as possible, representative litter was collected for each species from every region to avoid any bias in root traits associated with a particular region or land-use (i.e. grazing regime) (Anderson et al. 2007; see root traits in Table S2) and finally pooled across provenances. Living and dead roots were collected from soil-vegetation turfs 20 cm deep, washed free of adhering soil, air-dried, pooled and then coarsely chopped before being packed into litterbags (Smith et al. 2014). A sub-set of pooled root material was retained to determine morphological and physiochemical traits and nutrient contents (see below). Nylon mesh litterbags (8 × 8 cm, mesh size 50 µm to prevent in-growth of living roots) each contained 1.5 ± 0.005 g root litter.

Decomposition of root litter across land-use types

In early June 2016, the influence of land-use type, tree canopy, litter species and termites on savanna root decomposition was investigated by burying 456 litterbags (4 species × 5 regions × 4 replicate block (3 in Seronera) × 3 canopy types × 2 termite treatments). At each location, all four species were buried ~10 cm deep with all litterbags placed horizontally. Underneath trees, burial locations were either north or south facing from a tree to ensure similar aboveground herbaceous productivity. Trees with sub-canopies with dense woody thickets or severe human or elephant damage were avoided. Litterbags were buried between 30 and 50 cm away from the tree trunk. A double layer of 1 mm stainless steel mesh was used to exclude larger *Macrotermitinae* species from half of all litterbags. Unfortunately, on collection this metal mesh had partly or completely disintegrated in almost all locations, so the presence or absence of termites was determined by visual observation of holes in litterbags and sheeting (and sheeting fragments) surrounding root material, the soil cast made by termites during foraging (Sileshi et al. 2010, Sitters et al. 2014, Veldhuis et al. 2017). Termite presence was further quantified by scanning all litterbags to estimate percentage area of holes (see below). Nevertheless, potential differences in decomposition due to the

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metal mesh were statistically investigated during analyses. Additionally, we collected soil from each burial location and pooled this by block to determine soil texture following the hydrometer method, where sieved (<2 mm, removing stones) and pulverized soil was shaken in sodium hexametaphosphate 5% dispersing solution before being transferred to sedimentation cylinder determining percentage of clay, silt and sand content by timed suspension of an ASTM 152H-Type hydrometer (Gee and Bauder 1986). Soil organic carbon content was also determined by the Walkley-Black method using wet oxidation refluxing in a mixture of potassium dichromate, sulphuric acid and phosphoric acid and determining organic carbon gravimetrically by absorption in soda lime (Kalembsa and Jenkinson 1973). Both soil properties were determined due to previous studies highlighting that termite foraging can depend on soil texture and soil organic carbon (Jones 1990, Sileshi et al. 2010). Litterbags remained in the soil for one year and were collected at the end of May 2017.

To put the decomposition rates of the selected four grass in a wider ecological context, we incubated root litter of a further nine species, including a range of functional groups, in a common garden experiment. At six locations outside tree canopies around Seronera (Figure 1), root litterbags were buried containing: *Bothriochloa insculpta* (grass), *Crateostigma plantagineum* (forb), *Digitaria macroblephara* (grass), *Heteropogon contortus* (grass), *Indigofera volkensii* (legume), *Mariscus amourpus* (sedge), *Microchloa kunthii* (grass), *Pennisetum mezianum* (grass) and *Solanum incanum* (forb). A total of 78 litterbags, six replicates per species, were buried with and without metal mesh for one year.

Decomposition of litter near termite mounds

A shorter-term cafeteria-style experiment was established near low subterranean termite mounds (*Macrotermitinae*: *Odontotermes*) to investigate soil fauna selectivity of litter. In March 2017, root and leaf litter from *Cynodon* and *Themeda* as well as *Indigofera*, *Solanum* and *Zea mays* (crop) and *Zea mays* stems were all buried near six termite mounds (4.8 – 7.5 m diameter, < 0.5 m height) around Seronera. Double wrapped polypropylene litterbags (5 × 5 cm, mesh size 1 mm to allow micro- and mesofauna access) were prepared and contained 1.5 ± 0.005 g litter. Two trenches, one north and the other south facing, were dug approximately two meters from the edge of the termite mound at a depth of ~ 8 cm. A single litterbag for each species and litter type was then buried at a random position along the two trenches but spaced at least 10 cm apart. Litterbags were incubated for two months during the long wet season and harvested in May 2017.

Measuring termite intrusion into root litterbags

Due to the damage and disintegration of metal mesh designed to exclude termites, presence and absence of termites was determined by scanning nylon mesh litterbags for holes. A randomly selected standard 5 × 5 cm section of every litterbag was scanned in colour to a 400 dpi resolution on white background using a Xerox Workcentre 7845 (Webster, New York, USA). After scanning, holes in the litterbags were white so scans were converted into binary black and white image and the percentage area of holes calculated using ImageJ 1.49v (National Institute of Health, USA). Based on the number of pixels, all images with at least one hole larger than 0.3 mm in diameter were defined as having had termites present as this is the mean maximal width for the majority of termite species in the Serengeti (Catherine Parr. pers. comm.) whereas holes of smaller area were assumed to be small tears or noise and classified as termite absence. The median hole size was 0.55 mm² suggesting the majority of holes were created by termites rather than larger millipedes or isopods, yet larger macro-fauna may have been involved in the decomposition of roots for litterbags with larger holes. Mean total area of holes per litterbag was 12.2 ± 1.7 mm² (mean ± SE) and the highest area of holes per litterbag was 346.2 mm² within a scanned area of 2500 mm² for each litterbag. Furthermore, our measure of termite intrusion using holes was cross-validated with field observations of the presence of translocate soil for sheeting around root litter. Both measures significantly corroborated with one another with 99.4% chance of correctly identifying termite absence (exact binomial test: n = 154, p < 0.001) and 77.7% chance of correctly identifying termite presence (exact binomial test: n = 367, p < 0.001) (Conover 1971). Across the experiment 55% of all litterbags collected were defined as having termite intrusion.

Root litter analysis

Chemical and morphological traits commonly used as predictors of plant litter decomposition were analysed on roots before being decomposed. These traits included total root nitrogen (N) and phosphorus (P), mean root diameter (MRD), specific root length (SRL) and specific root area (SRA). Root traits were measured on three replicate samples prior to pooling, apart from N and P of eight common garden species that were pooled for chemical analysis (Table S2). Total N and P concentrations were measured via Kjeldahl digestion methods: N was determined by digestion in H₂SO₄ and titrated by NH₂SO₄; and, for P litter was acidified in ammonium molybdates solution and combined with ascorbic acid for colouring and analysed using spectrophotometry (Allen 1989). Morphological traits were measured from 0.5 g of air-dried material spread on acetate sheets and scanned. Root segments were hand-traced and analysed using SmartRoot (<https://smartroot.github.io>). Root diameter and surface area estimated in this study are likely to be lower than standard trait approaches on fresh material, yet it has been shown that the colour contrast

following drying enhances detection of fine roots for these species (Anderson et al. 2007) and determining traits on air-dried material reflects the quality of the air-dried material being decomposed. Root N and P are expressed as mg g^{-1} , MRD in mm, SRA as $\text{m}^2 \text{g}^{-1}$ and SRL as m g^{-1} (Table S2).

Measures of litter decomposition

After collection, litter was extracted from litterbags, oven dried at 70°C for 48 hrs and weighed (± 0.001 g). All litter was ashed for 4 hrs at 550°C to account for the collection of soil during incubation. Five samples for each species and litter type that had not been buried were ashed to derive an undecomposed ash content. The difference in ash-free weight before and after incubation was used to calculate litter mass loss and expressed as a percentage.

Statistical analysis

Roots from 426 litterbags recovered after one year of decomposition across land-uses surrounding Serengeti (out of 456 buried) were used in the statistical analysis. The effects of land-use, litter species, termite exclusion and litterbag holes, tree canopy and soil texture on root litter mass loss were explored using a linear mixed model (LMM) with restricted maximum likelihood estimations (REML). Due to significant co-linearity between soil properties (Table S1), only sand was used as covariate in the model given its stronger correlation with root decomposition. All other covariates, as well as two-way interactions were initially included as fixed terms in the model. The random structure, reflecting the experiment design, was burial location of the four species litterbags nested within block and then region. Final models were simplified following Akaike's information criterion (AIC), removing terms from the full model to improve the model likelihood and lower AIC value. Fixed variables were retained if significant in likelihood ratio tests (LRTs). For the final model, significance of each term was assessed contrasting models using Maximum Likelihood (ML) with and without fixed factors to generate *P*-values (Bolker et al. 2009, Zuur et al. 2009).

To evaluate the selectivity of soil fauna for root litter across land-uses, litter species, tree canopy and soil texture, we compared presence and absence of litterbag holes using a generalized linear mixed models (GLMM) following a Bernoulli distribution with logit transformation. The initial model structure and simplification was the same as outlined above with addition of a zero inflation term in the model due to an overabundance of zeros. The final model was further validated: firstly by checking for over-dispersion of residuals, and secondly by simulating 10,000 datasets based on the model coefficients and comparing the frequency of simulated and observed data for presence and absence of litterbag holes

(Zuur et al. 2016). In our final model residuals were not over-dispersed and had a good fit between simulated data and observed data, yet there was a slight under-estimation of presence of holes compared to observed values. Results from this analysis are presented as the probability of termite intrusion derived from the odds ratio.

Further analyses of the common garden experiment and the termite cafeteria-style experiment were determined using GLMMs. In the common garden experiment separate models were performed for species and individual root traits due to issues with collinearity (correlation matrix Figure S2) and in all models block was the single random term. While in the termite cafeteria-style experiment species and litter type (root, leaf and stem) were fixed terms and termite mound was the random term. In both experiments, root litter mass loss was high for some species (i.e. almost 100%) leading to over-estimation of mass loss using Gaussian distributions. Therefore we used a beta distribution, first converting mass loss values to between 0 and 1 and then back transforming values to percentage confided between 0 and 100%. For all models in our analysis, *P*-values for significant differences within terms were obtained through multiple contrasts as a function of least square means (Lenth 2016). All analyses were carried out in R version 3.3.3 (R Foundation for Statistical Computing, 2016) with LMM and GLMM models tested using the ‘lmer’, ‘glmer’ and ‘glmmadmb’ functions in lme4 (Bates et al. 2015) and glmmADMB (Fournier et al. 2012) packages in R.

RESULTS

Root litter mass loss and land-use

There was no significant difference in annual litter mass loss for roots buried in savanna wildlife protected areas compared to pastureland. Instead, species differed in rates of root decomposition and these differences were consistent across land-uses (Table 1; Figure 2). In the absence of holes in litterbags, *Chloris pycnothrix* and *Cynodon dactylon* had significantly higher mass loss averaging 54% and 52% respectively, compared to *Panicum maximum* and *Themeda triandra* averaging 45% and 45% loss (Table 1). Figure 2). However, rates of root decomposition of *Chloris* and *Cynodon* did not significantly differ, nor were there differences between decomposition of *Panicum* and *Themeda*. Furthermore, there was a significant negative correlation between root mass loss and soil sand content (Table 1) with a decline in 0.3% root mass loss for every 1% increase in sand content, totalling 14.4% difference across the experiment (range in sand percentage was between 37 and 85%; Table S1). In comparison, the largest average difference between species litter types was smaller ≈9%, but species differences were present across all soil texture types.

Termite intrusion into litterbags

Across land-uses, there was a significantly higher probability of termite intrusion into litterbags (i.e. the presence of holes in litterbags) underneath leguminous trees compared to non-legume trees and outside the tree canopy (GLMM: $d = 9.31$, $df = 2$, $P = 0.009$; Figure 3). On average, litterbags buried underneath leguminous trees had over 20% higher probability of termite intrusion compared to non-legume trees and outside tree canopies. However, likely differences in termite presence across tree canopies did not significantly impact root litter decomposition (Table 1). The probability of litterbag termite intrusion varied with root litter from different plant species (GLMM: $d = 9.66$, $df = 3$, $P = 0.046$) with *Themeda* having significantly lower probability of termite intrusion (43%) than other litter species (Figure 3; *Chloris* 57%, *Panicum* 59% and *Cynodon* 65%). Although the significance of species difference in termite presence probabilities was statistically weaker these did reflect termite driven species differences in root litter mass loss (Table 1; Figure 2).

Impact of termites intrusion on root litter mass

When present, termites had the greatest impact on *Panicum* root litter, increasing mass loss by 13% followed by 8% for *Cynodon* and 7% for *Chloris* above microbial decomposition (Figure 2; Table 1). However, the presence of termites made no significant difference to *Themeda* root litter mass loss (Figure 2). This lower mass loss for *Themeda* with termite present was consistent for both roots and leaves as seen when burying litter adjacent to termite mounds (Figure 4a). *Themeda* root and leaf litter decomposed significantly slower than all other species buried near termite mounds (Table 1; Figure 4a); however, root litter mass loss for *Themeda* was not the lowest when compared to the other nine wild savanna herbaceous species buried in the common garden (Figure 4b). Also noteworthy was that even in close proximity to termite mounds, roots decomposed slower than aboveground pools losing on average 20% less mass (Figure 4a).

Termites, species traits and root decomposition

In the common garden experiment we also found both species differences (Table 2) as well as significant differences with and without termites depending on species identity (Table 2; Figure 4b). Root N, Root P and MRD were all positively correlated with overall rates of root decomposition, but none of these traits were able to explain the interaction between plant species and termites (Figure 5; Table 2). The strongest predictor seemed to be root N and this relationship was weakened by the leguminous herb *Indigofera*

volkensii with high root N content but moderate mass loss (Figure 5a; Table 2). For root N the overall positive relationship was more similar to that prediction for microbial only decomposition (without termites) if the model included the interaction term (Figure 5a). The overall positive relationships for root P and MRD seemed driven by outliers (Figure 5b, c) and there was no statistically significant relationship between SRA and SRL and root decomposition (Table 2). Unfortunately for the common garden experiment, as our metal mesh was compromised we had little control over number of litterbags with and without termites. Thus, these relationships between litter mass loss, termites and traits should be interpreted with caution as some species are represented by a single litterbag (Figure 4 and Figure 5). Saying this, the proportion of litterbags with termite intrusion did not systemically vary in relation to any measured root trait.

DISCUSSION

Across savanna land-use types with contrasting fire and large mammalian herbivore regimes, we found little difference in rates of root litter decomposition. Instead, belowground decomposition is likely to be altered indirectly by land-use via changes in plant species composition, highlighting the importance of indirect effects controlling litter and nutrient cycling in savannas (Buitenwerf et al. 2011, Davies et al. 2013, Sileshi et al. 2010). Similarly the probability of termite intrusion into litterbags was not directly affected by land-use. However, selectivity by termites further contributes to species differences in root decomposition with both additive and non-additive effects in the presence of termites. This differs from several studies that suggest that termites are generalist detritivores of aboveground litter in savannas (Collins 1981, Manlay et al. 2004, Veldhuis et al. 2017), even in the Serengeti (Freyman et al. 2010). At the same time, larger soil macro-fauna, such as millipedes and isopods, may have contributed to plant species differences in rates of root litter decomposition, particularly in litterbags with larger holes, yet more work is necessary to understand how different savanna soil fauna regulate root decomposition. Combined, the results from our study further underscore the importance of accounting for root decomposition in order to formulate general principles regulating plant litter decomposition.

In termite-dominated savannas, several studies identify how herbivory and fire modify species composition and vegetation pattern formation feeding back to the regulation of ecosystem processes (Buitenwerf et al. 2011, Okullo and Moe 2012, Sileshi et al. 2010) (Figure 6). Yet, little is known about the relative strengths of these land-use driven feedbacks in savanna systems, particularly for belowground nutrient cycling. Our results highlight the over-arching importance of both plant species (and litter quality) and interactions with termites as selective foragers in determining rates of root decomposition.

Termites may interact with land-use processes to generate spatial heterogeneity of the plant-soil system (Figure 6). It has been widely observed that fungus-growing *Macrotermitinae* mounds are dominated by *Cynodon* with a rarity of other savanna grasses, notably *Themeda* (Sileshi et al. 2010). Close to termite mounds fire intensities are lower due to higher soil moisture and mammalian herbivory is greater due to higher nutrient concentrations thereby fostering plant species communities differences on mounds and away from mounds (Buitenwerf et al. 2011, Moe et al. 2009, Okullo and Moe 2012). Our findings suggest that termites will promote spatially explicit land-use effects where savanna swards dominated by *Chloris*, *Cynodon* and *Panicum* will likely lead to stronger ‘fertility islands’ of termite mounds due higher consumption of these termite preferred species, whereas greater cover of *Themeda* may weaken this effect but generate greater local heterogeneity of more root material decomposing *in situ* underneath plants (Figure 6). Termite selective foraging then mediates the impact of land-use on root decomposition indirectly via species composition. For example, *Themeda* leaf litter decomposes 30% slower than other species, as shown here, and is more combustible than other species (Simpson et al. 2016) and this will increase fuel loads away from mounds. Subsequent higher fire frequencies would further promote the dominance of *Themeda* and more root decomposition away from mounds (Figure 6). For aboveground litter, the interplay between herbivory, fire and microbial decomposition are well documented in the literature (Cornelissen et al. 2017, Grootemaat et al. 2015). Yet greater consideration of termites is required in developing a savanna land-use - decomposition framework, particularly considering how termites contribute to variation in decomposition and the spatial redistribution of nutrients belowground.

Plant species and macro-fauna driven differences in litter decomposition are usually mediated through variation in morphological, chemical and physiological traits. Despite differences in rates of root litter decomposition by termites and microbes across species, this was not corroborated by any traits measured as part of this study. Perhaps this signifies the importance of other root traits delineating termite and microbial species differences. For instance, polyphagous wood-litter termites’ preference for dung increases with higher carbon to nitrogen ratio, while microbe preference is for the opposite (Freyman et al. 2008, Sitters et al. 2014). Meanwhile for bamboo stems, both termite and microbial decomposition are negatively related to increasing woody density with termites even consuming thicker bark to obtain less dense stems (Liu et al. 2015). Thus, traits governing termite and microbe decomposition may depend upon plant organ or litter type. However, in our system traits governing termite and microbial decomposition could also be shared both belowground and aboveground, as in the case of *Themeda triandra* roots and leaves. Low rates of microbial and termite decomposition of *Themeda* could be due to high dry matter contents in both plant parts (Liu et al. 2015). Alternatively, *Themeda* plant tissue contains

high concentrations of the aromatic compound hydroquinone (a smoke-derived germination stimulant) (Kamran et al. 2017), which has been shown to be an arrestant to termites (Raina et al. 2005) and therefore likely has a negative influence on microbial gut flora and free-living microbes. To better predict savanna species litter decomposition, broader screening of root and leaf traits is necessary with greater consideration of termites and microbes.

Despite several studies on the interaction between savanna trees and termites, our study is the first to our knowledge to experimentally disentangle this interaction on root decomposition. We demonstrate that higher termite activity underneath leguminous trees does not result in greater root litter mass loss. From the termites' perspective, greater foraging opportunities exist underneath leguminous trees with greater herbaceous productivity and organic matter inputs (Belsky et al. 1989, Blaser et al. 2013). Supporting this we found enhanced termite, but not microbial, decomposition of *Panicum maximum*, a typically tree sub-canopy specialist (Figure S1) (Belsky 1994, Ludwig et al. 2004a,b). This suggests a termite preference for removing root litter from underneath leguminous trees. Yet, from the leguminous tree's perspective termite litter removal potentially reduces the availability of much needed litter derived nutrients (e.g. phosphorus) (Cramer et al. 2010). Likewise termites can be problematic for trees by directly damaging the tree or inhibiting recruitment; hence one benefit of forming a tree-ant symbiosis is that ants prey upon termites (Palmer 2003, Pringle 2014). Thus, one possible explanation for the lower than expected root litter decomposition for all herbaceous root litter under leguminous trees, despite higher probability of termite intrusion into litterbags, is that ants interrupt termite foraging.

In summary, we found that root decomposition in savannas depends on the interaction between litter type and termites rather than the direct influence of fire and large mammalian herbivores. In African savannas, multiple drivers can determine herbaceous species composition; for instance, herbaceous communities differed under tree canopies compared to outside them. Thus, similarly to land-use, tree canopies can indirectly influence the quality of root litter. This will have a stronger impact on root litter decomposition compared to elevated incidences of termite foraging underneath trees. Nevertheless, in savannas roots are less likely to be removed by fire than aboveground litter, so root decomposition will depend more on termite and microbial decomposition pathways. Evidence from our study suggests that interactions between termite and microbial decomposition vary according to species, differing from the typical view that termites, particularly fungus growers, are generalist detritivores that enhance decomposition. This may reflect a difference in the dominant termite taxa involved in root decomposition such as soil feeders as opposed to wood-feeding termite species that are associated with leaf, wood and dung decomposition (Freyman et al. 2008). Alternatively, root litter may have a broader range of traits

and qualities compared to other litter types (Freschet et al. 2017), thus expanding the range of potential species interaction with either additive or non-additive effects of termites on decomposition. In terms of root litter, our study demonstrates that greater consideration should be given to herbaceous plant species composition in termite occupied savannas to understand the spatial heterogeneity of belowground nutrient cycling.

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FIGURE LEGENDS

Figure 1. Root decomposition experiment locations inside (red symbols) and outside wildlife (yellow symbols) management areas surrounding the Serengeti National Park, Tanzania. Contrasting land-uses are surrounding the Serengeti ecosystem year since last fire (MODIS burn area product) and livestock cattle density (km^{-2}) for district adjacent to the park. For the main map, light grey areas demark protected areas dominated by wildlife where human settlement and livestock is not permitted.

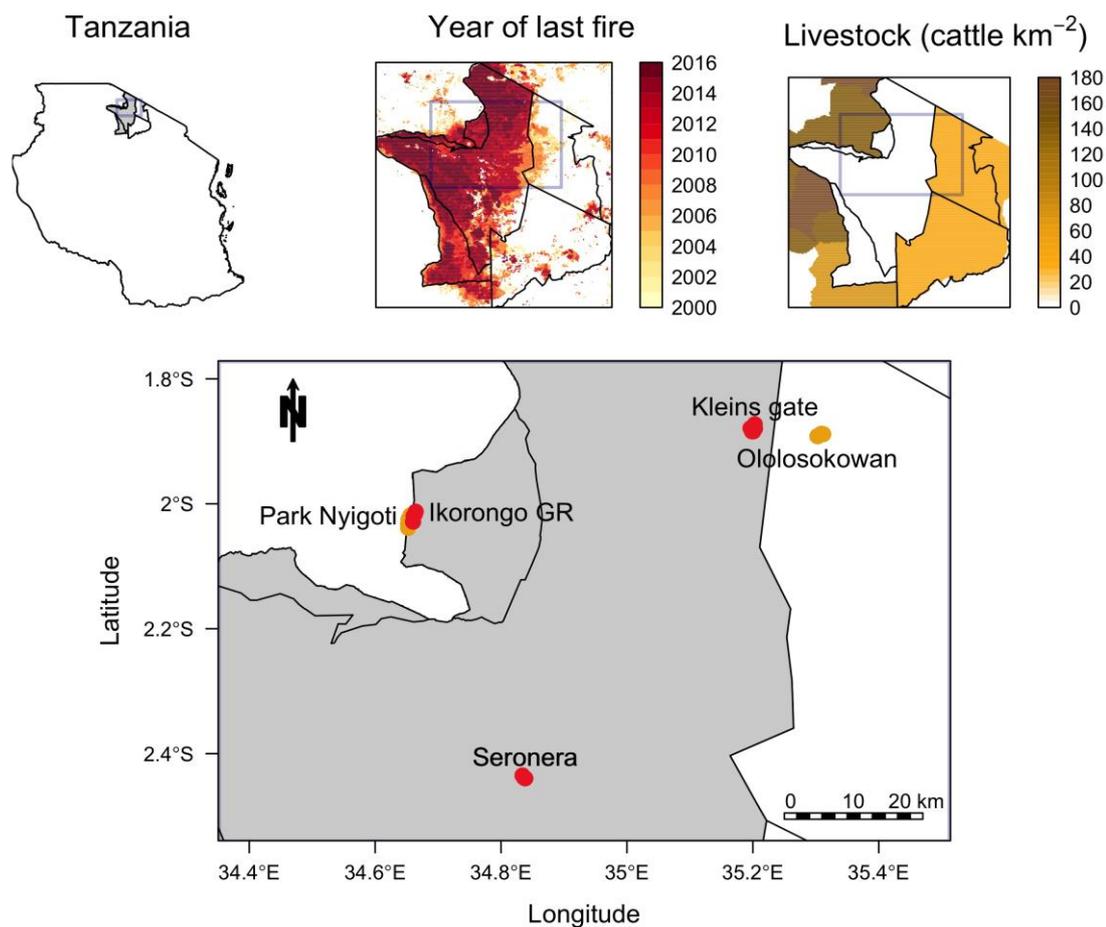


Figure 2. Annual root litter mass loss (ash-corrected) for four common savanna grass species in the absence (filled symbol) and presence (hollow symbol) of termites and buried on pastureland (circles) and wildlife dominated protected areas (squares). Root litter species include *Cynodon dactylon* (light green), *Chloris pycnothrix* (light blue), *Panicum maximum* (dark green) and *Themeda triandra* (red). Root mass loss has been averaged across different tree canopy types. Error bars ± 1 standard error.

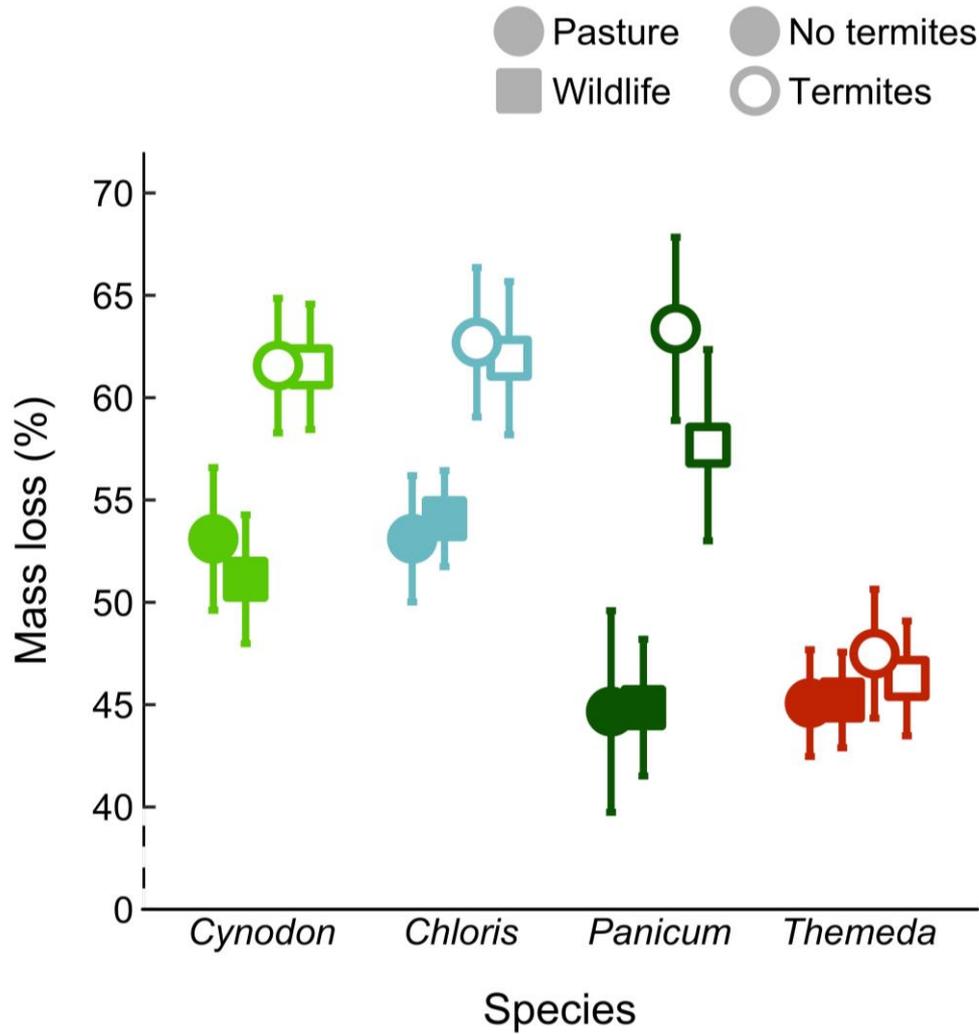


Figure 3. Probability of a termite intrusion into litterbags (indicated by holes in litterbags and soil castes around root litter) for four common savanna grass species across tree canopies. Root litter species include *Cynodon dactylon*, *Chloris pycnothrix*, *Panicum maximum* and *Themeda triandra*. Litterbags were buried under leguminous trees (dark grey circles), non-legume trees (grey squares) and outside the tree canopy (light grey triangles). Predicted probabilities of termite intrusion into litterbag have been generated based on odds ratio derived from a generalized linear mixed model and averaged across land-uses. Error bars \pm 1 standard error.

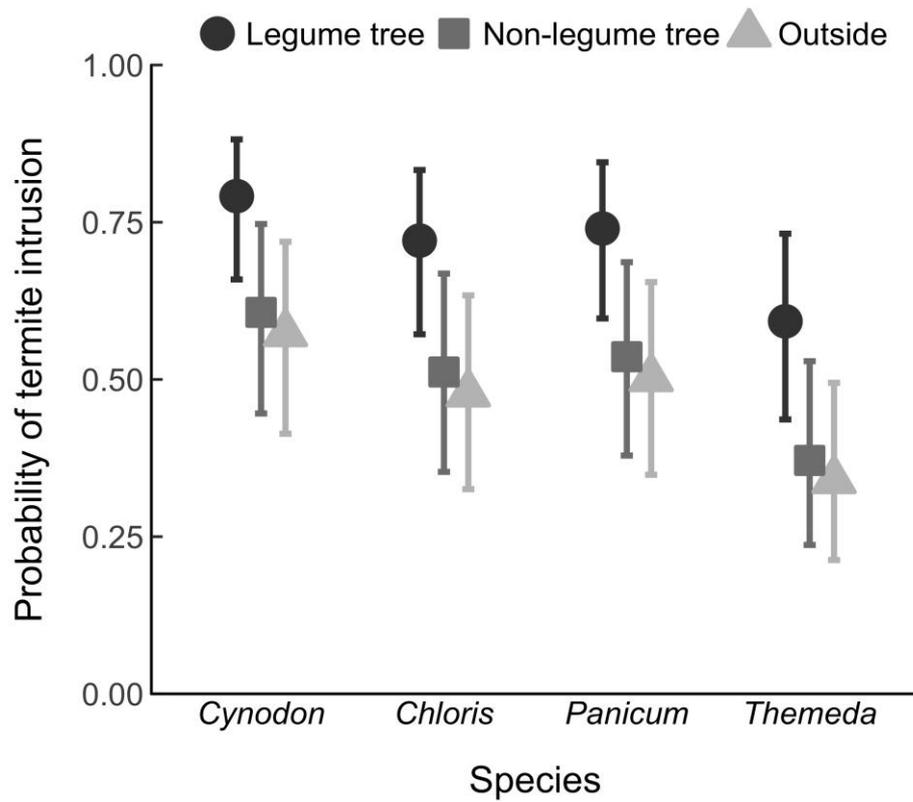


Figure 4. Mass loss of species litter (a) near termite mounds and (b) within a common garden in central Serengeti. For the termite cafeteria-style experiment (a) leaves are open squares, roots open circles and stems open triangles. Focal species across the study experiments are coloured whereas all other species are coloured in grey and include: *Bothriochloa insculpta*, *Chloris pycnothrix* (light blue), *Crateostigma plantagineum*, *Cynodon dactylon* (light green), *Digitaria macroblephara*, *Heteropogon contortus*, *Indigofera volkensii* (pink), *Mariscus amourpus*, *Microchloa kunthii*, *Panicum maximum* (dark green), *Pennisetum mezianum*, *Solanum incanum* (blue), *Themeda triandra* (red) and *Zea mays* (yellow). Symbol sizes for the common garden experiment (b) are proportional to number of litters (1 -5 litterbags) without (filled symbols) and with termite (open symbols). Error bars ± 1 standard error.

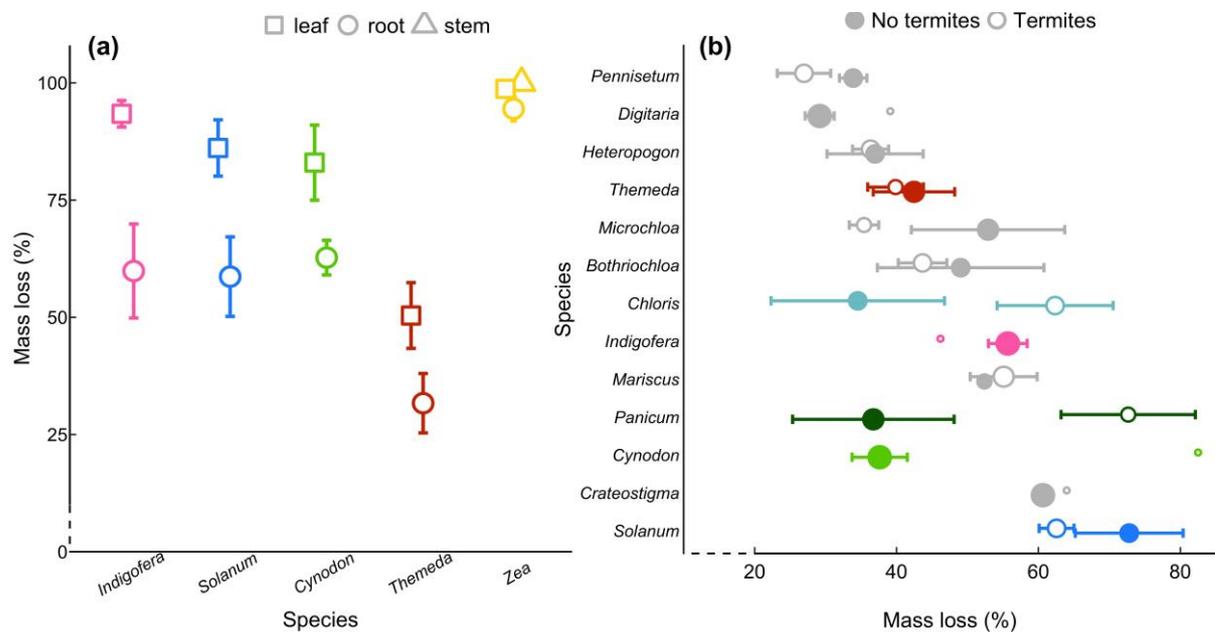


Figure 5. Root traits predicting common garden root decomposition: (a) root nitrogen (mg g^{-1}), (b) root phosphorus (mg g^{-1}) and (c) mean root diameter (mm). Focal species across the study experiments are coloured whereas all other species are coloured in grey and include: *Chloris pycnothrix* (light blue), *Cynodon dactylon* (light green), *Indigofera volkensii* (pink), *Panicum maximum* (dark green), *Solanum incanum* (blue) and *Themeda triandra* (red). Symbol sizes for the common garden experiment (b) are proportional to number of litters (1 - 5 litterbags) without (filled symbols) and with termite (open symbols). Solid black lines represent significant overall predications for root traits fitted with a generalized linear mixed model. Predictions for mass loss without termites (grey dashed line) and with termites (grey dotted line) are shown separately. Error bars ± 1 standard error.

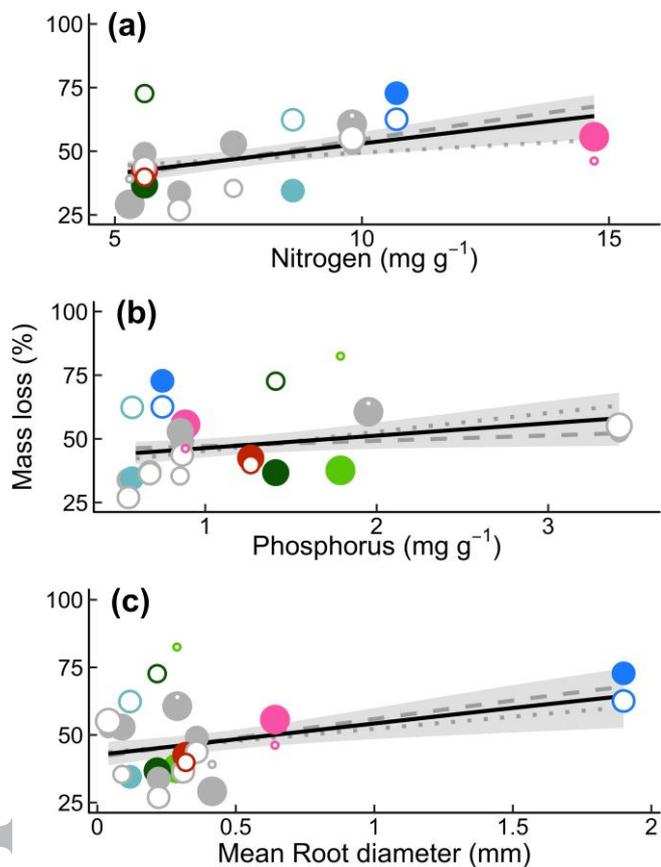


Figure 6. Conceptual model visualising the regulation of root decomposition by herbivory, fire, plant species and termites in savannas. Alternative scenarios are presented with termites as generalists and non-generalists (i.e. selective foragers). The impact of fire and herbivory vary with proximity to termite mounds: near mounds bioturbation and higher decomposition rates promote wetter soil conditions and higher nutrient availability and thus reduce fire frequency and increase herbivory. This in turn, alters plant species composition near and far away from mounds. For a scenario with non-generalist termite, these plant species differences influence rates of root litter removal by termites (dashed dark grey arrows) due to higher foraging of preferred species close to mounds (i.e. *Cynodon dactylon*) and avoidance of pyrophilic plant species (i.e. *Themeda triandra*) away from mounds. Less litter removal away from mounds enhances fire frequencies, while greater removal near mounds promotes nutrient cycling and herbivory. In the non-generalist termite scenario, greater rates of root decomposition (black circles belowground) can occur away from mound *in-situ* underneath plants whereas if termites are generalist the majority of root litter decomposes inside mounds.

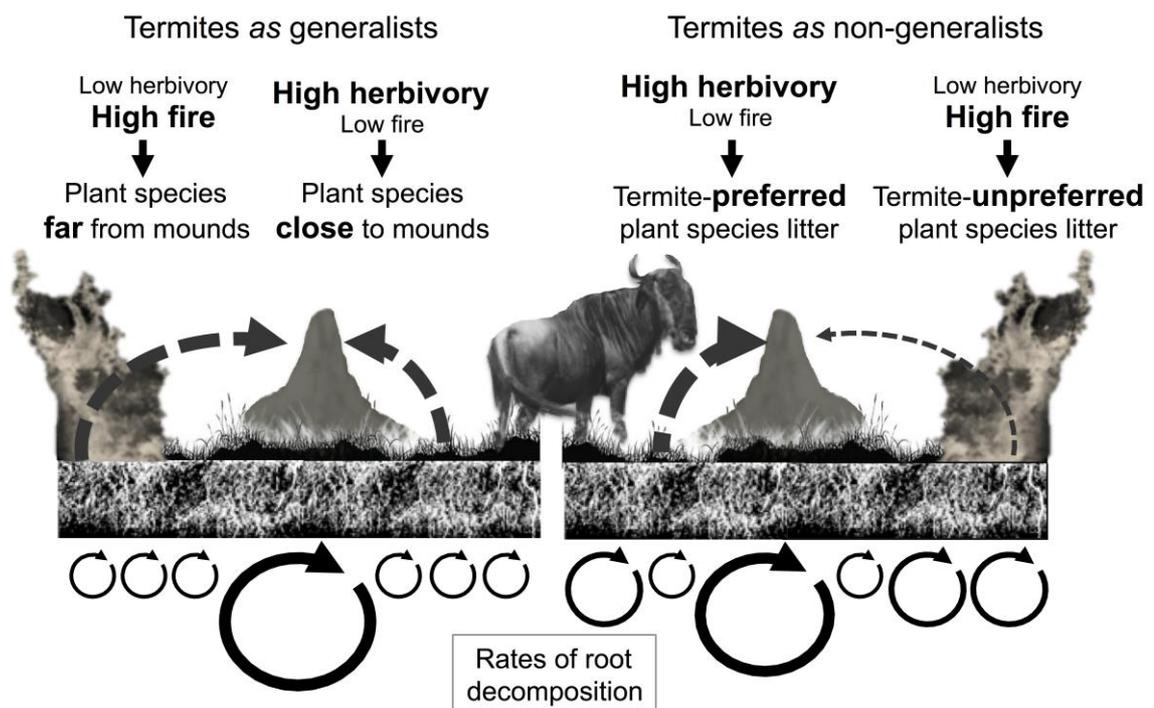


TABLE LEGENDS

Table 1. Effects of species, termite intrusion into litterbags, sand texture and plant pools (roots, leaves and stems) and their interactions on litter mass loss using linear mixed models from the land-use type and cafeteria-style experiments. Contrasting models with and without variables following likelihood ratio test have been used to generate *P*-values. Species and termite intrusion into litterbags *P*-values have been derived through comparison with the model without the interaction term.

Experiments	Variables	X^2	d.f.	<i>P</i>
Land-use	Species	33.10	3	<0.001
	Termite intrusion	14.69	1	<0.001
	Sand	6.53	1	0.011
	Species × termite intrusion	8.87	3	0.031
		Deviance	d.f.	<i>P</i>
Termite cafeteria	Species	56.4	4	<0.001
	Plant pool	29.7	2	<0.001

Table 2. Effects of species and traits, termite intrusion into litterbags and their interactions on litter mass loss using separate generalized linear mixed models within the common garden experiment. Contrasting models with and without variables following likelihood ratio test has been used to generate p-values. Species and termite hole *P*-values have been derived through comparison with the model without the interaction term.

	Species or root trait			Termite intrusion (presence/absence)			Species or trait interaction with termites		
	Deviance	d.f.	<i>P</i>	Deviance	d.f.	<i>P</i>	Deviance	d.f.	<i>P</i>
Species	34.76	12	<0.001	0.05	1	0.825	43.85	12	<0.001
Root nitrogen	13.63	1	<0.001	0.02	1	0.883	1.83	1	0.177
Root phosphorous	4.29	1	0.038	0.06	1	0.808	1.31	1	0.252
Mean root diameter	8.61	1	0.003	0.02	1	0.878	0.43	1	0.513
Specific root area	0.32	1	0.570	0.04	1	0.837	2.75	1	0.097
Specific root length	0.56	1	0.423	0.07	1	0.794	0.37	1	0.543