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Morphological Traits as Predictors of Litter Decomposition in Agroforestry Tree Species of Rwanda

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Abstract

This study examines the influence of key morphological traits and how the leaf traits are essential for understanding the strategies of biomass decomposition at the leaf trait level. Leaf thickness, leaf area, specific leaf area, and leaf dry matter content were morphological traits, and lignin and tannin were quality traits affecting litter decomposition, measured across six agroforestry tree species. We applied linear mixed models to quantify the effects of these traits on decomposition, treating species as random effects to account for inter-species variability. Principal Component Analysis revealed that the first principal component, which explains 45.6% of the total variance, is strongly associated with leaf thickness and dry matter content. These traits emerged as the primary drivers of variance in litter decomposition rates. The second principal component, accounting for 26.4% of the variance, is primarily influenced by Leaf Area and Lignin content, indicating their significant roles in the secondary variation observed among species. The total linear mixed model, incorporating all morphological traits, provided a significantly better fit than the reduced model, as indicated by a Chi-square test ($p < 0.05$). This suggests that combining morphological traits is crucial for understanding litter decomposition dynamics. Moreover, the results highlight species-specific differences in trait effects, emphasizing the need for tailored management strategies in agroforestry systems to optimize nutrient cycling. These findings contribute to a deeper understanding of the factors influencing litter decomposition, offering practical insights for selecting tree species that enhance soil fertility through improved decomposition rates. The study underscores the importance of considering both trait variability and species identity to optimize agroforestry practices for sustainable land management.

Keywords: *Litter decomposition, agroforestry, morphological traits, nutrient cycling, soil fertility*

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Introduction

Litter decomposition is a critical process in nutrient cycling, influencing soil fertility, carbon sequestration, and overall ecosystem productivity (Giweta, 2020). The rate at which leaf litter decomposes is influenced by a variety of factors, including environmental conditions (Canessa *et al.,* 2021), microbial activity (Palomino *et al*., 2023), soil properties (Matos *et al*., 2019; Su *et al.,* 2021; Zan *et al.*, 2022) and the intrinsic properties of the litter itself. Among these inherent properties, the morphological traits of leaves play a significant role in determining how quickly and effectively litter decomposes. (Gao *et al.,* 2016; Liao *et al.,* 2022).

Morphological traits such as leaf thickness (LT), leaf area (LA), specific leaf area (SLA), and leaf dry matter content (LDMC) are key indicators of a leaf's physical structure and chemical composition, both of which influence decomposition rates (Cornwell *et al*., 2008; Fortune *et al*., 2009; Kazakou *et al.,* 2009). The leaf area is the critical determinant for capturing light and is directly associated with biomass production (Cheng *et al*., 2022). SLA (leaf area/leaf mass) is an essential parameter for plant growth modelers because it determines how much new leaf area to deploy for each unit of biomass produced. Under elevated CO_2, any storage of the extra carbohydrate in the leaves or any reallocation of biomass to thicker leaves would tend to increase leaf mass more than leaf area, thereby decreasing SLA (Kimball *et al*., 2002). Leaves with a high SLA, which indicates a large area relative to dry weight, tend to decompose faster due to their greater surface area available for microbial colonization and their typically lower lignin content (Osono and Takeda 2004; González-Paleo *et al*., 2022). Conversely, leaves that are thicker and heavier may decompose more slowly, as they often contain more structural components like cellulose and lignin, which are resistant to microbial breakdown (Tan *et al*., 2020) and tend to have low nutrients, accompanied by long leaf lifespan, but species with relatively higher SLA are expected to grow fast (Weemstra *et al*., 2021).

Many studies have explored the relationship between tree species traits and litter decomposition. Aponte (2012) found that the quality of leaf litter, particularly its nitrogen, calcium, and manganese content, significantly influenced decomposition rates. Similarly, Bai (2022) observed that the chemical composition of litter, including nitrogen and polyphenol concentrations, affected decomposition and nutrient release. Bhalawe (2013) further highlighted the role of climatic factors in influencing decomposition rates, with *Tectona grandis* and *Dendrocalamus strictus* showing higher rates of weight loss and nutrient release. Gupta (2023) emphasized the importance of developing robust indices for predicting litter quality and decomposition patterns, particularly in agroforestry systems. These studies underscore the complex interplay of morphological and chemical traits in shaping litter decomposition and nutrient release patterns.

Despite these traits' importance, limited research focuses solely on the morphological predictors of litter decomposition. Most studies have combined chemical trait assessments with decomposition experiments (Chen *et al*., 2021; Cassart *et al*., 2020), leaving a gap in our understanding of how morphological traits alone can predict decomposition rates. The decomposition rate of tree litter, influenced by the plant material's morphology, varies among agroforestry species (Keerthika *et al*., 2024).

Understanding these traits is essential for predicting the litter decomposition rates of tree litter (Rawat *et al*., 2020), thus enabling farmers and land managers to make informed decisions about tree species selection and management practices in agroforestry systems. Agroforestry presents a promising approach to improving soil health (Fahad *et al*., 2022), but there is limited research on how the morphological traits of different tree species influence litter decomposition and nutrient release.

This study addresses this gap by providing a detailed morphological characterization of leaves from selected agroforestry tree species and examining their role in litter decomposition under two contrasting conditions: low altitude, dry (Kayonza) and high altitude, wet (Musanze) sites. There is no definitive answer whether chemical or physical traits predominantly influence decomposition rates, as both are closely linked and reflect the plant's overall strategy. By documenting traits such as leaf thickness, leaf area, SLA, and leaf dry matter content (Onipchenko *et al*., 2023), this research will provide insights into the decomposition potential of these species. This knowledge is crucial for informing species selection in agroforestry systems, particularly in regions where improving soil fertility is an essential challenge, by referring to the potential at the leaf trait level.

By characterizing the morphological traits of leaves from various agroforestry species, we can predict their decomposition potential and, consequently, their effectiveness as sources of green manure. This study investigates the morphological traits of selected agroforestry tree species and their relationship with litter decomposition rates in study sites. We tested the following hypothesis:

(1) Interactions between leaf morphological traits (e.g., leaf area and

LDMC) significantly influence litter decomposition rates, varying across species.
(2) Leaf morphological traits significantly affect the decomposition rates of agroforestry tree litter, as indicated by changes in lignin and tannin content.

Methodology

Study sites

The research was conducted in two ecologically distinct districts in Rwanda: Musanze district (Kinigi and Nkotsi sectors) in Northern Province and Kayonza district (Gahini sector) in Eastern Province (Fig.1). These locations were deliberately chosen to capture the diversity of climatic conditions and soil types present in Rwanda, which are crucial for understanding the morphological characteristics of the selected agroforestry species.

Musanze district (1° 29' 59.42" S, 29° 38' 5.89" E) is located within the volcanic highlands, with an average elevation of 2000 m. a.s.l. The region experiences a subtropical highland climate (classified as Aw according to the Köppen-Geiger climate classification system; Beck *et al.*, 2020), characterized by mean annual precipitation ranging from 1400 to 1800 mm and temperatures between 13°C and 20°C ((Twahirwa *et al.*, 2023). The rainfall pattern is bimodal, with primary rainy seasons occurring from March to May and September to December.

The soils are predominantly Mollic Andosols, formed from volcanic deposits, which are particularly susceptible to erosion due to intensive cultivation on steep slopes and inadequate soil conservation practices. This region was chosen because the agroforestry species under morphological characterization (Section: Selection of tree species) are prevalent here and play a significant role in local agroecosystems.

In contrast, Kayonza district (1°51' S, 30°39' E) lies in the eastern part of the country at a lower average altitude of 1573 meters a.s.l. This area features a tropical savanna climate (also classified as Aw by the Köppen-Geiger system; Beck *et al.*, 2020), with annual precipitation ranging from 900 to 1500 mm and temperatures from 15°C to 26°C (Uwimbabazi *et al.*, 2022). The region experiences a rainy season, which is less pronounced than the Northern Province. Kayonza was selected for this study because it hosts dominant species and is widely distributed across the landscapes under investigation (Section: Selection of tree species). The contrasting environmental conditions between these two regions provide a robust framework for understanding how different climatic factors influence the morphological traits of the selected tree species.

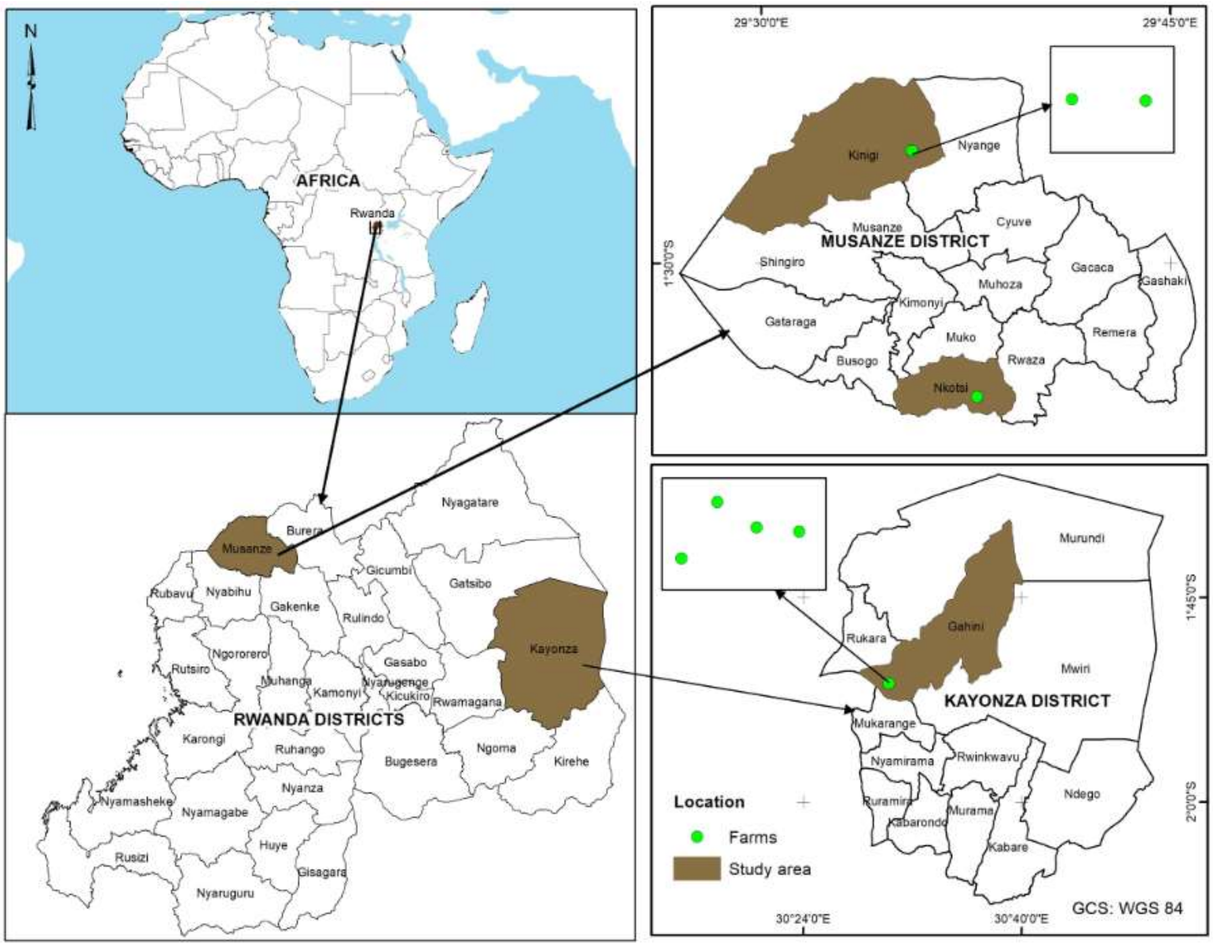

Figure 1: Location of the two contrasting selected study areas

Selection of tree species and traits of interest

Six selected agroforestry tree species represent a different functional group comprising *Alnus acuminata* and *Calliandra calothyrsus* (exotic N_2-fixing trees), *Eucalyptus globulus* and *Grevillea robusta* (exotic non-N_2-fixing), and *Markhamia lutea* and *Croton megalocarpus* (native non-N_2-fixing trees). These species were explicitly targeted due to their dominance in the respective regions, their ecological and economic importance to local farming communities, and their distribution where selected species occur in almost all the landscapes either in or around the farms. This diversity allows for a comprehensive analysis of how varying species characteristics influence leaf morphology and subsequent litter decomposition rates.

The study focused on a range of morphological traits. These leaf functional traits are plants' most common and essential morphological characteristics and play critical physiological roles in plant growth and production; they are sensitive to ecological processes, especially litter decomposition. These leaf functional traits include:

- Leaf thickness (LT): Measured to determine leaves' durability and decomposition potential. Thicker leaves decompose more slowly due to their higher lignin content and lower surface area-to-volume ratio (Niinemets, 1999).
- Leaf area (LA) and Specific leaf area (SLA) are critical indicators of leaf efficiency in photosynthesis and nutrient use. SLA, defined as leaf area per unit dry mass, is inversely related to leaf toughness and decomposition rates and determines the reproductive strategy of the plant (Tecco *et al*., 2013).
- Leaf fresh weight and dry weight: These measurements help in understanding the leaves' water content and overall biomass, which can affect decomposition speed.
- Leaf dry matter content (LDMC): The oven-dried weight of a leaf divided by its fresh leaf area indicates plant resource use and is related to leaf lifespan (Pakeman *et al*., 2011). Higher LDMC is often associated with slower decomposition rates due to stricter cell walls.
- Root collar diameter, diameter at breast height (DBH), and Height: Although primarily morphological, these traits can provide additional context for understanding growth patterns and biomass allocation in different species, indirectly affecting leaf production and litter quality.
- Lignin and Tannin

Measurements of morphological traits

In early September 2022, ten mature and healthy individual trees of each species were selected randomly from each site to measure leaf functional traits. The selected individuals within a species had similar features, such as root collar diameter, height, and DBH. According to Cornelissen *et al*. (2003), 100 to 200 fully expanded and sun-exposed leaves were collected from each individual. Twenty leaves were chosen randomly from each individual to measure leaf area, leaf fresh weight, and leaf dry weight, and later LDMC and SLA were calculated. Five leaves per individual tree were randomly taken to measure LT. The root collar diameter and DBH were also measured from the same selected individual trees.

Root collar diameter (cm) was measured at the base of the tree trunk using a caliper, and DBH (cm) measured 1.3 meters above the ground using a diameter tape. Height (cm) was measured using a clinometer. Leaf thickness (mm) was measured using a micrometer digital vernier caliper. Fresh weight (FW) (g) was measured using a precision balance, and leaf dry weight (DW) (g) was measured after oven drying at 70°C for 48-72 h. LDMC (mg/g) was calculated as the ratio of leaf dry weight to fresh weight. Leaf Area (mm^2) was measured using the Petiole Pro mobile application, a tool designed for rapid and accurate leaf area assessment, as described in Schrader *et al*. (2017). Fresh, intact leaves were collected and placed on a flat, contrasting background to ensure clear visibility. A smartphone equipped with the Petiole Pro app was used to capture images of the leaves, with calibration pads provided by the app's developers used for precise scale calibration. These pads, placed alongside the leaves during image capture, ensured the app could accurately calculate the leaf area. This method allowed for efficient and non-destructive leaf area measurement across multiple samples while maintaining high accuracy and consistency with established methodologies. Lastly, SLA was calculated as the leaf area divided by its dry weight.

Chemical traits measurements

Sample leaves were taken from the same leaves collected from the individual trees used to measure morphological traits. Leaves were air-dried at 25°C for three days, grounded, and processed for chemical characterization (lignin and condensed tannin content). Lignin concentrations were determined following the Klason method after hydrolysis with 72% H_2SO_4 (Dence, 1992), and condensed tannin concentrations were extracted in hot 50% aqueous methanol (Makkar and Becker, 1998).

Statistical analysis

Associations among the six green leaves and litter traits studied were analyzed by principal component analysis (PCA) (R Core Team, 2022) to identify significant contributors to the characteristics. The FactoMineR and factoextra packages of R studio (R-4.3.3 version) were used to quantify the respective contributions of green leaf to explore the predictive power of these traits on litter chemical characteristics (lignin and condensed tannin). A linear mixed model was fitted using the linear function from the lme4 package in R, and estimates of parameter effects and variance components for both fixed and random effects in the model were provided.

The analyses used lignin and tannin as strong characteristics influencing litter decomposition as the response variable, with leaf traits (LA, SLA, LDMC, and LT) as fixed effects. Species were modeled as a random effect to account for inter-species variability. All the variables were tested for normality and log-transformed before analysis.

To validate our model, we compared a complete model, which included all fixed effects, to a reduced model, excluding one or more fixed effects, using a Chi-square test (likelihood ratio test) to determine whether adding additional parameters significantly improved the model fit.

The full and null models were determined and validated by the Akaike Information Criterion (AIC) and Chi-square (likelihood ratio test) (Kasali, 2013).

Results

Morphological traits

For the Kayonza site, *Calliandra calothyrsus* and *Croton megalocarpus* showed higher SLA compared to *Grevillea robusta* (Fig. 2). This suggests that the later species may have thicker, denser leaves that decompose more slowly.

In Musanze site, on the other hand, *Markhamia lutea* exhibited the highest SLA than *Alnus acuminata* and *Eucalyptus globulus,* suggesting that these species have more challenging, thicker leaves that resist decomposition. For Leaf Area (cm^2), the study results revealed that the species in Kayonza exhibit relatively more minor leaf areas, with *Calliandra calothyrsus* showing the smallest value. *Croton megalocarpus* and *Grevillea robusta* also display similar leaf area ranges (Fig.2).

For Leaf thickness, in Kayonza, *Grevillea robusta* and *Croton megalocarpus* have relatively thicker leaves compared to *Calliandra calothyrsus, which* exhibited the thinnest leaves (Fig.2). For Musanze *Markhamia lutea* shows relatively thin leaves, which may indicate faster decomposition rates compared to *Alnus acuminata* and *Eucalyptus globulus* (Fig.2).

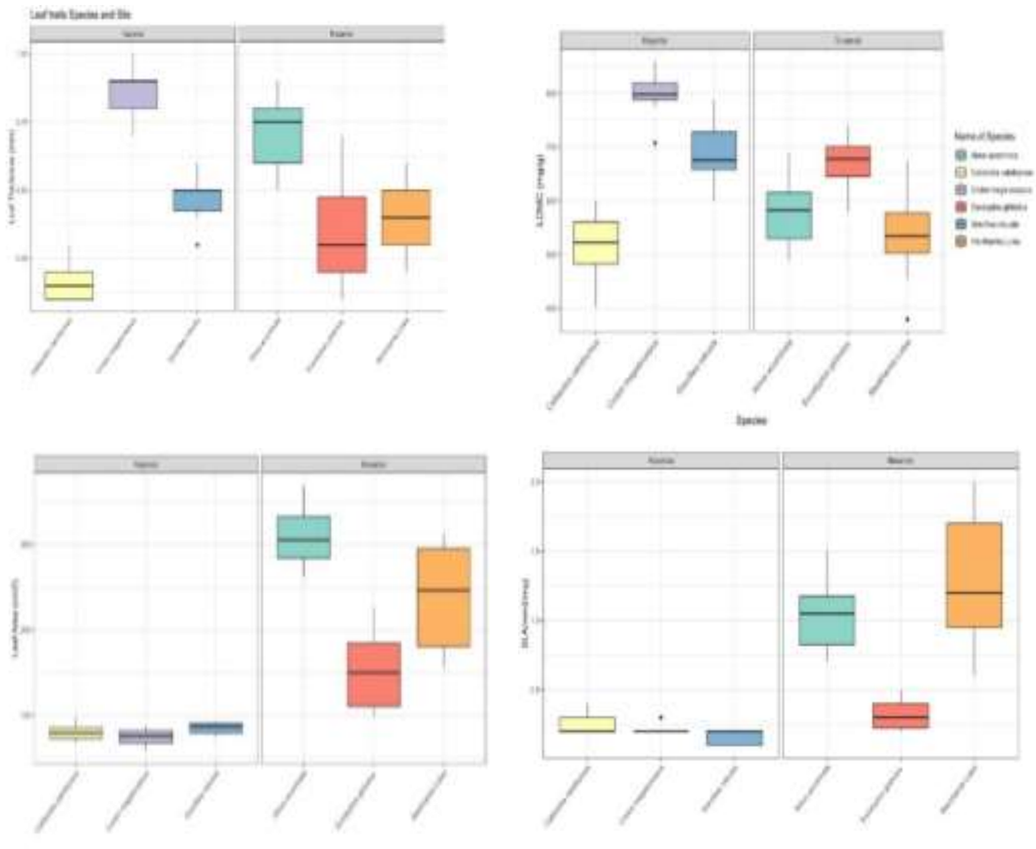

Figure 2: Values of leaf trait recorded for six agroforestry tree species in two contrasting regions of Rwanda (Kayonza, the semi-arid, and Musanze, the humid highland). Alnus acuminata and Calliandra calothyrsus (exotic N_2-fixing trees), Eucalyptus globulus and Grevillea robusta (exotic non-N_2-fixing), and Markhamia lutea and Croton megalocarpus (native non-N_2-fixing trees). Green Leaf traits ((Leaf area (LA) cm^2, Specific leaf area (SLA) $cm^2\ g^{-1}$, Leaf dry matter content (LDMC) $mg\ g^{-1}$, and Leaf thickness mm. The center line indicates the median and the upper and lower box height indicates the interquartile range.

For LDMC, in Kayonza site, the results revealed that *Grevillea robusta* and ***Croton** megalocarpus* exhibit relatively high LDMC values compared to *Calliandra calothyrsus* with much lower LDMC (Fig.2). In Musanze, *Eucalyptus globulus* exhibits a relatively high LDMC, *Alnus acuminata* displays moderate LDMC values than *Markhamia lutea* with lower LDMC.

Association amongst leaf traits, lignin, and tannin

The PCA plot provides insights into the relationships between leaf morphological traits and chemical characteristics that influence litter decomposition rate, specifically lignin and tannin. Principal Component 1 (PC1) explains 45.6% of the total variance, capturing the majority of the variation in the dataset. In comparison, Principal Component 2 (PC2) accounts for an additional 26.4%, indicating a secondary source of variation (Fig. 3). Together, these components explain about 72% of the variability, making them informative in understanding the key factors driving litter decomposition. Specific Leaf Area (SLA) and Leaf Area (LA) are also positively correlated, contributing to the variance along PC1 (Fig. 3), indicating that species with larger leaves and higher SLA tend to decompose faster, as these traits are typically associated with quicker nutrient release.

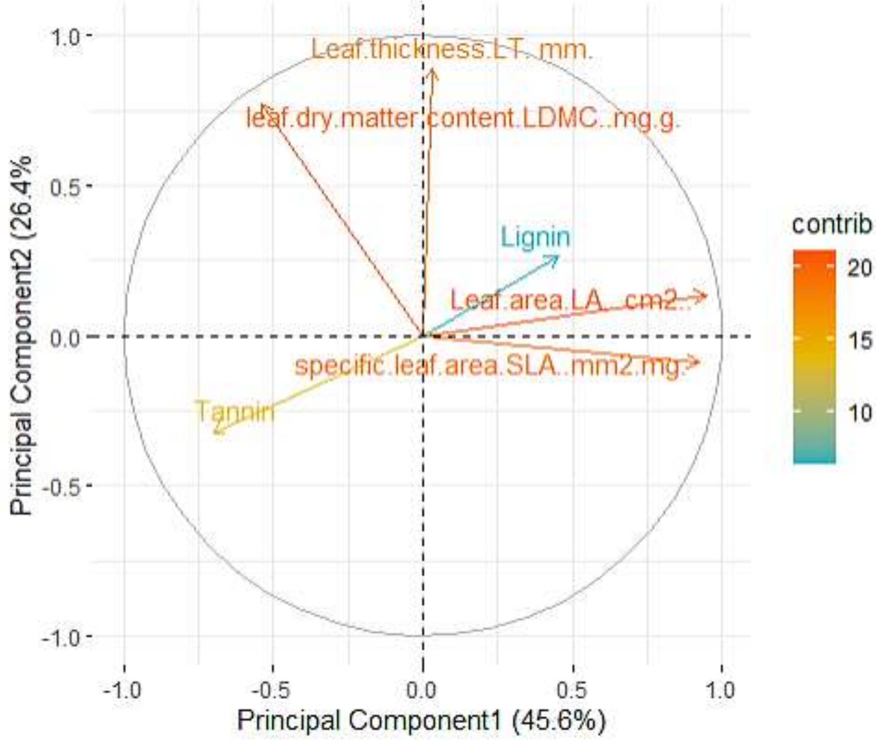

Figure 3: A principal component plot of leaf and litter traits of six different agroforestry tree species in two contrasting study areas. PC1 represents the direction that explains the most significant amount of variance. It captures the most prominent pattern in the differences among study species, with variability associated with Leaf thickness and LDMC. PC2 represents insight into the next level of variation. It captures the variation in data explicitly related to Lignin and Leaf Area differences.

Lignin, on the other hand, loads strongly along PC2, highlighting its role in explaining variability distinct from the other traits, particularly in species

where lignin content plays a significant role in decomposition. Tannin shows a weaker association, contributing less to the total variance and inversely related to the factors influencing PC2 (Fig. 3).

The REML analysis, based on the four significant contributors (identified through PCA above) as fixed effects with lignin and tannin as a response variable, showed that the leaf traits best-explained variation in the decomposition process (Table1).

Table 1. ***Results of linear mixed model (LMM) analysis of litter decomposition in both study areas***

linear mixed model					
Response variable	**Fixed effect trait**	**Estimate**	**variation explained %**	**Std. dev**	
Lignin	Leaf thickness	-9.69679	20.2		
	Leaf area	0.0619214	51.2		
	Leaf Dry Matter Content	0.02931	24.9		
	Specific Leaf Area	-2.40828	1.8		
Tannin	Leaf thickness	-1.1730	3.1		
	Leaf area	-0.01073	15.9		
	Leaf Dry Matter Content	-0.00006	0		
	Specific Leaf Area	-0.4950	0		
	Random effect				
	Marginal R2				
Model statistics for the null and full model					
Model Parameter	**Df)**	**AIC**	**LogLik**	**Chi-square**	**(p-value)**
Null Model					
Lignin	0	400.637	-199.318		
Tannin	0	258.846	-128.423		
Full Model					
Lignin	4	379.177	-184.588	29.46	<0.001
Tannin	4	236.335	-113.178	30.491	<0.001

Linear regression models were fitted to see the effects of crucial leaf traits LA, LT, LDMC, and SLA on lignin and tannin content, which are used as indicators of decomposition (Table 1). Both lignin and tannin were modeled as response variables. Model comparison was performed between the full model (including all predictors) and the null model (without predictors) to determine the strength of these relationships**.** For the lignin Model**,** the whole model had lower AIC than the null model, indicating a better fit. The Chi-Square test yielded a value of 29.46**,** with a p-value < 0.001**,** indicating that the predictors significantly improved the model. For the tannin model, the whole model also performed significantly better than the null model, with a low AIC compared to the null model. The Chi-Square test yielded a value of 30.491**,** with a p-value < 0.001**,** indicating that the predictors significantly improve the model.

By fitting the lignin full model with all predictors, the results highlighted which traits strongly influence lignin and tannin based on their contribution to the total explained variance. For the lignin model, the study traits LA explained 51.2% of the variance in lignin, which means LA had a strong influence on lignin content, LT had 20.0%, LDMC had 24.9%, and SLA explained only 1.88% of the variance. For the tannin full model, results predicted that LA explained 15.9% of the variance in tannin; LT had affected 3.1% of tannin content, while LDMC and SLA traits are not good predictors of tannin content.

Leaf traits predicting decomposition

LT**,** LDMC**,** LA**,** and SLA affect Lignin and Tannin decomposition indicators. Using multiple linear regression, results indicated that the effect of LA showed a significant positive impact on lignin content with a standardized coefficient of **0**.0619 and a p-value < 0.001, suggesting that larger leaves are associated with higher lignin levels. LT significantly affected lignin with a sizeable standardized coefficient of **-**9.6968 and a p-value of 0.010**,** indicating that thicker leaves are associated with lower lignin content (Table 2).

Table 2. ***Multiple regression analysis to assess relationships between decomposition parameters (Lignin and Tannin) and leaf traits (LA, LT, LDMC, and SLA) across study species leaves collected in September 2022***

Predictors	**Lignin**			**Tannin**		
Leaf Trait	**(Std Coef)**	**p-value**	**Effect**	**Std Coef**	**p-value**	**Effect**
LA	0.06	<0.001	Positive	-0.01	0.019	Negative
LT	-9.7	0.01	Negative	-1.17	0.296	Negative
LDMC	0.03	0.005	Positive	0	0.826	Negative
SLA	-2.41	0.436	Negative	-0.5	0.598	Negative

LA: Leaf area, LT: Leaf thickness, LDMC: Leaf dry matter content, SLA: Specific leaf area

LDMC also had a significant positive effect on lignin, with a standardized coefficient of 0.0293 and a p-value of 0.005**,** meaning leaves with higher dry matter content had more lignin. While the SLA showed a negative effect on lignin, with a standardized coefficient of -2.4083**,** the p-value of 0.436 indicated that this effect was not statistically significant (Table.2). For tannin as a litter decomposition indicator, the effect leaf traits showed that LA had a substantial adverse impact on tannin content, with a standardized coefficient of -0.01 and a p-value of 0.019, meaning that larger leaves tend to have lower tannin content. LT had a negative effect on tannin, with a standardized coefficient of -1.17, but this effect was not statistically significant **(**p-value = 0.296**).** LDMC and SLA showed no significant impact on tannin content, with small standardized coefficients of 0 and -0.5 and p-values of 0.826 and 0.598, respectively.

Discussion

Morphological traits

Specific Leaf Area (SLA) is recognized as a critical morphological trait that influences litter decomposition rates, with higher SLA typically associated with faster decomposition due to increased surface area and lower tissue density. In our study, SLA emerged as a significant predictor of decomposition, aligning with prior research demonstrating SLA's role in promoting faster nutrient cycling through enhanced microbial activity (Liu *et al*., 2018). This relationship is particularly evident in species such as *Markhamia lutea*, where the high SLA observed in the humid highland of Musanze suggests an accelerated contribution to nutrient cycling, consistent with studies showing that species with higher SLA, such as *Markhamia*, decompose more rapidly and release nutrients at a faster rate (Rawat *et al*., 2020).

The variations in SLA between species reflect adaptive strategies to environmental pressures. Our study found significant differences in SLA between the humid highland of Musanze and the semi-arid region of Kayonza, with species in Musanze exhibiting significantly higher SLA. This is consistent with findings from Canessa *et al*. (2021), who noted that environmental conditions, particularly temperature and moisture availability, impose intense selective pressures on plant traits. In semi-arid regions, species such as *Calliandra calothyrsus* displayed lower SLA, an adaptation to reduce water loss and avoid heat stress, as observed in similar studies (Yang *et al*., 2022). Such adaptations, while beneficial for water conservation, may also influence decomposition rates, as smaller leaves with lower SLA decompose more slowly due to their structural properties and lower microbial activity on the leaf surface.

In contrast, species like *Alnus acuminata* and *Markhamia lutea* in the humid highlands displayed much larger leaves and higher SLA, characteristic of environments with abundant moisture that allow for more significant leaf expansion and increased photosynthetic capacity (Liu *et al*., 2021). Larger leaves facilitate faster decomposition due to greater exposure to microbial decomposers and may also interact with other traits, such as leaf thickness and lignin content, which play crucial roles in decomposition dynamics. For instance, *Eucalyptus globulus*, despite having a moderate SLA, is known for its relatively thicker leaves, which may slow down decomposition but contribute to long-term soil organic matter accumulation (He, Y. *et al*., 2024). This aligns with the broader understanding that thicker, lignin-rich leaves, like those of *Grevillea robusta* and *Alnus acuminata*, decompose more slowly, but their slower breakdown provides a steady release of nutrients and enhances soil structure over time (Berg and McClaugherty, 2020).

Leaf Dry Matter Content (LDMC) further corroborates this relationship, as species with higher LDMC, such as *Grevillea robusta* and *Eucalyptus globulus*, tend to decompose more slowly due to their more challenging, thicker leaves. This slower decomposition is beneficial in agroforestry systems where long-term soil organic matter accumulation is critical for improving soil fertility and structure (Oyebamiji *et al*., 2024). In contrast, species with lower LDMC, such as *Calliandra calothyrsus*, exhibit faster decomposition rates, contributing to quicker nutrient cycling, which is advantageous in systems requiring rapid fertility replenishment (Sileshi *et al*., 2020).

Interestingly, *Alnus acuminata*, an N-fixing species, displayed moderate LDMC values, suggesting an intermediate decomposition rate. This species' ability to fix nitrogen makes it a valuable contributor to both organic matter accumulation and nitrogen input, further supporting its role in agroforestry systems to enhance soil fertility (Lebrazi and Fikri-Benbrahim, 2022).) Similarly, *Markhamia lutea*, with its faster leaf breakdown and lower LDMC, has the potential to enhance nutrient cycling more rapidly; these results align with those of Wigley *et al*., 2016), making it a critical species for restoration efforts in humid environments such as Musanze.

Markhamia lutea, as a native species, is particularly notable in our study for its rapid decomposition rate, which underscores its potential to contribute to quicker nutrient release and enhanced nutrient cycling in humid environments. These findings highlight the importance of native species like *Markhamia* in land restoration initiatives, where their conservation and promotion can play a crucial role in supporting sustainable ecosystem management. By integrating native species into restoration projects, we can harness their ecological benefits for nutrient cycling and promote biodiversity and the resilience of ecosystems (Dhyani *et al*., 2020).

Our findings highlight the complex interactions between morphological traits like SLA, LDMC, and leaf area in determining decomposition rates and nutrient cycling. While species with higher SLA and lower LDMC, such as *Markhamia lutea* and *Calliandra calothyrsus*, contribute to rapid nutrient release, species with thicker leaves and higher LDMC, like *Grevillea robusta* and *Alnus acuminata*, play a crucial role in long-term soil carbon storage and fertility improvement. These results underscore the importance of integrating both fast- and slow-decomposing species in agroforestry systems to optimize short-term fertility and long-term sustainability (Gupta *et al*., 2023).

The association between leaf traits, lignin, and tannin

In our study, Leaf Thickness (LT) and Leaf Dry Matter Content (LDMC) exhibited strong loadings along the first principal component (PC1), highlighting their role as key drivers of variability among species (Fig. 2.3). This axis primarily represents a continuum between traits associated with slow decomposition, such as thick, tough leaves, and fast-decomposing, nutrient-rich leaves. Species with thicker, denser leaves, characterized by higher LT and LDMC, are positioned positively along PC1, indicating that these traits are closely linked to slower decomposition rates. Thicker leaves and higher LDMC are generally associated with tougher leaf structures, which are more resistant to microbial attack, thereby delaying nutrient release during decomposition. These results align with the findings of previous studies that suggest thick, lignin-rich leaves decompose more slowly due to their increased structural complexity (Stewart *et al*., 2015). Lignin, in particular, is known for its recalcitrance to microbial degradation, and its positive association with LDMC and LT further supports the idea that species with thicker, lignified leaves exhibit slower decomposition rates. The resistance of lignified tissue to microbial breakdown requires more time and energy for decomposers to process, effectively slowing down nutrient cycling. The strong correlation between lignin content and the structural leaf traits suggests that lignified, dense leaves contribute to slower litter decomposition and prolonged nutrient immobilization (Liao *et al*., 2022).

Interestingly, SLA emerged as a trait on the opposite side of PC1, indicating an inverse relationship with traits like LT, LDMC, and lignin. High SLA, characterized by thinner leaves with a larger surface area relative to their weight, is often associated with faster decomposition due to the ease of microbial colonization and breakdown. This aligns with the worldwide leaf economics spectrum (Donovan *et al*., 2011), where species with high SLA typically exhibit faster growth and nutrient cycling. However, in our analysis, SLA showed a notable inverse relationship with tannin content (Fig. 2.3), suggesting that species with higher SLA tend to have lower tannin concentrations. Tannins inhibit microbial activity and slow down decomposition, implying that fast-growing species with high SLA may offset their rapid leaf turnover by reducing tannin concentrations to promote faster decomposition (Zhao *et al*., 2022).

Similar to the outcomes reported by (Ramos *et al*., 2021), our results showed that species displaying larger LA often decompose more quickly due to the increased surface area available for microbial colonization. The relationship between LA and tannin content further complicates the decomposition process. While larger leaves provide more opportunities for microbial activity, species with higher LA may exhibit lower tannin concentrations, facilitating faster nutrient release. Conversely, species with smaller leaves (low SLA and low LA) may rely on higher tannin production as a defense mechanism to slow down decomposition. These results are consistent with prior findings highlighting the trade-offs between leaf size, nutrient content, and chemical defenses in regulating decomposition rates (Pan *et al*., 2022).

Our principal component analysis provides valuable insights into the functional traits that regulate litter decomposition. Species with higher LT, LDMC, and lignin content will likely contribute to slower decomposition rates, prolonging nutrient release and enhancing long-term carbon storage in soils. On the other hand, species with higher SLA and larger LA decompose more rapidly, promoting faster nutrient cycling but contributing less to long-term organic matter accumulation. This dynamic underscore the importance of trait combinations in shaping decomposition processes and nutrient cycling across ecosystems (Prescott and Vesterdal, 2021).

Leaf traits predicting decomposition

LA showed a significant positive effect on lignin content, with larger leaves associated with higher lignin concentrations (Table 2.2). The positive association between LA and lignin content could be due to larger leaves having more structural material, which tends to be lignified and less degradable (Cornwell *et al*., 2008). In contrast, LT significantly affected decomposition, indicating that thicker leaves are associated with lower lignin levels (Stewart *et al*., 2015). This inverse relationship suggests that species with thicker leaves may compensate for their physical robustness by investing less in lignin production, which is consistent with studies showing that leaf thickness often relates to toughness rather than chemical resistance (Cornelissen *et al*., 2003; Kazakou *et al*., 2006). Despite the physical strength provided by thicker leaves, our results imply that lignin concentration, a key chemical defense mechanism, may be reduced in these species, potentially leading to faster decomposition compared to thinner but more lignified leaves.

LDMC also significantly positively affected lignin content, suggesting that denser leaves with higher dry matter are more lignified and decompose more slowly. LDMC correlates with leaf toughness and resistance to decomposition (Fortunel *et al*., 2009), and our results confirm that species with higher LDMC are likely to contribute to slower nutrient cycling due to their higher lignin content. This finding aligns with previous research that has demonstrated the importance of LDMC in predicting litter decomposability, as denser leaves are more difficult for microbial decomposers to break down (Cornwell *et al*., 2008; Ueda et al., 2022). SLA exhibited a negative but non-significant effect on lignin content, suggesting that while thinner, larger-surfaced leaves may contribute to faster decomposition, their influence on lignin levels was not statistically significant in our study.

When examining tannin content, LA had a significant negative effect, meaning species with larger leaves tend to have lower tannin concentrations. Tannins inhibit microbial decomposition by binding proteins and reducing microbial activity (Cornelissen *et al*., 2003; Wieder *et al*., 2009). Interestingly, LT also had a negative but non-significant effect on tannin content, which could imply that thicker leaves, although physically challenging, do not necessarily rely on tannin as a chemical defense mechanism. This finding is consistent with studies that suggest thick leaves are more dependent on structural defenses, such as cellulose and lignin, rather than tannins, for resistance to decomposition (Rahman *et al.*, 2013). Neither LDMC nor SLA significantly affected tannin content, highlighting the complexity of leaf trait interactions and their influence on decomposition.

Conclusions

Among the studied leaf traits in predicting litter decomposition, LA, LT, and LDMC were significant predictors of lignin content, while LA was the primary predictor of tannin content. Confirming that species with more extensive, denser leaves contribute to slower decomposition and prolonged nutrient cycling. Conversely, SLA exhibited a weak but negative association with lignin, consistent with the notion that thinner, more delicate leaves decompose more quickly, although their influence on lignin concentration was not significant in this study. The inverse relationship between LA and tannin content suggests that species with larger leaves invest less in tannin-based defenses, further promoting faster decomposition. These findings offer valuable insights into how functional traits influence decomposition dynamics and nutrient cycling, particularly in agroforestry systems where sustainable soil management is critical. Future studies should explore how these leaf traits interact with environmental factors to influence decomposition across different ecosystems.

Author contribution

VM, ABT, CPM, SMSM, and NIM conceptualized and designed the study; VM collected data for the study; VM analyzed and interpreted the data; VM wrote the first version of the manuscript; CPM read and modified the draft manuscript with substantial inputs.

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