Leaf litter Decomposition dynamics across a Recovering Tropical Forest in the lowland Ecuadorian Chocó

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Abstract

Litter decomposition by arthropods, microbes, and fungi is a key ecosystem process in tropical forests, yet its response to forest disturbance and recovery remains poorly understood. To investigate decomposition dynamics across forest succession, we conducted an experiment in the Ecuadorian lowland Chocó (Esmeraldas) using a chronosequence approach. We deployed above- (AG) and belowground (BG) litterbags in 32 plots spanning active cacao plantations and pastures (age 0), secondary forests (1–38 years), and old-growth forest. AG litterbags (5 mm mesh) allowed arthropod access, while BG litterbags restricted decomposition to microbial activity. Each contained standardized leaf litter from five common tree species: *Pourouma bicolor*, *Brosimum utile*, *Compsoneura atopa*, *Vochysia macrophylla*, and *Trema micrantha*. Litterbags were collected at three time points, with replacements every 45 days. We examined decomposition drivers by modeling litter mass loss (%) against forest age and environmental factors. AG decomposition was analyzed in relation to tree aboveground biomass, surface temperature, leaf litter biomass, elevation, and terrain slope, while BG decomposition was assessed with soil pH, soil C:N, terrain slope, soil moisture, and soil temperature. Additionally, we tested how small-scale disturbances and large animal exclusion affected decomposition (PREX) using four treatments: control (C), fenced (CF, exclusion of large ground-dwelling animals), perturbed (P, removal of litter and understory vegetation in 100 m²), and perturbed-fenced (PF, combined litter removal and animal exclusion). AG decomposition rates increased with forest succession but followed a U-shaped pattern in plots recovering from cacao land-use, with a mid-succession decline and higher rates in old-growth forests. Key drivers included surface temperature, elevation, and tree aboveground biomass, with temperature varying significantly depending on land-use history. BG decomposition was unaffected by forest age, decreased with C:N, and showed a bell-shaped response to soil moisture. Large animal exclusion (CF) had no effect, whereas perturbation (P, PF) significantly altered decomposition. Notably, decomposition in P plots showed dynamic recovery, whereas in PF plots, mass loss remained suppressed throughout the 135-day study, emphasizing the role of large animals in facilitating ecosystem recovery.

Keywords: litterbags, tropical ecosystem processes, forest regeneration, Ecuadorian Chocó, chronosequence

Introduction

Litter decomposition is a fundamental ecological process in forests, driving the breakdown of fallen leaves and organic material on the forest floor through the activity of bacteria, fungi, insects, and other invertebrates (Q. Liu et al. 2005; Makkonen et al. 2012). Decomposition plays a pivotal role in nutrient cycling, thus supporting the fertility and productivity of forests (Long et al. 2021). Understanding decomposition dynamics is particularly relevant in regenerating tropical forests, which are expanding globally due to land abandonment and secondary succession (Heinrich et al. 2021). Secondary tropical forests have become critical for carbon storage and biodiversity conservation, yet their recovery trajectories vary depending on past land-use, disturbance history, and environmental conditions (Poorter et al. 2016). To grasp changes in nutrient cycling and forest productivity during forest regrowth, it is imperative to discern the impact of local environmental factors on litter decomposition. In lowland tropical rainforests, variations in local climatic conditions and soil characteristics occur within regions, across landscapes and at the forest microhabitat level (Paudel et al. 2015; Ostertag et al. 2022), due to differences in land-use legacy (Foster et al. 2003a; Kallenbach and Stuart Grandy 2015), disturbance level (Röder et al. 2024), or successional stage (Attignon et al. 2004; Stone et al. 2020). These factors in turn strongly influence tree species diversity and thus determine litter quality (Sánchez-Silva et al. 2018) and shape the decomposer community structure (Ashford et al. 2013), both of which are primary drivers of decomposition rates (Cornwell et al. 2008; García-Palacios et al. 2013).

Findings from previous chronosequence studies (Powers et al. 2009; Paudel et al. 2015; Sánchez-Silva et al. 2018; Morffi-Mestre et al. 2023) indicate that decomposition rates tend to increase with forest recovery. Forest age is a critical factor influencing litter decomposition, with an increase in decomposition rates as aboveground biomass accumulates (Lohbeck et al. 2015). This "vegetation quantity effect" suggests that the recovery of ecological processes, including decomposition, is closely tied to biomass accumulation. Mature forests, with their more stable microclimates and developed decomposer communities, support higher decomposition rates, likely due to more favourable microhabitats and enhanced microbial activity in more structurally complex environments (Sniegocki et al. 2022). Forest structure also influences microclimate and it is well-established that temperature and water availability are the two main controlling factors of decomposition, both above- (Salinas et al. 2011) and belowground (Aerts 1997). The importance of soil moisture is particularly pronounced in tropical forests, where temperature is less limiting and soil microbial activity is primarily moisture-driven (Meir et al. 2008; Schaap et al. 2024). However, past land-use exerts a lasting influence on forest structure and soil properties (Chazdon 2003), which can affect decomposition trajectories over time.

Historical agricultural activities, such as cultivation and grazing, can lead to soil compaction, nutrient depletion, and shifts in plant species composition (van der Sande et al. 2022), which in turn affect litter quality, decomposer communities and decomposition rates. Soil moisture acts as a key modulator of decomposition rates, exhibiting a non-linear relationship with microbial activity (Sierra, Malghani, and Loescher 2017). On one hand, increased moisture enhances soil organic carbon solubility and diffusion, facilitating microbial uptake and reducing physiological stress (Moyano, Manzoni, and Chenu 2013; Manzoni et al. 2014). On the other hand, excessive moisture reduces oxygen availability, which slows aerobic decomposition (Skopp et al., 1990; Keiluweit et al., 2016). Similarly, soil pH influences microbial communities and enzymatic activity, with decomposition rates varying across pH gradients (Sellan et al. 2020). Additionally, soil C:N ratios can have contrasting effects on decomposition, exhibiting ecosystem-dependent variability, with mass loss increasing with C:N in forests but decreasing in grasslands (Blanco et al. 2023). Topography also influences decomposition via its effects on soil properties and microclimate, with some studies reporting increased decomposition on steeper slopes due to enhanced solar exposure, temperature, and soil aeration (Hu et al. 2020), while others found no significant effects (Ma et al. 2024). Elevation is often closely associated with temperature and forest composition (Sinha et al. 2018), thus affecting leaf litter decomposition, especially aboveground (Bohara et al. 2020).

Forest clearance activities, including biomass removal and logging, modify microclimate, soil properties, disrupt decomposer communities and hinder litter breakdown (Latterini et al. 2023). However, large-scale experimental manipulations of decomposition in tropical forests remain rare. A long-term litter manipulation experiment in mature forest in Panama, revealed that litter removal alters soil conditions, reduced nutrient cycling, and impacted soil fauna and microbial communities (Sayer, Tanner, and Lacey 2006; Ashford et al. 2013). However, the immediate effects of perturbations associated with tropical land-use change on decomposition dynamics remain uncharacterised. To address this, we studied the impacts of vegetation removal and large animal exclusion on leaf litter decomposition in the Chocó Forest in Ecuador, a lowland tropical forest known for its remarkable biodiversity and ecological importance. We used a space-for-time approach (described in Escobar *et al.*, 2024), to examine how disturbance and environmental factors interact to influence above- and belowground decomposition along a chronosequence of naturally regenerating tropical forest with different land-use legacies. To mimic the low-impact forest degradation commonly observed in extensive areas of neotropical forests (Matricardi et al. 2020), we simulated two types of small-scale disruptions (100 m^2) representing human interventions such as selective logging (Sagarin and Pauchard 2010) or hunting (Dirzo et al. 2014; Granados et al. 2017) within forest areas at different stages of recovery from larger-scale disturbances (>1 ha).

By investigating the *in situ* effects of small-scale disturbances on decomposition along a forest recovery chronosequence, our study addressed three key objectives: (1) To elucidate variations in leaf litter decomposition across stages of forest regeneration from different land-uses. (2) To identify how changes in key abiotic drivers during forest regrowth influence leaf litter decomposition. (3) To experimentally assess the recovery of decomposition following a strong pulse perturbation and evaluate the relative importance of large animal activity on decomposition dynamics.

We hypothesised that: (1) Decomposition rates will increase with forest recovery, but the trajectory will differ between land-use legacies. H2) Differences in decomposition dynamics with forest age and between land-use legacies will be explained by distinct environmental conditions and soil properties, whereby decomposition rates will increase with temperature and be highest at moderate soil moisture levels. H3) Small-scale pulse disturbances simulating forest clearance and large animal exclusion will inhibit decomposition and the impact of perturbation will increase with forest age.

Materials and Methods

Study design

This study formed a crucial component of the *Reassembly* Research Unit (www.reassembly.de), which focuses on elucidating the dynamics of tropical forest recovery. Situated in the northwest of Ecuador, within the lowland Chocó Forest, the study area includes the Canandé reserve of the Jocotoco Foundation, the Tesoro Escondido reserve and the nearby villages of La Yuca and Hoja Blanca (Province of Esmeraldas). Detailed information on the *Reassembly* study design is given in the respective site description (Escobar et al. 2024). The *Reassembly* chronosequence comprises study plots representing various stages of forest regeneration (Escobar et al. 2024), spanning active cacao plantations and pastures to 38-year-old regenerating forests originating from these respective land-use legacies. For our study we selected a subset of 32 plots consisting of three active cacao plantations, three active pastures, 18 forests at various stages of recovery (nine with cacao plantation legacy and nine with pasture legacy) and eight old-growth forests. The active cacao plots encompass two different land management practices, two plots with organic agroforestry cultivation and one conventional plantation encircled by pasture lands. Forest recovery is represented by forest age, defined as the number of years since the last human use of the study plot, and the age distribution of the plots was as uniform as possible across land-use legacies (Table S1). Finally, the old-growth forests provide a benchmark for undisturbed forest conditions; they were chosen based on the absence of evidence for past anthropogenic exploitation, determined from historic data provided by the Jocotoco Foundation.

Perturbation-Recovery Experiment (PREX)

To study the short-term response of decomposition processes to disturbance, we conducted a Perturbation-Recovery Experiment (PREX). We established four 10 x 10 m subplots within each forest plot long the chronosequence. In March 2022, we applied one of four treatments to each subplot: 1) complete removal of litter, dead wood, lianas, seeds, shrubs, and understory plants with < 25 cm stem circumference at 1.3 m above the ground (P_o), 2) large animal exclusion by surrounding the area with a *c.* 1 m high shade-cloth fence (CF); 3) combined perturbation and fencing (PF_o); and 4) undisturbed open controls (C). In March 2023, we repeated the P_o and PF_o treatments in 4 x 10 m areas inside the previously perturbed subplots. All new vegetation with stem circumference of < 25 cm, and all dead wood and leaf litter were removed from a 4 m x 10 m area in the old-growth and regeneration subplots, and a 2.5 m x 8 m area in the active agriculture. Because of the sloped terrain of some plots, a barrier was placed between the newly perturbed area and the one perturbed the year before to protect from eventual mudslides. Therefore, the re-perturbation added two more treatments to the experiment: open perturbed treatment (P) and perturbed-fenced (PF).

Decomposition experiment

To investigate the influence of forest age, land-use legacy and small-scale disturbance on decomposition dynamics, we measure litter mass loss during three consecutive 45-day post-disturbance regeneration stages. We used litterbags to measure decomposition above- and belowground: aboveground (AG) litterbags measured 20 cm x 20 cm and were made with 0.5-cm plastic mesh; belowground (BG) litterbags measured 5 cm x 5 cm and were made with 0.5 mm mesh. We used a standard litter mix (with equal proportions of species) to encompass a range of leaf traits (Table S2) and plant species present across the chronosequence (Figure S1). To create the litter mixture, we collected and chopped leaves (to ca. 5 x 5 cm for litterbags and to 5 x 5 mm for teabags) from five local common trees: *Pourouma bicolor* (Urticaceae), *Brosimum utile* (Moraceae), *Compsoneura atopa* (Myristicaceae), *Vochysia macrophylla* (Vochysiaceae), *Trema micrantha* (Cannabaceae). To measure aboveground decomposition, we defaunated the leaves by freezing at -18°C for at least 30 days, thoroughly mixed an equal mass of leaves per species to guarantee a uniform distribution, and then placed 30 g (± 0.01 g) of the standardised mix in each AG litterbag. For belowground decomposition, we oven dried the leaves at 70 °C to constant weight, then placed 0.2 g (± 0.001 g) of each litter from each species in each BG litterbag to give a total of 1 g litter dry mass.

In March 2023, we deployed the first set of litterbags on the same day the perturbation treatment was applied. We placed one AG litterbag and two BG litterbags (c. 10 cm depth) in all four PREX subplots per plot. We used two BG litterbags per subplot as buried bags were more likely to be damaged or lost. After an incubation period of 45 days, we collected all litterbags and placed a new set of bags in each subplot and we repeated this process after 90 days. Thus, we measured decomposition at 45, 90 and 135 days post-perturbation, in four subplots within 32 study plots, giving a total of 386 AG litterbags and 772 BG litterbags.

Upon retrieval, the litterbags were thoroughly cleaned of soil and root material and then dried to constant weight at 70°C. The final dry weight was used to determine mass loss and decomposition was expressed as the percentage dry mass loss (%) of litter over each 45-day incubation. For belowground decomposition, mass loss of the two BG litterbags was averaged. A total of 62 AG and 67 BG litterbags were either lost or discarded because the litterbags had been damaged or because the content was indistinguishable from soil and roots, leaving 324 AG litterbags and 705 BG litterbags for analysis.

Environmental data

To characterise each plot, we used data on slope, tree aboveground biomass, elevation, litter standing crop, soil surface temperature, soil temperature at 0-6 cm depth, and soil pH, soil moisture and soil C:N ratio from 0-10 cm depth. We measured soil temperature and soil moisture with TMS-4 data logger (TOMST s.r.o., Prague, Czech Republic) following the field method described by Wild *et al.* (2019). We summarised the daily measurements by calculating the 95th percentile for each study plot for the study period of our decomposition experiment (see Supplementary Methods for further detail). For soil pH and C:N ratio, eight soil samples were collected at 0-10 cm depth at the margins of each plot and later mixed to form a single composite sample of 800-1000 g. Within a week of collection, 300 gr to 500 gr of the mixed sample was oven-dried to constant weight at 40°C. Dry samples were stored in sealed plastic bags. Soil pH was measured in a 1:1 soil-water solution and soil total C and N content was measured by elemental analysis (Elemental Analyzer FlashSmart, Thermo Fisher Scientific, Italy). To determine litter standing crop, we collected litter from four 50 x 50 cm areas of each plot prior to the first perturbance event. The litter was dried to constant weight to obtain dry mass. Tree aboveground biomass was estimated on individual tree DBH and height measurements (Chave et al. 2014) upon a detailed botanical survey on the study plots. Elevation and slope were extracted as topography DEM for plot centroids using bilinear interpolation. The topographical and botanical data for each plot was collected as part of the *Reassembly* project and the respective methods and datasets can be found in the site description (Escobar et al. 2024).

Statistical analysis

Data analysis was conducted in R version 4.3.1 (R Core Team 2023, www.r-project.org). To test the effect of forest age and land-use legacy on litter decomposition, we constructed generalised linear mixed models (GLMM, gaussian family) with the lme4 package (Dawber 2009; Bates et al. 2015) using mass loss in the control treatments in separate models for above- and belowground decomposition. For aboveground decomposition, litter mass loss was square-root-transformed to meet modelling assumptions. The models included forest age, land-use legacy (pasture or cacao) and time - or regeneration stages - since the start of treatments (45, 90 or 135 days) as fixed effects. The interaction term between forest age and land-use legacy was also included to capture differences in forest successional trajectories. We specified the study plots as a random intercept. To estimate fixed and random effects, we utilized the lmerTest package (Kuznetsova, Brockhoff, and Christensen 2017) and we assessed the significance of fixed effects with the *Anova* function in the car package (Fox and Weisberg 2018) based using Type II sums of squares and an F-test. If time since the start of treatments had no significant effect ($p > 0.05$) on a given response variable, we assumed that there was no seasonal variation across three regeneration stages. For the AG litterbags, exploratory data analysis revealed a possible quadratic relationship between mass loss and forest age, we therefore constructed models with and without the quadratic term and conducted a likelihood ratio test (*anova* function in stats package) to test the model fit. We calculated the Akaike Information Criteria (AIC) and selected the best model based on the lowest AIC. Finally, we used the DHARMa package (Hartig 2016) for model diagnostics, and to test for overdispersion or zero inflation.

To evaluate how microhabitats vary with forest succession, we used generalised linear models to model environmental factors as a function of forest age, excluding old-growth forests. We assessed correlations among these variables and excluded LAI due to its high correlation with tree aboveground biomass, retaining only the latter (Table S2). To determine whether these environmental factors explain differences in decomposition with forest age or land-use legacy, we used generalised linear models to model mass loss from the control treatments as a function of five environmental variables per sample position measured across all 32 study plots (Table S3), including old-growth forests. Predictors were log- or square-root transformed if necessary to meet modelling assumptions. The models were constructed and tested as described above.

Finally, to evaluate the impact of perturbation (P and PF treatments) and large animal exclusion (CF and PF treatments) on mass loss, we performed Kruskal-Wallis tests to compare the experimental treatments to the control for every regeneration stage and sample position. To measure the effect sizes, we calculated epsilon squared (ε^2) as:

$$\varepsilon^2 = \frac{H-k+1}{n-k} \tag{Eq. 1}$$

Where *n* the total sample size (64), *k* is the number of groups being tested (2, control and one treatment) and *H* is the Kruskal-Wallis statistic (Vogt and Johnson 2016).

The high variation and non-normality of the data from both AG and BG samples did not allow model fitting against the continuous variable of forest age.

For easier visualisation, we calculated treatment effects (TE) as response ratios:

$$(TE_i)_{days} = \frac{(Mass\ Loss\ \%\ CF, P\ or\ PF)_i}{(Mass\ Loss\ \%\ C)_i} \tag{Eq. 2}$$

Where CF, P and PF are the experimental treatments and C is the control subplot in each study plot *i*, and regeneration stage (45, 90 or 135 days).

We report significant results at $p < 0.05$ and non-significant trends at $p < 0.1$.

Results

1. *Decomposition dynamics across the chronosequence*

Mass loss (%) was the highest in the old-growth forest, followed by cacao plantations, and was lowest in pastures (Table S4). The differences in mass loss among land-use types was greatest for AG litterbags.

Aboveground mass loss generally increased with forest age (ANOVA, $F_{2,17} = 5.5$, $p = 0.014$). At sites with cacao legacy the pattern of mass loss followed a quadratic trend (Cacao × Age^2, $p = 0.011$) with lower decomposition rates in the middle of the chronosequence (Figure 1), but this trend was not apparent in the sites with pasture legacy (Pasture × Age^2, $p = 0.212$), and there was no overall difference in decomposition between land-use legacies.

There was a trend of declining belowground mass loss with forest succesional stage (β = -0.04, SE = 0.07, p = 0.600), but the effect was not significant (Figure 1) and land-use legacy had no detectable influence.

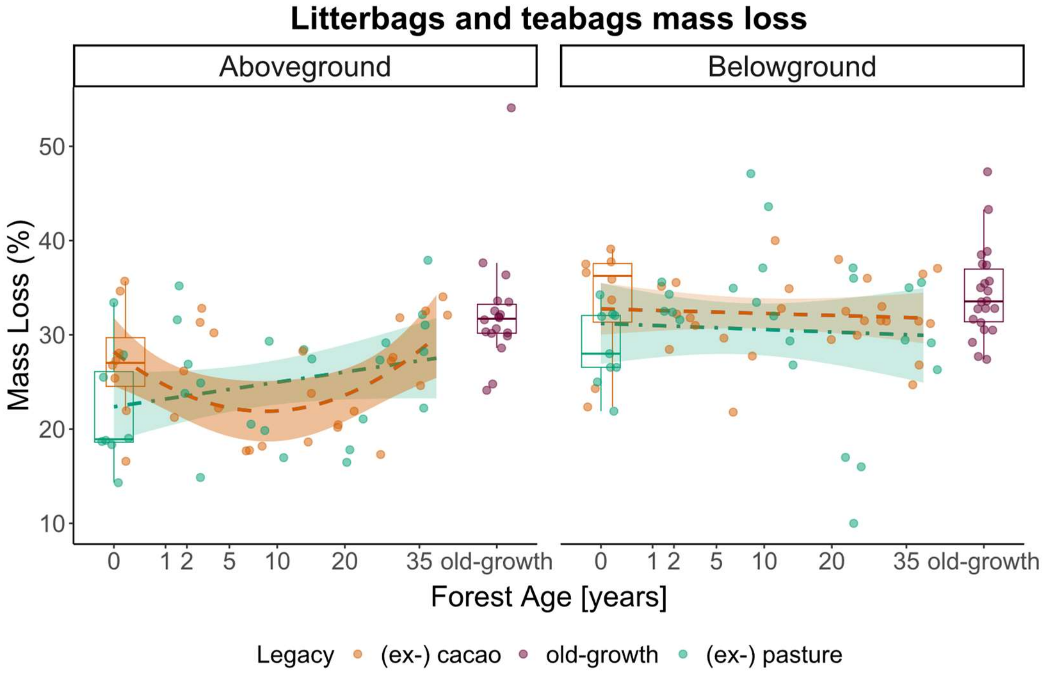

Figure 1 Leaf litter decomposition dynamics measured above- and belowground at three time points along a chronosequence of tropical forest regeneration from two different land-uses, where dots represent mean mass loss per subplot and timepoint; lines with shading represent modelled relationships with confidence intervals and their opacity stands for statistical significance; green is pasture, orange is cacao plantation and purple is old-growth forest; old-growth forests were not included in the models and the data distribution is instead represented by boxplots, showing medians, interquartile range and whiskers.

2. *The influence of environmental factors*

Environmental variables differed markedly among sites along the regeneration gradient (Figure 2). Tree aboveground biomass and leaf area index (LAI) increased with forest age, a trend that persisted in old-growth forests. Elevation and soil C:N also increased with successional stage but exhibited lower values in old-growth forests. Temperature and pH decreased with forest age, stabilizing at lower levels in old-growth stands. Leaf litter standing crop followed a bell-shaped trajectory, increasing until mid-chronosequence before declining, with old-growth forests showing lower biomass than the peak. Legacy effects were most pronounced early in the chronosequence, as pastures lacked leaf litter due to minimal tree cover.

A detailed description of the results of the individual measured variables and their respective trends with forest age is reported in the Supplementary Information (Table S5 -Table S10).

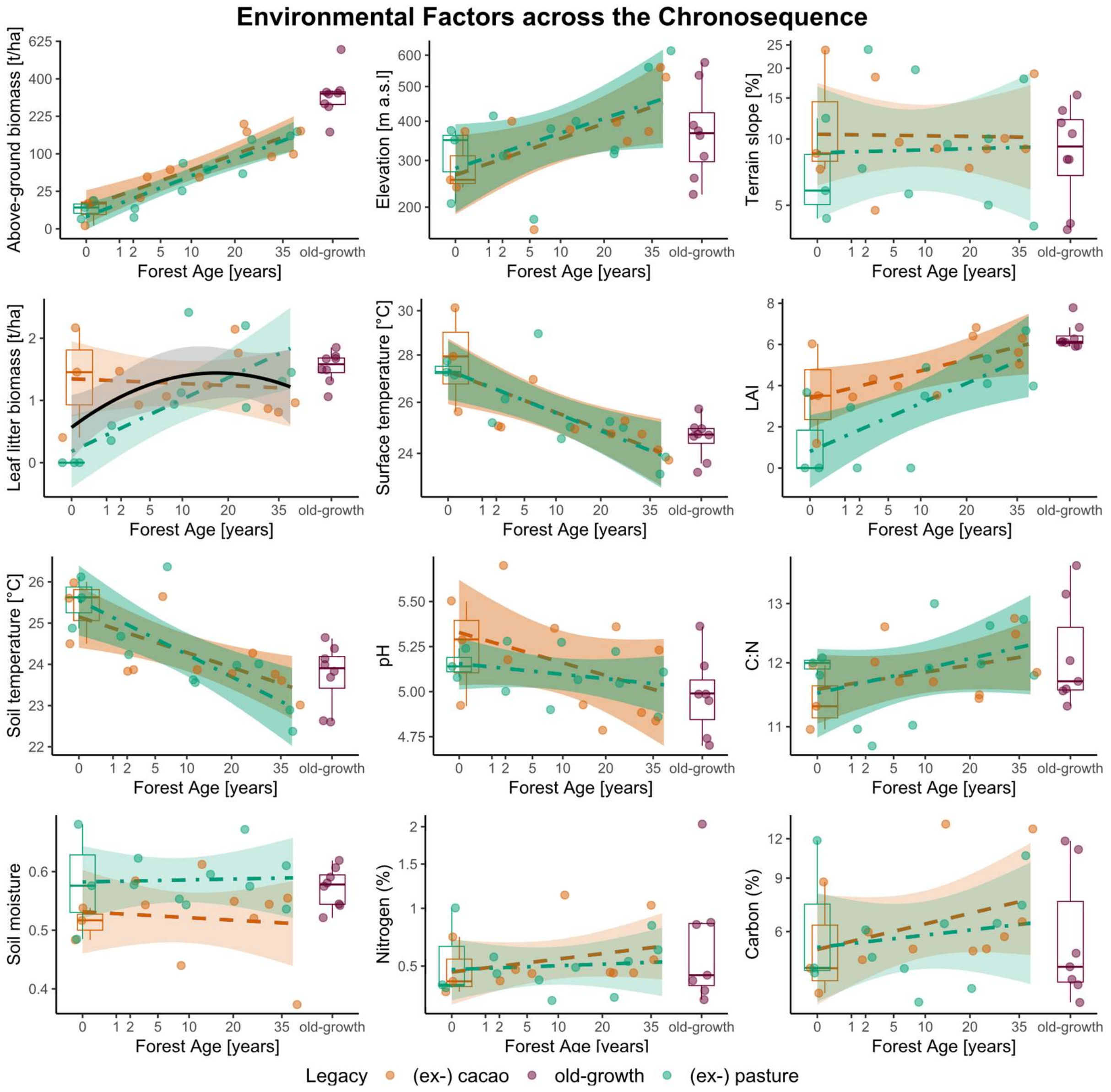

Figure 2 Environmental and soil variables measured along the studied chronosequence; lines with shading represent modelled relationships with confidence intervals and their opacity stands for statistical significance; leaf litter biomass showed a bell-shaped relationship with forest age; in all models we differentiated the trends of each variable with forest age by land-use legacy; green is pasture, orange is cacao plantation and purple is old-growth forest; old-growth forests were not included in the models and the data distribution is instead represented by boxplots, showing medians, interquartile range and whiskers;

Aboveground mass loss was related to differences in tree aboveground biomass, surface temperature and elevation across plots, whereas belowground mass loss was related to soil C:N and soil moisture (Table S11).

Aboveground mass loss increased linearly with tree aboveground biomass ($\beta = 0.04$, $p = 0.005$) and elevation ($\beta = 0.07$, $p = 0.03$) but exhibited a bell-shaped relationship with leaf litter biomass ($\beta = -2.00$, $p = 0.023$, ANOVA $p = 0.061$), with peak values at a leaf litter biomass of approx. 1 t/ha. Aboveground mass loss declined with increasing surface temperature ($\beta = -3.14$, $p = 0.022$) but the relationship differed among land-use legacies ($p = 0.012$), whereby mass loss increased with soil surface temperature in old-growth forests but declined in plots with cacao and pasture legacy (Figure 3). Both above- and belowground mass loss increased with slope, though the trend was not significant. Belowground mass loss declined with increasing soil C:N ($\beta = -35.67$, $p = 0.033$) but showed a bell-shaped relationship with soil moisture ($\beta = -26.73$, $p < 0.001$, ANOVA $p = 0.001$), with peak mass loss at a soil moisture of c. 0.57 (unitless, scale 0 - 1). Belowground mass loss also tended to increase with soil temperature, but the relationship was not significant across or within land-use legacies.

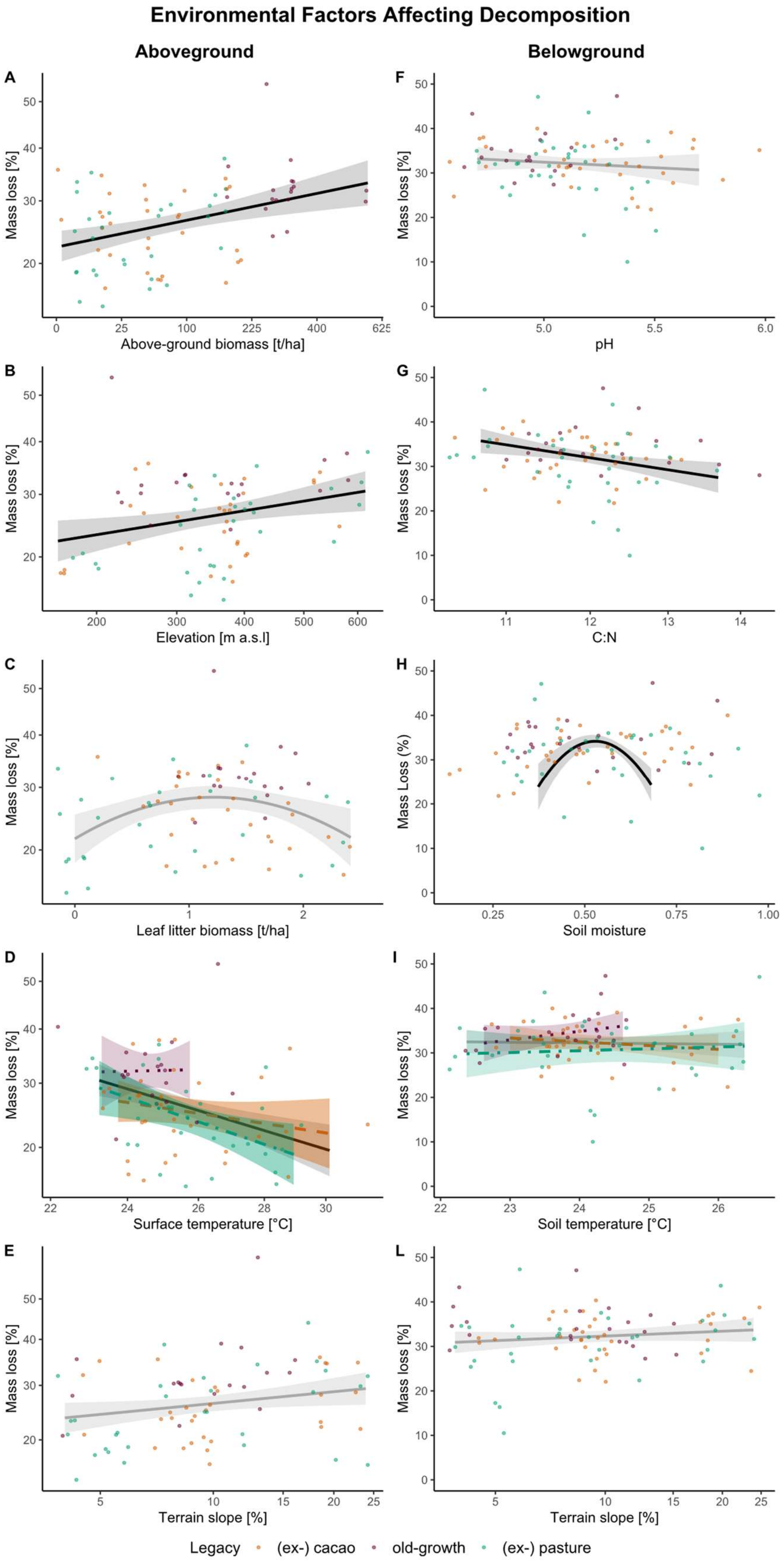

Figure 3 Leaf litter decomposition as a function of microhabitat variables for above- (A-E) and belowground (F-L) litterbags, respectively; the individual trends for each legacy are depicted for surface temperature and soil temperature; models were fitted on transformed data; the opacity of the regression lines and respective confidence intervals stands for statistical significance.

3. *Effects of small-scale perturbation and animal exclusion*

In the control treatment, neither above- nor belowground mass loss differed over time. The effects of the three PREX treatments on above- and belowground mass loss was overall comparable (Figure 4, Table S12). Fencing had no influence on mass loss at any time point from the disturbance event. Perturbation inhibited mass loss both above- ($p < 0.001$, $\varepsilon^2 = 0.20$) and belowground ($p = 0.022$, $\varepsilon^2 = 0.07$) after 45 days and only aboveground after 90 days from disturbance ($p < 0.001$, $\varepsilon^2 = 0.22$). The perturbed-fenced treatment had a similar effect on belowground mass loss, resulting marginally lower than the control only after 45 days ($p = 0.095$, $\varepsilon^2 = 0.03$). However, aboveground mass loss was strongly decreased at all three regeneration stages in the perturbed-fenced treatment (45 days: $p = 0.004$, $\varepsilon^2 = 0.12$; 90 days: $p < 0.001$, $\varepsilon^2 = 0.23$; 135 days: $p = 0.002$, $\varepsilon^2 = 0.14$).

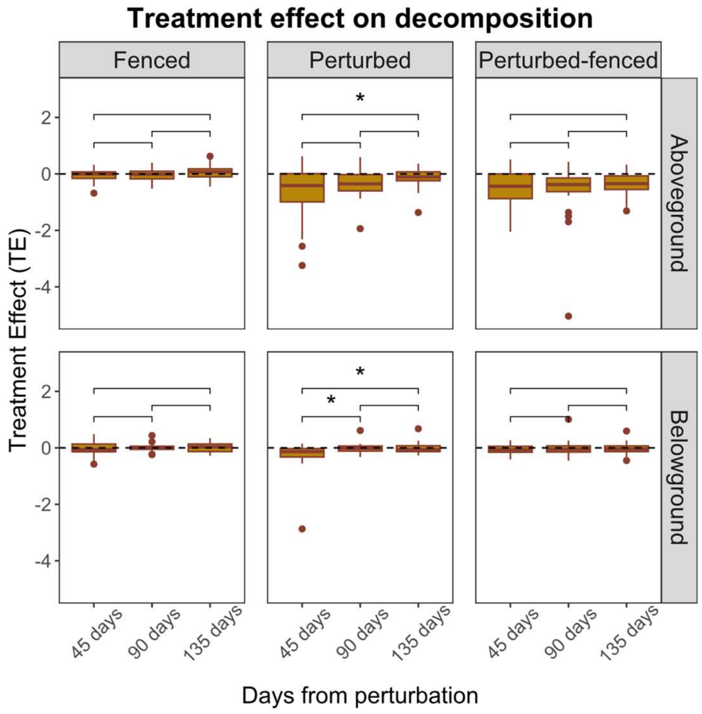

Figure 4 Treatment effect (TE) on above- and belowground decomposition in the experimental treatments (CF, P and PF) at the three time points from the PREX event; the dashed black line at TE = 0 indicates no effect of CF, P nor PF hence when mass loss in the treatment was the same as the control, TE < 0 when mass loss in the treatment was less than the control, TE > 0 when mass loss in the treatment was higher than the control; the significancy levels refer to the p-values of the Wilcoxon rank-sum test to compare the TE in the three time points (days from perturbation) within each treatment and sample position.

Discussion

Our study revealed that land-use legacy influences the recovery of decomposition processes during forest regeneration, while animal exclusion impacts the recovery of these processes in response to small-scale "pulse" disturbances. In accordance with our first hypothesis, the changes in mass loss during secondary forest succession followed different trajectories at sites with pasture legacy compared to former cacao plantations. We also found evidence to support our second hypothesis that differences in decomposition along the forest chronosequence would be related to microclimate. Finally, as hypothesised, perturbations representing initial land clearance inhibited litter decomposition. However, although large animal exclusion had no immediate effect on litter mass loss, the effects of small-scale perturbations persisted for longer in fenced treatments, indicating that animal activity could accelerate the recovery of decomposition processes after land clearance. Here, we discuss the relevance of our results for the regeneration of forest ecosystem functions after land abandonment.

1. Land-use legacy influences decomposition dynamics during forest regeneration

Our results demonstrate that land-use legacy influences aboveground litter decomposition during forest regrowth. The U-shaped relationship between decomposition and regeneration time from former cacao plantation use, with lower mass loss in the middle of the chronosequence, suggests that the decomposition process may slow down during certain stages of forest recovery, possibly due to shifts in both the decomposer community and microclimatic conditions (Guariguata and Ostertag 2001; Paudel et al. 2015). Although we did not assess decomposer communities in this study, work on temperate forest succession showed that the richness and density (individuals/m^2) of each functional groups of arthropods declined from early to mid-successional stages, and then increased again towards mature forest habitats (Deng et al. 2022), which mirrors the pattern we observed for litter mass loss. Lower mass loss in mid-successional stages may also be linked to transient changes in vegetation structure (Gessner et al. 2010), soil chemistry (van der Sande et al. 2022), or nutrient availability as the forest transitions from earlier successional stages to a more complex system (Sánchez-Silva et al. 2018; Thom and Keeton 2020).

By contrast, the linear increase in mass loss with forest age at sites with pasture legacy, reflects the pronounced changes in microclimate and habitat conditions during the transition from open pasture to closed canopy forest. The harsh microclimatic conditions in the pastures, characterized by high temperatures, lack of canopy cover, and intense solar radiation, create an environment that is unfavourable for decomposer communities (Lorenzo et al. 2014). Accordingly, litter decay rates are often lower in pastures compared to forest sites (Stone *et al.*, 2020; Röder *et al.*, 2024) and increase as ecosystems regenerate. In our study, the soil surface temperature in open pastures was up to 11ºC higher than in forest sites (Table S5), and no shade nor protection from any tree canopy (Table S8), supporting our second hypothesis about the importance of changes in microclimate for the recovery of decomposition processes during forest regrowth. Overall, we demonstrate that land-use legacy can have long-lasting impacts on ecological processes, though their effects may become less pronounced as forests mature (Foster et al. 2003b).

In contrast to surface processes, belowground decomposition tended to decline with successional age, and was not influenced by land-use legacy, indicating that belowground litter decay follows a different trajectory of recovery compared to aboveground decomposition. Soil microbial communities could be less responsive to forest regeneration, likely more constrained by factors such as soil pH (bacteria (Rousk et al. 2010) and fungi (Shi et al. 2019)) or nutrient content (Cornwell et al. 2008) and less bound to aboveground structural properties. However, also belowground samples consistently exhibited the lowest decomposition rates in the active pastures.

Greater mass loss in active cacao plantations compared to pasture sites reflects differences in soil and microclimatic conditions under these two land-use types. It has been shown that nitrogen fertilisation in plantations (Gill, Schilling, and Hobbie 2021) and higher pH (Luizão, Luizão, and Proctor 2007; Corre, Beese, and Brumme 2003) can enhance microbial activity, thereby increasing decomposition. Although nitrogen (%) levels in active cacao plantations were similar to those in pastures at our sites, pH was noticeably higher in the plantations (Figure 2, Table S9). Additionally, cacao fields have a litter layer on the soil surface, which reduces evaporation and maintains a more favourable microclimate for decomposition (Sayer 2006) and acts as a buffer against harsh environmental conditions (R. Liu et al. 2021). In our study, active cacao plantations had fairly high leaf litter standing crop (up to 2 t/ha, Table S7) thereby confirming the protective role of the litter layer. Finally, pastures are commonly associated with soil compaction, nutrient depletion, and diminished microbial activity (Souza et al. 2013), all of which significantly reduce decomposition potential.

2. *Microclimate and soil properties drive decomposition dynamics across the chronosequence*

Among the abiotic factors examined, tree aboveground biomass showed a strong positive relationship with AG decomposition ($p = 0.005$), reinforcing the “vegetation quantity effect” observed in previous studies (Lohbeck et al. 2015). The accumulation of tree aboveground biomass promotes favourable microhabitats for decomposers by increasing organic matter inputs, enhancing moisture retention, and moderating temperature fluctuations, all of which enhance microbial activity. In our study, temperature in old-growth forest remained stable, ranging between 23-26°C (Table S5), and higher LAI indicated a dense canopy providing shade and protection (Table S8), further supporting the vegetation quantity effect. Consequently, as tree biomass accumulates in regenerating forests, decomposition rates tend to increase, reflecting the recovery of ecological processes tied to forest structure. This pattern aligns with broader findings that forest age is strongly correlated with tree aboveground biomass in secondary forests (Chazdon et al. 2006; Poorter et al. 2016; Ojoatre et al. 2024; Escobar et al. 2024), further supporting the hypothesis that forest recovery enhances decomposition processes.

Surface temperature emerged as one of the most influential drivers of AG decomposition. In old-growth forests, decomposition rates were positively correlated with temperature, consistent with findings that temperature boosts microbial and enzymatic processes that facilitate decomposition (Salinas *et al.*, 2011), even when moisture levels remain constant across sites (Esquivel et al. 2020). However, in regenerating forests with cacao and pasture legacies, decomposition rates declined with increasing temperature, suggesting that past land-use induced structural changes, such as reduced canopy cover in early regeneration plots (Table S8). Notably, surface temperatures in active plots and early pasture regeneration sites (forest age = 2 years) frequently exceeded 30°C (Table S5). These results align with studies highlighting the role of canopy closure in regulating microclimates that are conducive to decomposition (Wallace *et al.*, 2018). Elevated surface temperatures are often linked to decreased soil moisture (Foster et al. 2003b), a pattern observed in our active cacao plots, where soil moisture was consistently lower than the other sites (Table S6).

Elevation is also often closely associated with temperature and forest composition (Sinha et al. 2018). Our elevation range was quite narrow, spanning between 159-615 m a.s.l. The site-specific microclimate within such short gradient likely affected the positive relationship we observed between elevation and decomposition. In particular, mid- to high-elevation forest plots provided more favourable conditions for decomposition, such as increased humidity and moderate temperatures that reduce desiccation. Similarly, a study in the forests of Mount Kilimanjaro observed a peak in decomposition rates at mid-elevations, hence confirming a significant positive effect of both temperature and humidity on decomposition (Röder et al. 2024).

With respect to leaf litter biomass, our results suggest an optimal standing crop for maximizing decomposition, as indicated by a bell-shaped relationship with samples’ mass loss (Figure 3). Low litter biomass may limit decomposition due to insufficient substrate availability, while excessive litter could indicate constrained decomposer activity, possibly due to unfavourable microclimatic conditions or resource imbalances. This aligns with findings from the Gigante Litter Manipulation Project, which demonstrated that experimental litter additions did not necessarily enhance decomposition rates (Sayer, Tanner, and Lacey 2006).

Soil moisture and C:N ratio emerged as key drivers of BG decomposition, consistent with studies highlighting water availability and nutrient constraints, particularly nitrogen and phosphorus, as fundamental controls on decomposition (Pausas and Bond 2020; Wieder, Cleveland, and Townsend 2009). In our plots, soil C:N ranged between 10.7-13.7, with overall lower values observed in the agricultural plots (Table S9). Decomposition rates declined with increasing soil C:N, likely mirroring the challenge microbial decomposers face in accessing nitrogen as a nutrient source to sustain carbon metabolism in the litter (Blanco et al. 2023). Generally, soils with higher C:N ratios and more complex carbon compounds support slower decomposition rates than nutrient-rich substrates (Schaap et al. 2024). Decomposition exhibited a bell-shaped response to soil moisture, with mass loss peaking at an optimal level before declining as excess water created anoxic conditions that inhibited microbial activity. In tropical wet forests, this threshold is easily reached, as observed in our study, where active pastures—lacking canopy cover and root biomass—became overly saturated (Table S6) and exhibited the lowest decomposition rates. A boreal forest study similarly demonstrated that high moisture levels primarily limit decomposition by depleting oxygen availability (Sierra, Malghani, and Loescher 2017). Conversely, active cacao plots exhibited very low soil moisture levels, likely due to increased evaporation.

Soil temperatures were consistently lower than surface temperatures (Table S5), highlighting the buffering effect of soil and residual humidity in mitigating extreme aboveground conditions, particularly in early successional plots. Although not statistically significant, temperature was positively correlated with decomposition, supporting the well-established theory that higher temperatures accelerate microbial activity and organic matter breakdown (Aerts 1997).

3. *Forest clearance inhibits decomposition and exclusion of large animal slows down recovery*

Our results indicate that the presence of the fence alone had no substantial effect on decomposition. At first glance, this appears to contradict our initial hypothesis that mechanical disturbance by large animals would accelerate decomposition by promoting litter fragmentation and soil mixing.

However, the perturbation treatments (P and PF) strongly reduced aboveground decomposition following disturbance, supporting our hypothesis and aligning with previous research showing that disturbances—such as logging or clearing—can slow decomposition by disrupting decomposer communities and altering soil conditions (Laigle et al. 2021; Latterini et al. 2023). In the P treatment, decomposition rates initially decreased (45 days) but gradually recovered to control levels after 135 days, suggesting a process of recovery over time. In contrast, no such recovery was observed in the perturbed-fenced (PF) treatment, indicating that excluding large animals inhibited decomposition recovery within the experiment's timeframe. This finding reinforces the crucial role of large animals in forest nutrient cycling by promoting litter breakdown, soil mixing, and bioturbation. Thus, large animals appear to play a key role in facilitating post-disturbance decomposition recovery, offering novel insights into their ecological function in tropical forests.

Belowground decomposition also showed an initial decline following disturbance (after 45 days), but the effect size was relatively small (P: $\varepsilon^2 = 0.07$, PF: $\varepsilon^2 = 0.03$) and diminished over time. This suggests that belowground decomposition is less sensitive to pulse disturbances and exhibits greater resilience overall.

Conclusion

Our study reveals the intricate and dynamic nature of leaf litter decomposition in regenerating tropical forests, emphasizing the profound and lasting influence of land-use legacy. We demonstrate that historical land-use not only shapes initial recovery trajectories but also continues to mediate ecosystem processes long after abandonment. In forests regenerating from cacao plantations, aboveground decomposition followed a non-linear pattern, suggesting complex interactions between decomposer communities, microclimate, and vegetation structure. In contrast, forests recovering from pastures experienced prolonged suppression of decomposition, highlighting the severe and persistent ecological constraints imposed by this legacy. Key environmental drivers emerged with distinct yet interconnected roles to forest age and land-use history.

We also provide novel insights into the role of large animals and disturbance in decomposition recovery. While simply excluding large animals did not alter decomposition, perturbation treatments significantly slowed the process. However, recovery occurred when animals remained present, whereas decomposition remained suppressed in the fenced treatment.

Together, these results deepen our understanding of decomposition as a multifaceted and context-dependent process, governed by the interplay of past and present ecological conditions. By demonstrating how vegetation structure, land-use legacy, and decomposer community resilience collectively shape decomposition, our study provides key insights into tropical forest recovery. These findings have direct implications for conservation and restoration strategies, emphasizing the need to account for historical land-use impacts, prioritize vegetation recovery, and recognize the ecological functions of fauna in promoting nutrient cycling. As tropical forests continue to regenerate under varying legacies and anthropogenic pressures, understanding these ecological processes becomes increasingly critical for guiding effective forest management and fostering ecosystem resilience.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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- Supporting information -

Supplementary Methods

Study design

Table S1. Study sites described by coordinates, forest age, successional stage and legacy.

Successional stage	*Legacy*	*Plot*	*Latitude*	*Longitude*	*Forest Age*
Active agriculture	Cacao	CA62	0.511049	-79.2121680	0
		CA63	0.544072	-79.14537496	0
		CA64	0.543444	-79.15454602	0
	Pasture	PA54	0.536930	-79.18835000	0
		PA55	0.497629	-79.22451722	0
		PA57	0.511013	-79.20914000	0
Forest regeneration	Cacao	CR02	0.501487	-79.22595296	2
		CR04	0.511733	-79.18950979	36
		CR05	0.511791	-79.20359239	12
		CR06	0.520722	-79.21059304	20
		CR09	0.472487	-79.22959582	6
		CR10	0.477183	-79.19957402	24
		CR11	0.477902	-79.20506568	31
		CR13	0.488969	-79.22612546	1
		CR14	0.49816	-79.13848336	38
	Pasture	PR19	0.552739	-79.17480114	6
		PR21	0.508066	-79.18989812	36
		PR22	0.509382	-79.20450795	12
		PR23	0.510839	-79.23202079	1
		PR26	0.526454	-79.20466318	22
		PR31	0.479072	-79.21007127	24
		PR33	0.489268	-79.22841179	1
		PR34	0.493511	-79.13792672	38
		PR36	0.51652	-79.1430608	9
Old-growth forest	Old-growth	OG38	0.513162	-79.19323403	NA
		OG39	0.521561	-79.19537184	NA
		OG41	0.529896	-79.18850798	NA
		OG42	0.538494	-79.17462629	NA
		OG45	0.477783	-79.20370404	NA
		OG46	0.478053	-79.19470296	NA
		OG49	0.531384	-79.14797897	NA
		OG52	0.545743	-79.13757074	NA

Decomposition experiment

Table S2. Leaf traits of plant species used in the decomposition experiment; the values reported consist of the mean of measurements taken from three different specimens per species.

Species	*C:N*	*Thickness b/r(mm)*	*Thickness a(mm)*	*Toughness 1(N)*	*Toughness 2(N)*	*Toughness 3(N)*	*Fresh weight (mg) / petiole*	*Fresh weight (mg)*	*Leaf + R:X DW (mg) / petiole*	*DW blade (mg)*
Brosimum utile	28.37	0.12	0.12	3.16	2.84	2.73	2920.67	116.67	19	958.33
Compsoneura atopa	37.35	0.25	0.24	4.63	4.66	4.86	7637.67	517.67	88	1898
Pourouma minor	24.61	0.2	0.2	3.54	3.5	3.23	29193.33	8718.33	2520.33	11412.67
Trema micrantha	15.57	0.13	0.12	1.15	0.97	1.02	993.67	60.67	11.33	332.67
Vochysia macrophylla	36.95	0.16	0.16	4.02	4.17	4.16	2071	86	19.33	440

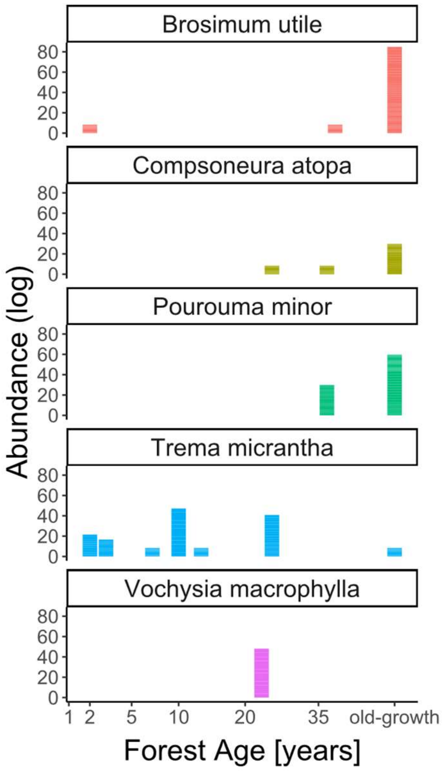

Figure S1. Occurrence of plants used for the natural leaf litter mix along the chronosequence.

Environmental data

Soil loggers TMS-4

Temperature and soil moisture data was recorded with soil loggers TMS-4 (https://tomst.com/web/en/systems/tms/tms-4/) following the method described by Wild.
During November-January 2023, we installed a soil logger TMS-4 in the soil of the three fenced treatments of the PREX experiment (CF, PF, PF$_o$). We left the soil logger in the field for at least 13 days, during which it recorded temperature values at three height levels (-6 cm, 0 cm (surface), and +12 cm). Due to the low number of loggers, loggers were installed in a subset of samples and moved to a new set of plots after at least 13 days had passed. Before we installed the logger in the PREX-PF, vegetation, and litter were removed in an area of 1m^2 and placed in the centre.

Leaf Area Index (LAI)

The leaf area index (LAI) was measured using an LAI 2200c device. For each plot, we collected four measurements per treatment. These measurements were then processed using the FV2200 software, which incorporates an open-sky reference for calibration. As LAI represents the ratio of leaf area to ground area, it is a unitless index (m^2/m^2).

Statistical analysis

Table S2. Correlation matrix among environmental variables.

Soil temperature	*Surface temperature*	*Elevation*	*Terrain slope*	*LAI*	*Leaf litter biomass*	*Tree aboveground biomass*	*Soil moisture*	*pH*	*C:N*
1	0.971	-0.589	-0.205	-0.59	-0.434	-0.442	-0.061	0.233	-0.291
0.889	1	-0.493	-0.173	-0.615	-0.389	-0.475	-0.012	0.236	-0.261
-0.567	-0.539	1	0.115	0.27	0.203	0.233	0.186	-0.302	0.507
-0.173	-0.153	0.006	1	-0.003	-0.012	-0.083	0.12	0.327	-0.19
-0.381	-0.4	0.132	0.02	1	0.433	0.586	-0.194	-0.271	0.238
-0.273	-0.238	0.117	0.03	0.276	1	0.377	0.031	0.011	0.314
-0.429	-0.481	0.218	0.041	0.493	0.35	1	0.048	-0.358	0.109
-0.132	-0.115	0.196	0.091	-0.131	0.021	0.028	1	-0.058	0.083
0.245	0.257	-0.225	0.186	-0.202	0.015	-0.216	-0.083	1	-0.233
-0.231	-0.188	0.24	-0.006	0.076	0.138	0.118	0.061	-0.117	1

LAI showed a moderate correlation with tree aboveground biomass and temperature therefore we decided not to include it in the main text. Elevation was also partially correlated with temperature (surface: $r^2 = 0.564$, soil: $r^2 = 0.591$) but we kept it as a main predictor.

Table S3. Predictors of each individual model for both sample positions; we reported the transformation we applied to each respective variable.

Sample position	*Variable*	*Transformation*
Aboveground	Above-ground biomass	sqrt
	Elevation	sqrt
	Leaf litter biomass	NA
	Temperature (surface)	log
	Terrain slope	log
Belowground	pH	NA
	Soil C:N	log
	Soil moisture	NA
	Temperature (soil)	NA
	Terrain slope	log

We also included LAI, Nitrogen (%) and Carbon (%) in the analysis, though not in the final results. Nitrogen (%) was square-root transformed and Carbon (%) was log- transformed.

Supplementary Results

1. *Decomposition dynamics across the chronosequence*

Table S4. Mass loss (%) data from both above- (AG) and belowground (BG) litterbags measured in the control treatments.

Plot ID	*Legacy*	*Mass loss (%)*	*Position*	*Days from PREX (Regeneration Stage)*
CA62	Cacao	37.5	BG	45
CA62	Cacao	33.7	BG	90
CA62	Cacao	22.35	BG	135
CR04	Cacao	24.7	BG	45
CR04	Cacao	31.45	BG	90
CR04	Cacao	26.8	BG	135
CR10	Cacao	31.5	BG	45
CR10	Cacao	29.95	BG	90
CR10	Cacao	36	BG	135
OG38	Old-growth	30.5	BG	45
OG38	Old-growth	35.7	BG	90
OG38	Old-growth	30.55	BG	135
OG45	Old-growth	38.5	BG	45
OG45	Old-growth	29.2	BG	90
OG45	Old-growth	34.65	BG	135
PA54	Pasture	26.55	BG	45
PA54	Pasture	28	BG	90
PA54	Pasture	31.95	BG	135
PR21	Pasture	NA	BG	45
PR21	Pasture	29.45	BG	90
PR21	Pasture	35	BG	135
PR31	Pasture	36	BG	45
PR31	Pasture	NA	BG	90
PR31	Pasture	37.1	BG	135
PR26	Pasture	16.47	AG	45
CR06	Cacao	21.90	AG	45
PR22	Pasture	NA	AG	45
OG52	Old-growth	28.60	AG	45
OG49	Old-growth	32.14	AG	45
PR36	Pasture	16.97	AG	45
PA55	Pasture	18.68	AG	45
OG41	Old-growth	29.87	AG	45
PA54	Pasture	18.35	AG	45
CA62	Cacao	16.58	AG	90
PA57	Pasture	14.31	AG	90
PR21	Pasture	31.04	AG	90
CR14	Cacao	34.03	AG	90
OG42	Old-growth	31.92	AG	90
PR19	Pasture	NA	AG	90
OG45	Old-growth	NA	AG	90
CR11	Cacao	31.81	AG	90
OG46	Old-growth	30.21	AG	90
OG38	Old-growth	36.35	AG	135
OG39	Old-growth	37.63	AG	135
CA63	Cacao	34.64	AG	135

CR02	Cacao	22.23	AG	135
CR13	Cacao	21.23	AG	135
PR33	Pasture	14.86	AG	135

2. *The influence of environmental factors*

Surface temperature did not exceed 26°C in the old-growth forests, whereas it reached values above 30 °C in the active plots and early pasture regeneration (forest age = 2). Soil temperatures at 6 cm depth were overall lower than surface temperatures and only exceeded 30° C in one active cacao and one active pasture plots (Table S5).

Volumetric soil moisture values are reported in a scale of 0 to 1 (Table S6). Soil moisture ranged from 0.49 to 0.69, which falls within the range reported for comparable tropical moist forests (Veldkamp *et al.*, 2003).

Leaf litter biomass ranged between 0 in the active pastures to more than 2 t/ha in early regeneration sites. However, leaf litter biomass production was highest in the old-growth forest and in the younger forest stages (Table S7), regardless of the agricultural legacy.

Tables S8 and S9 report measurements of Leaf Area Index, soil pH, and C:N, respectively.

Above biomass, elevation and terrain slope are reported in the site description (Escobar *et al.*, 2024).

Table S5. Surface and soil temperatures (°C) measured by the TMS-4 loggers.

Plot ID	*Microhabitat*	*Min*	*Q5*	*Median*	*Mean*	*Q95*	*Max*
CA62	*Surface*	19.75	21.875	23.5	24.84404	31.92813	42.5625
	Soil	22.5625	23.75	25	25.22695	27.375	29.25
CA63	*Surface*	22.3125	22.875	24	24.125	25.6875	26.875
	Soil	23.375	23.5625	24.0625	24.04762	24.5	24.75
CA64	*Surface*	22.125	22.875	24.5	26.17827	34.05625	40.375
	Soil	24.375	24.75	26.5	26.9656	30.25	32.75
CR02	*Surface*	21.375	21.875	23.4375	24.02588	27.625	31.375
	Soil	23	23.4875	24.375	24.58083	26.1875	26.625
CR04	*Surface*	20.625	21.5	22.5	22.63484	24.125	25.5
	Soil	21.3125	21.75	22.375	22.41659	22.9125	23.25
CR05	*Surface*	20.375	21.75	23	23.26339	25.375	26.6875
	Soil	22.25	22.75	23.375	23.44644	24.125	24.375
CR06	*Surface*	21.1875	22.10313	23.125	23.27716	24.625	25.375
	Soil	22.125	22.5	23.25	23.20657	23.77188	24.125
CR09	*Surface*	22.5	23	24.625	25.06575	28.09063	30.8125
	Soil	24	24.375	25.25	25.40656	26.625	27.25
CR10	*Surface*	21.5625	22.05938	23.4375	23.6082	25.37813	28.125
	Soil	22.5	22.87188	23.5	23.58709	24.375	24.625
CR11	*Surface*	21.5	21.9375	23.375	23.57345	25.57813	30.5
	Soil	22.625	22.9375	23.625	23.70862	24.57813	25
CR13	*Surface*	21.5	22.08438	23.25	23.4171	25	25.875
	Soil	22.375	22.625	23.375	23.40004	24.125	24.25
CR14	*Surface*	20.8125	21.25	22.25	22.39863	23.91875	24.875
	Soil	21.5	21.75	22.25	22.30007	22.875	23.125

OG38	*Surface*	20.75	21.5	22.5	22.70959	24.25	24.9375
	Soil	21.4375	21.9375	22.625	22.66875	23.25	23.5
OG39	*Surface*	21.25	21.62188	22.375	22.50859	23.81563	24.375
	Soil	21.625	21.8125	22.25	22.28571	22.75	23
OG41	*Surface*	21.625	21.9375	23.125	23.24356	24.59375	25.1875
	Soil	22.3125	22.5625	23.25	23.23422	23.75	24
OG42	*Surface*	21.625	22.25	23.25	23.44583	25	25.625
	Soil	22.5	22.89688	23.5	23.55078	24.25	24.375
OG45	*Surface*	21.375	21.875	23.25	23.39931	25	26
	Soil	22.375	22.75	23.4375	23.45343	24	24.25
OG46	*Surface*	21.875	22.25	23.75	23.7782	25.25	28.8125
	Soil	22.875	23.05625	23.75	23.7782	24.375	24.625
OG49	*Surface*	22.375	22.625	23.625	23.64506	24.75	25.375
	Soil	23.25	23.375	23.8125	23.80974	24.125	24.375
OG52	*Surface*	22.625	23.0625	24.125	24.25335	25.75	26.625
	Soil	23.8125	24	24.5	24.4828	25	25.125
PA54	*Surface*	20.75	22.625	25	26.67799	35.08125	40.1875
	Soil	23.5	24.25	26.8125	27.4048	32.125	33.375
PA55	*Surface*	20.75	21.625	23.5	24.95715	32.5	37.75
	Soil	22.875	23.6875	25.1875	25.66703	28.875	30.875
PA57	*Surface*	19.875	22	23.875	25.23151	32.925	39.5
	Soil	23.125	24.5	25.75	25.93012	27.875	28.75
PR19	*Surface*	21.875	23.3125	24.75	25.79572	30.34688	34.125
	Soil	24.25	24.875	25.875	25.99615	27.375	27.875
PR21	*Surface*	20.75	21.5	22.5	22.64653	24.25	25.5
	Soil	21.25	21.75	22.4375	22.43077	23	23.375
PR22	*Surface*	20.4375	21.875	22.9375	23.22746	25.17188	27.875
	Soil	22.5	23	23.5	23.5614	24.125	24.5
PR23	*Surface*	21.375	21.9375	23.0625	23.21637	25	27.375
	Soil	21.9375	22.1875	23.625	23.40217	24.375	24.875
PR26	*Surface*	20.4375	21.75	23.25	24.66065	32.075	40.125
	Soil	22.5	23.5	24.9375	25.33482	28.3375	29.875
PR31	*Surface*	21.5	21.875	23.5	23.6894	25.75	27.75
	Soil	22.75	23.0625	23.8125	23.88777	24.75	25.125
PR33	*Surface*	21.875	22.5625	24.3125	25.83905	34.4125	41.25
	Soil	23.75	24.375	25.875	26.25406	29.125	30.625
PR34	*Surface*	20.5625	20.875	21.875	21.98316	23.5	24.5
	Soil	21.5	21.625	22.0625	22.08362	22.5625	22.875
PR36	*Surface*	21.875	22.25	23.375	23.60834	25.625	26.75
	Soil	22.9375	23.0625	23.75	23.75898	24.5	24.75

Table S6. Soil moisture measured by the TMS-4 loggers after correcting by temperature, soil texture and density. Scale is 0 to 1.

Plot ID	*Min*	*Q5*	*Median*	*Mean*	*Q95*	*Max*
CA62	0.502	0.505	0.511	0.512	**0.524**	0.539
CA63	0.521	0.522	0.526	0.526	**0.534**	0.540

CA64	0.475	0.476	0.501	0.498	**0.520**	0.531
CR02	0.513	0.517	0.526	0.526	0.534	0.536
CR04	0.514	0.515	0.517	0.519	0.526	0.536
CR05	0.562	0.565	0.569	0.571	0.586	0.598
CR06	0.528	0.531	0.535	0.535	0.540	0.541
CR09	0.441	0.451	0.475	0.473	**0.488**	0.518
CR10	0.523	0.524	0.530	0.530	0.536	0.538
CR11	0.533	0.535	0.538	0.538	0.542	0.543
CR13	0.582	0.588	0.601	0.605	0.634	0.658
CR14	0.588	0.590	0.594	0.594	0.597	0.597
OG38	0.556	0.560	0.567	0.572	0.589	0.594
OG39	0.621	0.632	0.638	0.640	0.649	0.651
OG41	0.493	0.500	0.515	0.524	0.561	0.568
OG42	0.550	0.553	0.617	0.610	0.649	0.651
OG45	0.566	0.566	0.575	0.577	0.593	0.601
OG46	0.549	0.549	0.556	0.556	0.565	0.567
OG49	0.486	0.535	0.565	0.563	0.585	0.640
OG52	0.437	0.440	0.453	0.456	0.491	0.516
PA54	0.516	0.525	0.534	0.534	**0.547**	0.559
PA55	0.642	0.653	0.667	0.668	**0.686**	0.693
PA57	0.545	0.556	0.565	0.564	**0.571**	0.574
PR19	0.504	0.507	0.596	0.578	**0.625**	0.633
PR21	0.542	0.544	0.548	0.552	0.564	0.565
PR22	0.583	0.584	0.589	0.589	0.592	0.594
PR23	0.351	0.387	0.564	0.543	**0.625**	0.652
PR26	0.584	0.602	0.624	0.623	0.643	0.672
PR31	0.562	0.574	0.587	0.586	0.596	0.602
PR33	0.544	0.570	0.585	0.582	**0.593**	0.594
PR34	0.597	0.598	0.606	0.607	0.618	0.620
PR36	0.477	0.512	0.524	0.534	0.587	0.596

Table S7. Measurements of leaf litter standing crop in each plot. Biomass and respective standard deviation are expressed in t/ha.

Plot ID	*Leaf litter biomass (t/ha)*	*Std dev*
CA62	2.16725	1.155059
CA63	1.4545	0.318285
CA64	0.4035	0.260165
CR02	0.926	0.204967
CR04	0.81325	0.272944
CR05	1.24075	1.139832
CR06	2.1425	1.461609
CR09	1.066	0.286804
CR10	1.76225	0.513767
CR11	0.868	0.360212
CR13	1.469	0.506208

CR14	0.962	0.396594
OG38	1.8515	0.251288
OG39	1.6725	0.483242
OG41	1.668	0.645435
OG42	1.061	0.152737
OG45	1.7095	0.185113
OG46	1.49625	0.231783
OG49	1.3155	0.357631
OG52	1.491	0.310327
PA54	0.001	0
PA55	0.001	0
PA57	0.033	0.064
PR19	0.9385	0.706749
PR21	1.451	0.413538
PR22	2.415	1.404879
PR23	0.597	0.427491
PR26	2.201333	1.199183
PR31	0.88575	0.224898
PR33	0.35325	0.292477
PR34	1.31	0.296789
PR36	1.12525	0.384261

Table S8. Measurements of the Leaf Area Index (LAI) in the plots (only control treatments reported).

Plot	*LAI*
CA62	0
CA63	6.03
CA64	3.51
CR02	4.33
CR04	6.3
CR05	3.53
CR06	6.41
CR09	3.97
CR10	6.82
CR11	5.62
CR13	3.45
CR14	5.04
OG38	5.91
OG39	6.14
OG41	6.83
OG42	5.94
OG45	6.11
OG46	6.09
OG49	6.27
OG52	7.78
PA54	0
PA55	0
PA57	3.67

PR19	0
PR21	6.67
PR22	4.88
PR23	2.94
PR26	4.1
PR31	5.3
PR33	0
PR34	3.98
PR36	3.5

Table S9. Measurements of soil pH and C:N ratio.

Plot ID	*pH*	*Nitrogen (%)*	*Carbon (%)*	*C:N*
CA62	5.50	0.73117	8.74422	11.959
CA63	5.29	0.33260	3.64507	10.959
CA64	4.92	0.39637	4.48336	11.311
CR02	5.18	0.47472	5.98120	12.599
CR04	4.84	1.03162	12.86983	12.475
CR05	4.93	1.13972	13.32875	11.695
CR06	4.79	0.45099	5.15558	11.432
CR09	5.35	0.44769	5.23768	11.699
CR10	5.36	0.45642	5.24398	11.489
CR11	4.88	0.45263	5.76786	12.743
CR13	5.70	0.40018	4.80750	12.013
CR14	5.23	0.54675	6.47654	11.846
OG38	4.99	0.84249	11.09373	13.168
OG39	4.70	0.86266	11.80498	13.684
OG41	4.99	0.40113	4.53839	11.314
OG42	5.36	2.03694	3.91361	11.551
OG46	4.95	0.33992	4.09149	12.037
OG49	4.74	0.43685	5.05931	11.581
OG52	5.14	0.28778	3.36770	11.702
PA54	5.24	0.35701	4.31359	12.083
PA55	5.08	0.37495	4.49874	11.998
PA57	5.14	1.00473	11.85641	11.801
PR19	4.90	0.40570	4.47160	11.022
PR21	4.86	0.83392	10.61424	12.728
PR22	5.07	0.49073	6.37939	13.000
PR23	5.0	0.44721	4.90319	10.964
PR26	5.22	0.30048	3.79200	12.620
PR31	5.05	0.53362	6.39526	11.985
PR33	5.28	0.56704	6.07561	10.715
PR34	5.11	0.62304	7.35087	11.798
PR36	5.27	0.28348	3.37725	11.914

Table S10. Model summaries and ANOVA (Type II) test results of the models of each environmental variable with forest age as predictor.

Response variable	*Predictor*	*β*	*SE*	*p*	*ANOVA (Df, F, p-value)*		
Tree aboveground biomass	Forest Age	0.25	0.03	**< 0.0001**	1	67.18	**< 0.0001**
Leaf litter biomass	Forest Age^2	-1.59	0.61	**0.002**	2	4.78	0.019
Temperature (surface)	Forest Age	0.00	0.00	**< 0.0001**	1	23.21	**< 0.0001**
Temperature (Soil)	Forest Age	-0.05	0.01	**< 0.0001**	1	24.25	**< 0.0001**
Terrain slope	Forest Age	0.00	0.01	0.9	1	0.012	0.914
Elevation	Forest Age	0.14	0.03	**0.0004**	1	16.98	**0.0004**
Soil moisture	Forest Age	0.00	0.00	0.6	1	0.26	0.615
pH	Forest Age	-0.01	0.00	0.07	1	3.73	0.066
C:N	Forest Age	0.00	0.00	**0.05**	1	4.44	**0.047**
Carbon (%)	Forest Age	0.01	0.00	0.1	1	2.94	0.100
Nitrogen (%)	Forest Age	0.00	0.00	0.2	1	1.94	0.178
LAI	Forest Age	0.09	0.02	**0.001**	1	14.53	**0.001**

Table S11. Model summaries and ANOVA (Type II) test results of the individual models of mass loss (%) as a function of the respective predictors (here we reported the fixed effects).

Aboveground predictors	*β*	*SE*	*p*	*ANOVA (Df, Df.res, F, p-value)*			
Tree aboveground biomass	0.04	0.01	**0.005**	1	28.4	9.5	**0.005**
Elevation	0.07	0.03	**0.030**	1	30.1	5.2	**0.030**
Leaf litter biomass^2	-2.00	0.83	**0.023**	2	27.1	3.1	0.061
Temperature (surface)	-3.14	1.29	**0.022**	1	26	5.9	**0.023**
Legacy				1	23.7	5.2	**0.013**
* Legacy				1	25.7	0.8	0.474
Terrain slope	0.34	0.20	0.095	1	26.9	3.0	0.096

Belowground predictors	*β*	*SE*	*p*	*ANOVA (Df, Df.res, F, p-value)*			
pH	-2.69	3.82	0.486	1	28.8	0.5	0.486
Soil C:N	-35.67	15.93	**0.032**	1	31.1	5.0	**0.033**
Soil moisture^2	-26.73	6.62	**< 0.001**	2	27.2	8.4	**0.001**
Temperature (soil)	0.35	0.86	0.689	1	28.6	0.2	0.689
Legacy				1	25.8	1.2	0.318
* Legacy				1	26.4	0.5	0.635
Terrain slope	1.49	1.81	0.416	1	30.2	0.7	0.416

3. *Effects of small-scale perturbation and animal exclusion*

Table S12 Kruskal-Wallis test results and epsilon squared to measure effect size of the test significancy.

Treatment	*Position*	*Days from Perturbation*	*p value*	*ε^2*
Fenced	Aboveground	45	0.853	-0.016
		90	0.476	-0.008
		135	0.272	0.003

	Belowground	45	0.943	-0.016
		90	0.644	-0.013
		135	0.501	-0.009
Perturbed	Aboveground	45	**< 0.001**	**0.196**
		90	**< 0.001**	**0.216**
		135	0.311	0.000
	Belowground	45	**0.022**	**0.069**
		90	0.207	0.010
		135	0.354	-0.002
Perturbed-fenced	Aboveground	45	**0.004**	**0.121**
		90	**< 0.001**	**0.225**
		135	**0.002**	**0.141**
	Belowground	45	0.095	0.029
		90	0.168	0.015
		135	0.207	0.010

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