NUTRIENT CONTEXT AND LITTER COMPOSITION CONTROL WETLAND PLANT ROOT DECOMPOSITION

By

Daphka Joseph

July, 2025

Director of Thesis: Dr. Ariane Peralta, PhD

Major Department: Biology

ABSTRACT

Anthropogenic factors such as deforestation and agriculture have both a direct and indirect impact on nutrient availability and carbon (C) storage potential. Deposition of nutrients can modify plant uptake of nutrients, plant composition (quality of litter), soil microbial communities, plant-microbe associations and availability of organic C and nitrogen. Decomposition rates are dependent on a few of these factors such as plant composition, moisture availability, and concentrations of carbon to nitrogen (C:N) ratio in soils and litter. In this study, I addressed how nutrient enrichment influences the plant root decomposition rate and C storage potential. I hypothesized that if plant species vary in growth response to different nutrient context histories, then root decomposition rates will depend on nutrient context (long-term fertilization vs. ambient) and plant functional group (grass vs. shrub). I tested this hypothesis by conducting a long-term wetland ecology experiment at East Carolina University's West Research Campus in Greenville, North Carolina by measuring mass loss of different plant litters (grass *Chasmanthium laxum* and shrub *Rhus copallinum*) sourced from different soil nutrient histories and buried in different soil environmental conditions and associated microbial communities. Additionally, I analyzed C:N ratios for both plant litter and soil collected from mowed fertilized and mowed unfertilized plots. Results revealed that plant root litter C:N ratios were different based on the interaction between plant type (grass vs shrub) and fertilization (fertilized vs unfertilized). Soil C:N ratio showed a difference across fertilization. The variation in fertilization effects on soil C:N ratio and root litter properties influenced plant root litter decomposition to different degrees. Percent mass loss of grass roots was similar across all main effects of fertilization, plant nutrient history, and mesh size, while there was a difference across buried plots and mesh size (µm) for the shrub roots. Shrub roots buried in fertilized plots within 200 µm nylon mesh bags had higher percent mass loss than those buried in unfertilized plots within 20 µm nylon mesh bags due to the access of macrofauna allowed with the larger mesh size. Additionally, shrub roots buried in fertilized plots within 200 µm nylon mesh bags had higher percent mass loss compared to unfertilized plots. The soil C:N ratio combination with differences in root litter C:N ratio influenced rates of decomposition where shrub litter with high C:N ratios had faster decomposition rates when buried in fertilized soils (with lower soil C:N ratio), while grass litter with low C:N ratio had similar effects on both fertilized and unfertilized plots. This study provided insight into how nutrient enrichment influences native plant root decomposition rates and C storage potential by measuring mass loss of different plant litter sourced from different soil nutrient histories and showed higher mass loss rates led to greater soil C stocks. Since long-term fertilization increases the relative abundance of woody species such as *Rhus copallinum* over time, the higher mass loss of these roots could negate carbon gains from higher biomass due to root turnover outweighing the plant biomass inputs. Therefore, understanding the relationship between long-term fertilization and plant functional groups is important for evaluating plant root composition and its influence on microbial activity and the role wetlands can play in climate change mitigation.

NUTRIENT CONTEXT AND LITTER COMPOSITION CONTROL WETLAND PLANT ROOT DECOMPOSITION

A Thesis
Presented to the Faculty of the Department of Biology
East Carolina University

In Partial Fulfillment of the Requirements for the Degree
Master of Science in Biology

By
Daphka Joseph
July, 2025

Director of Thesis: Ariane L. Peralta, PhD
Thesis Committee Members:
Rachel Gittman, PhD
Scott Cory, PhD
Michael Brewer, PhD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Human activities play an important role in nutrient availability and carbon (C) storage potential. Anthropogenic factors such as deforestation, agriculture, and industrialization have indirect effects on atmospheric deposition of nutrients and directly through abiotic factors (Allison et al., 2013; Guignard et al., 2017). Deposition of nutrients onto historically nutrient-limited ecosystems can modify plant uptake rates of nutrients, plant structures (chemically, biologically, and physically), soil bacterial communities, and plant-microbe associations (Guignard et al., 2017). Specifically, resource availability of organic C and nutrients (nitrogen (N), phosphorus (P)) can influence rates of plant root decomposition to different degrees (Fennessy et al., 2008). For example, C can be transferred from the atmosphere by photo- and chemoautotrophic microbes and photosynthesizing plants (Gougoulias et al., 2014). The balance of the C fixed by phototrophs and C respired by heterotrophs dictates the C storage potential of ecosystems, and different abiotic (e.g., nutrient enrichment) and biotic factors (e.g., food web structure) can influence this C balance, which results in uncertainty in predicting climate change effects on C storage potential across ecosystems (Jackson et al., 2017).

Decomposition rates are dependent on the following factors such as temperature, moisture variability, quality of plant litter and C to N ratio (C:N) of organic material. (Bradford et al., 2015; Zhang et al., 2008). A C:N ratio of 24:1 to 30:1 is important for microbes to decompose organic material effectively. Higher C:N ratios indicate there is less N from organic matter available for microbes, meaning decomposition rates will be slower because microbes need more N to break down material (Zhang et al., 2020). Lower C:N ratios indicate more readily available N from organic matter, which allows for faster decomposition rates (Zhang et al., 2020).

Long-term enrichment studies are commonly conducted in terrestrial ecosystems such as grasslands (Harpole et al., 2016; Leff et al., 2015). These studies showed that the addition of nutrients (fertilizer) to soil decreased biodiversity of plant and bacterial communities and increased plant and microbial biomass. However, long-term nutrient enrichment studies at the terrestrial-aquatic interface could result in different patterns and consequences on C storage potential and climate change mitigation (Koceja et al., 2021; Zhou et al., 2023). The drying and ditching of wetland ecosystems is known to increase C losses due to increased decomposition rates and increases the biodegradation of soil organic carbon (SOC) (Zhou et al., 2023). Therefore, the additional stressor of soil drying could magnify nutrient effects on decomposition rates.

Nutrient and water availability influence patterns of microbial communities and rates of decomposition due to increased microbial activity and changes to plant biomass abundance and composition. Plant chemical composition and plant biomass also affect plant root decomposition rates, and available soil nutrient concentrations could modify rates in unaccounted-for ways. Therefore, soil moisture and SOC availability can affect both plant root production and rate of root decomposition (Von Haden & Dornbush, 2014). Although plant litter is a primary contributor to SOC stabilization, heterotrophic microbes that can access SOC, especially when primed after nutrient and/or labile C inputs, can further destabilize C storage. In this instance, ecosystems could shift from being C sinks to C sources (Wu et al., 2024). Plant communities undergoing higher nutrient uptake go through multiple processes such as faster growth rate, weakened cellular structures, less biomass to the roots, high nutrient concentrations, and high C storage potential (Bodker et al., 2015; Ma et al., 2023). Nutrient addition in soil can affect microbes by reducing microbial biomass and composition (Leff et al., 2015).

Decomposition is an important process that provides nutrients for many organisms through flows of energy. Decomposition releases those nutrients along with other elements such as C back into the air and soil through nutrient cycling and modified by microbes (Carlesso et al., 2019). Decomposition by heterotrophic microorganisms releases organic material from plants as a substrate, maintains some biomass, and then releases the rest back to the environment as carbon dioxide (CO_2) (Gougoulias et al., 2014). As land use and climate change influence flows of energy and nutrients, processes such as decomposition could be changing in ways that are not currently considered when estimating C stocks (Ontl & Schulte, 2012). For example, changes to soil moisture and nutrient concentrations could have fundamental impacts on plant and bacterial community patterns, physiology, and subsequent root decomposition rates (Von Haden & Dornbush, 2014). Plant roots have evolved to differentiate between beneficial, commensal or pathogenic microorganisms, which help shape microbial communities to target those specific plants (Jacoby et al., 2017).

To address the extent to which nutrient enrichment influences the plant root decomposition rate and C storage potential, I measured mass loss of different plant litters sourced from different soil nutrient histories exposed to soil belowground communities and soil microbes found in coastal plain wetland plots under ambient or enriched nutrient deposition. I hypothesized that if plant species vary in growth response to different nutrient context histories, then root decomposition rates will depend on nutrient context (long-term fertilization vs. ambient) and plant functional group (grass vs. shrub). Changes to plant root composition (measured as C:N ratio) will influence the activity of the resident soil microbial community. I predicted that (i) plant litter buried in wet fertilized plots with higher microbial activity will undergo more mass loss, especially for grass litter, compared to unfertilized plots with lower microbial activity. I also predicted that (ii) there will be more C storage measured in wet unfertilized plots than fertilized plots because of higher rates of mineralized organic C activating microbial decomposition and C loss. This study will also help improve the prediction of the C storage potential of coastal plain wetlands.

METHODS

Experimental Design

To test the hypothesis, a long-term wetland ecology experiment at the West Research Campus (WRC) at East Carolina University (ECU) was leveraged. In 2003, a 2×2 factorial experiment was applied to a tract of wet pine savannah habitat to support diverse plant communities. Experimental treatments represent two levels of nutrient enrichment (fertilized, unfertilized) and two levels of disturbance (mowed, unmowed), which are replicated on eight 20×30 m blocks (**Figure 1**). The nitrogen-phosphorus-potassium (N-P-K 10- 10-10) pellet fertilizer is applied 3× per year (45.4 kg ha^{-1} for each nutrient), and plots are mowed, and litter removed to simulate a fire disturbance (Goodwillie et al., 2020). Half the blocks adjacent to a ditch are drier parts of the wetland compared to the blocks away from the ditch. Annual plant (since 2004) and microbial (since 2014) community surveys are conducted to test the effects of fertilization, disturbance, and their interaction on wetland community compositions (Goodwillie et al., 2020). For the root litterbag experiment, two different plant functional groups were chosen because of their differing root litter composition and C:N ratios: shrub species (*Rhus copallinum*) and grass species (*Chasmanthium laxum*). Specifically, *Rhus copallinum* was chosen based on the relative abundance at WRC where the species had an increased relative abundance in mowed fertilized plots compared to unfertilized (**Figure 2**).

Root Litterbag Preparation and Processing

Nylon mesh bags allow microbes to break down the root litter, and this approach enables quantitative assessment of decomposition rate of a subset of decomposers. Litterbags were measured at 20 cm length x 10 cm height and made from two different mesh sizes; one with a nylon mesh size of 20 microns (μm) to allow only microbial access and one with a mesh size of

200 µm to allow soil invertebrates that are less than 200 µm in size to access root litter. Two types of root litter were prepared: *Rhus copallinum* (high C:N ratio) and *Chasmanthium laxum* (low C:N ratio). The root litter was rinsed to rid of soil and then oven-dried at 60 °C for 48 hours. After the drying process, the root litter was cut and measured into 2.00 grams (g) and distributed to each litterbag. There was a total of 128 litterbags (half of the 20 µm and half of the 200 µm). In the field, each wet mowed plot had root litter from all combinations of plant nutrient history (unfertilized, fertilized), mesh size (20 µm, 200 µm), and soil nutrient history (fertilized, unfertilized). Additionally, unmowed plots were not used in this quantitative assessment.

At random, 16 litterbags were buried in an unfertilized plot and 16 buried in a fertilized plot, which equaled 32 experimental units (**Figure 3**). To account for variation within plots, there were four litterbag technical replicates buried for a total of 128 experimental units at 5.08 cm. Litterbags remained in the field for a monitoring period of eight weeks from October 2025 to December 2025. After the monitoring period, root litter was retrieved at once and transferred to a sterilized surface for separation of contents from the original root litter including rocks and other soil particles. Seven litter bags were missing in the field and two litter bags had holes (one 200 µm unfertilized and one 20 µm unfertilized). Root litter was gently washed with tap water, placed in envelopes with identification written on the front side, and dried at 60°C for 48 hours. Afterwards, the root litter was weighed to 0.0001 grams to calculate the mass loss.

Collection and Preparation to Quantify Root Litter Quality

Additionally, the plant root litter samples were subset and ground for further elemental analysis to measure the concentrations of C and N of dried root litter. Rinsed and dried root litter were ground into a fine powder using a mortar and pestle, resulting in a homogenous mixture (**Figure 4**). After ensuring homogeneity, the fine root litter was placed in 1.5 mL microtubes with three subsamples for grass and shrub root litter buried in fertilized and unfertilized plots. Samples were sent to the Department of Biological and Agricultural Engineering at North Carolina State University's Environmental Analysis Lab for elemental analysis of C and N using combustion analysis.

Collection and Preparation to Quantify Soil Quality

To determine how the differences in root litter C:N ratio combined with soil C:N ratio influenced rates of decomposition, soil sampling and processing were applied. Soil samples were analyzed only from wet plots to align with the root litter collected from wet plots. Composite soil samples were collected from mowed/fertilized and mowed/unfertilized plots at WRC which had two soil cores (3.1 cm diameter, 12 cm depth) adjacent to three quadrats and each composite sample was passed through a 4 mm sieve, homogenized, dried at room temperature, and ground to a fine powder using a mortar and pestle. Then a subsample of each soil was analyzed for total C and N using a combustion elemental analyzer at the Environmental and Agricultural Testing Service laboratory (Department of Crop and Soil Sciences at North Carolina State University (Koceja et al., 2021).

Statistical Analyses

All statistical analyses were performed in the R statistical environment (RStudio 2024.09.0+375, R version 4.4.1, 2024). I used a nonparametric test (Kruskal-Wallis) for all response variables because it does not assume the data follows a normal distribution and Dunn's test to run comparison between more than two independent groups (Kruskal & Wallis, 1952). I used multiple R packages such as ggpubr (Kassambra, 2023) for customizing plots, ggplot2 (Wickham, 2016) for graphing data, tidyverse (Wickham et al., 2019) to import data, dplyr

(Wickham et al., 2023) for data wrangling, reshape (Wickham, 2007) for restructuring and aggregating data.

Boxplots were created to graph the mass loss percent of grass species and the shrub species, using ggplot and geom_boxplot to show how the data were distributed across litter source (fertilized, unfertilized), current nutrient conditions (fertilized, unfertilized), and mesh size (20, 200 μm). Additionally, I ran assumptions of normality and homogeneity of variances to check for normally distributed data and if variances of data were similar. For the grass species, the Shapiro-Wilk test was run to test whether the data deviated significantly from a normal distribution. The test calculated that the data closely resembled a normal distribution but the probability of obtaining the observed statistic was low, indicating a significant deviation from a normal distribution (W= 0.9554, p-value= 0.0281). Therefore, I ran the nonparametric Kruskal-Wallis test to compare the independent groups of fertilization, plant nutrient history, mesh size and the interactions on mass (2.1239, p-value= 0.5471). For the shrub species, the Shapiro-Wilk test calculated that the data closely resembled a normal distribution and the probability of obtaining the observed statistic was high, indicating no significant deviation from a normal distribution (W= 0.9677, p-value= 0.1068). To continue testing for homogeneity of variances, I ran Bartlett's test which tested the assumption of equal variances in different samples for the shrub species ($K^2 = 6.0665$, df = 3, p-value = 0.1084). Despite meeting assumptions of normality and homogeneity of variances for the shrub species, I ran the nonparametric Kruskal-Wallis test to compare the independent groups of fertilization, and mesh size and the interactions on shrub litter mass loss. After running Kruskal-Wallis, I ran Dunn's test to identify significant pairwise differences between buried plot and mesh size for the grass and shrub species. Since assumptions were violated for some of the response variables, I ran the non-parametric Kruskal-Wallis test to compare the independent groups of fertilization and plant types and their interactions on root percent C, root percent N, root C:N ratio, soil percent C, soil percent N and soil C:N ratio. After running Kruskal-Wallis, I ran Dunn’s test to identify significant pairwise differences between plant type and fertilization.

RESULTS

Fertilization Influenced Grass and Shrub Root Litter Quality in Distinct Ways

I evaluated the root litter quality represented by plant root percent C and N concentrations for grass and shrub roots collected from fertilized and unfertilized plots. Root percent C was marginally different across plant type and fertilization (Kruskal-Wallis: X^2= 7.1026, p-value = 0.0687). There were between group differences observed between fertilized grass roots and fertilized shrub roots (Dunn's test: Z = -2.3778, p.unadj = 0.0174; **Table 1**) and fertilized shrub and unfertilized shrub roots (Dunn's test: Z = 2.1513, p.unadj = 0.0315; **Table 1**). A general trend was observed, where shrub roots collected from fertilized plots had higher percent C than grass roots, while grass roots in unfertilized plots had a higher percent C compared to shrub roots (**Figure 5A**). Root percent N was different across plant type and fertilization (Kruskal-Wallis: X^2= 8.8129, p-value = 0.0319). There were between group differences observed between fertilized grass and fertilized shrub root percent N (Dunn's test: Z = 2.4387, p.unadj = 0.0147; **Table 2**) and unfertilized grass and fertilized shrub root percent N (Dunn's test: Z = 2.3252, p.unadj = 0.0201; **Table 2**). Grass roots collected from both fertilized and unfertilized plots showed a higher percent N than fertilized and unfertilized shrub roots (**Figure 5B**). Root C:N ratio was different across plant type and fertilization (Kruskal-Wallis: X^2= 9.1538, p-value = 0.0273). There group differences observed between C:N ratio of unfertilized grass roots and fertilized shrub roots (Dunn's test: Z = -2.1513, p.unadj = 0.0315). Specifically, shrub roots had a higher C:N ratio compared to grass roots sourced from fertilized plots only (Dunn's test: Z=-2.8307, p.unadj=0.0046; **Table 3**; **Figure 5C**).

Fertilization influenced concentrations of soil C and N. Results indicated that soil samples collected from fertilized plots showing higher percent C compared to unfertilized plots (Kruskal-Wallis: X^2 = 5.3333, p-value = 0.0209; **Figure 6A**), and soils from fertilized plots had higher percent N compared to those from unfertilized plots (Kruskal-Wallis: $X^2 = 4.744$, p-value = 0.0294; **Figure 6B**). Further, soil samples collected from fertilized plots had higher C:N ratio than soils from unfertilized plots (Kruskal-Wallis: $X^2 = 4.0833$, p-value = 0.0433; **Figure 6C**).

Fertilization Influences Decomposition Rates to Varying Degrees

Next, I evaluated how fertilization, plant nutrient history, and mesh size influenced mass loss of the *Chasmanthium laxum* grass root litter and the *Rhus copallinum* shrub root litter. Results indicated that the percent mass loss of the grass roots were similar across all main effects of fertilization, plant nutrient history, and mesh size (Kruskal-Wallis: $X^2 = 4.1985$, p-value= 0.7567; **Figure 7**). In contrast, fertilization and mesh size influenced mass loss of shrub root litter (Kruskal-Wallis: $X^2 = 9.7006$, p = 0.02129; **Figure 8**). Specifically, results showed more mass loss of shrub roots buried in nylon mesh bags of 200 µm in fertilized plots compared to roots buried in nylon mesh bags of 20 µm in unfertilized plots (Dunn's test: Z=2.9648, p.unadj=0.0030; **Table 4; Figure 8**). For shrub roots buried in 200 µm mesh bags, mass loss was higher in fertilized compared to unfertilized plots (Dunn's test: Z = 2.2123, p.unadj = 0.0269; **Table 4; Figure 8**).

Mesh Size Influences Decomposition Rates to Varying Degrees

There were no significant effects of plant species (grass and shrub) and distinct mesh sizes (20 µm and 200 µm) on percent mass loss (Kruskal-Wallis: $X^2 = 6.0275$, p-value = 0.1103), but a general trend emerged. Grass species buried in 20 µm nylon mesh bags had higher percent mass loss (26.5 %) than the shrub species (20.3 %) (**Figure 9; Table 5**). For roots buried in the 200 µm nylon mesh bags, the grass species had higher percent mass loss (28.2 %) than the shrub species (26.3 %) (**Figure 9; Table 5**). However, root litter buried in fertilized plots within 200 μm nylon mesh bags had higher percent mass loss than root litter buried in fertilized plots within the 20 μm nylon mesh bags (**Figure 9**).

DISCUSSION

Nutrient Enrichment Influence on Plant Root Decomposition and C Storage Potential

This study examined how nutrient enrichment influenced the plant root decomposition rate and C storage potential by measuring effects of fertilization, plant nutrient history, and mesh size on percent mass loss of plant litter after eight weeks. I hypothesized that if plant species vary in growth response to distinct nutrient contexts (i.e., nutrient history represented by plants sourced from fertilized or ambient plots), then species-specific root chemical composition differences (grass vs. shrub) will influence root decomposition rates and depend on contemporary nutrient enrichment (i.e., nutrient treatment represented by long-term fertilization vs. ambient conditions). After eight weeks of litter decomposition, data supported the hypothesis for the shrub species but not the grass species. For both grass and shrub, nutrient history of the plant (source treatment) did not influence litter decomposition. For the shrub species only, the contemporary nutrient conditions (buried plot treatment) and decomposer access to litter (mesh size) influenced decomposition of shrub roots.

Prior studies revealed contrasting results to those observed in the present study. For example, one study conducted a litterbag experiment in a grassland ecosystem and found that effects of fertilization and plant nutrient history influenced mass loss of roots derived from a native and non-native grass species *Lolium perenne*. This study provided some evidence that the root C:N ratios might change over time in (Fornara et al., 2020). Another study measured the interaction between litter type and nutrient enrichment using standardized teabags as model litterbag with different C:N ratios. Results revealed that nutrient enrichment enhanced decomposition rate of high C:N tea litter compared to ambient coastal plain wetland nutrient conditions, which matched results for the shrub species (Koceja et al., 2021). The contrasting results pertaining to fertilization effect on decomposition rates from this study compared to prior studies could be due to plant species chosen for the study, mesh size difference and the length of study. An additional study conducted a long-term experiment using a grass species (*Urochloa decumbens*) and different treatments (fertilization, no fertilization) showed factors such as addition of nutrients in soil from previous fertilization and low litter C:N ratio helped with faster decomposition rates (Coutinho et al., 2025).

The variation in nutrient effects on shrub root decomposition and grass root decomposition could be due to a difference of components such as litter chemical and physical composition. Litter quality (chemical characteristics) and root diameter (thickness of roots) are two factors that affect decomposition rates in different plant functional groups which is divided between fine (<2 mm root diameter) and coarse (>2 mm) roots (Fantozzi et al., 2024; Zhang & Wang, 2015). Fine roots are classified as non-woody roots responsible for nutrient and water uptake while coarse roots are woody roots responsible for plant stability (Fantozzi et al., 2024). One study observed that plant litter decomposed at different rates according to species. Specifically, fine root from graminoids and conifers decomposed faster than the coarse roots from broadleaf trees and shrubs (Zhang & Wang, 2015), while in another study noted that N concentrations are typically higher in fine roots such as *Poa sp.* compared to coarse roots such as *Artemisia sp.* (McLaren & Turkington, 2011). Additional articles found that decomposition rates were heavily influenced by litter quality or lignin concentration, N concentration and other components such as cellulose, sugars and phenols (Porre et al., 2020; Yang et al., 2022). The increased relative abundance of *Rhus copallinum* from my study conducted in a North Carolina coastal plain wetland provided insight as to why C loss rates are higher in fertilized plots compared to unfertilized. The difference in C loss rates were shown by the influence of root biomass and morphology between fine and coarse roots where coarse root turnover can depend on climate response (Zhang & Wang, 2015).

The soil C:N ratio in combination with the differences in litter C:N ratio influenced rates of decomposition in my study. Lower C:N ratio tend to support higher rates of decomposition due to N-rich environment while higher C:N ratio tend to have slower decomposition because of a N-poor environment where microbial abundance can be relatively low (Bai et al., 2023). Soil C:N ratio was lower from fertilized plots and higher in unfertilized plots. However, grass litter (low C:N ratio) decomposition rates were similar across both fertilized and unfertilized plots while shrub roots buried in fertilized plots (low soil C:N ratio) had faster rates of decomposition compared to unfertilized plots. One study found higher root mass loss in fertilized areas compared to unfertilized plots, primarily due to soil C accumulation (Fornara et al., 2020). In the present study, higher soil C:N ratio in unfertilized plots influenced shrubs roots by slower decomposition rates. Therefore, plant litter with higher C:N ratio added to low soil C:N ratio helped with faster decomposition rates based on the study (Bai et al., 2023). However, adding plant litter with high C:N ratio to soil with high C:N ratio can cause slower decomposition rates (Rejmánková, & Houdková, 2006), which was reflected in the shrub root litter buried in unfertilized plots.

Plant litter and soil composition can also dictate the rate of C loss via decomposition, which have ecosystem-scale implications for C cycling (Zhang et al., 2014). One litterbag study found that major C fluxes typically come from C input belowground, and plant species that undergo faster decomposition rates had higher CO_2 emissions based on field measurements (Zhang et al., 2014). Another litterbag study focused on decomposition and soil organic matter

(SOM) concentrations, indicating that quick decomposing roots contributed to a more stable SOM pool by observing mass loss of forest litter that becomes isolated in SOM or released as CO_2 (Prescott & Vesterdal, 2021). Additionally, microbial activity plays a role in litter C stability, where some proportion of grass litter C stabilized into SOM and not all lost through microbial respiration during early decomposition (Prescott & Vesterdal, 2021).

Mesh Size Influence on Plant Root Decomposition

Mesh size of litterbags can affect the rate of decomposition due to physical and biological factors (Lecerf, 2017). Since *Rhus copallinum* represented majority of coarse roots, the larger mesh size results in a higher diversity of decomposers getting access to coarse roots, which account for most of *Rhus copallinum* root biomass (Zhang & Wang, 2015). The findings from my study showed decomposition differences due to macrofauna access to root litter (200 µm mesh) compared to microbial (20 µm mesh) access alone. In prior studies, a 1 mm mesh size was used because of its exclusion of macrofauna (large detritivores) but inclusion of microbes, microfauna and mesofauna (Lecerf, 2017; Xie, 2020). To observe the direct and indirect effects of soil fauna on decomposition rates and mesh size, an experiment was conducted in a temperate grassland using a grass species *Agrostis capillaris* (Bradford et al., 2002). Mesh sizes less than 1 mm could prevent some of the microfauna and mesofauna from passing through the litterbag (Xie, 2020). On the other hand, mesh greater than 2 mm allowed macrofauna to participate in litter decomposition through both direct and indirect effects (Bradford et al., 2002; Xie, 2020). Decomposition rates based on various mesh sizes can differ based on habitats they are buried in. One litterbag study found that litter buried in various mesh sizes (1-2 mm) significantly affected decomposition rates in different habitats such as tropical forests, deserts and grasslands by examining mean annual temperature (MAT), mean annual precipitation (MAP), latitude, elevation, soil texture (Silver & Maya, 2001). There were a few papers that included measured evapotranspiration (AET), therefore the authors used data from nearby weather stations with similar latitude, longitude and elevation and the World Climate website to calculate (AET) (Silver & Miya, 2001). Overall, varied sizes in mesh bags can play a role in the access of microorganisms and affect decomposition rates.

Limitations and Future Directions

It is possible that the length of the experiment and the time of year the experiment was conducted masked expected treatment effects in my study. For example, the timing of the litterbag experiment occurred towards the middle of fall to the beginning of winter (October to December) when microbial activity is typically slower because of temperature sensitivity, resulting in decreased rates of enzymatic reaction and microbial growth (Knapp & Kerwyn, 2022; Torsekar et al., 2024). The length of study was eight weeks which added sufficient time for some microbes and detritivores to break down litter, however longer periods of time such as a few months up to a year could increase the breakdown process as shown by many decomposition experiments, which could change the results by showing an increase in the average mass loss (Adamcyk et al., 2019). If litter bags were buried in January and collected in April, there could be more mass loss based on a longer time frame and varying weather conditions from cold to warm. Results could vary during spring or summer months when temperatures are hotter and environments are wetter, leading to faster microbial activity and higher rates of decomposition (Canessa et al., 2020). Conducting my experiment during warmer seasons could result in larger differences in decomposition for litter buried, especially litter buried in the 20 µm mesh. Additionally, there was continuous rainfall during the experiment which could also play a role in an increase of relative microbial activity across fertilized treatments. Prolonged rainfall could alter the rate at which root litter decomposes by the presence or absence of large-bodied decomposers (Salamanca et al., 2003). One study conducted in an arid desert region to Mediterranean savannah-like shrubland using a grass species (*Stipa capensis*) showed that decomposition rates had a positive association with precipitation, and decomposers in the soil decomposed litter faster in cold wet winters compared to hot dry summers (Torsekar et al., 2024). Another study observed higher decomposition rates in areas with high precipitation such as a temperate lowland site which had an average of 63% mass loss compared to drier conditions and a shrub species (*Frankenia chilensis)* had about 97% mass loss (Canessa et al., 2020). In my study, when retrieving the litterbags after the prolonged rainfall, I noticed several detritivores such as earthworms that were attached to the surface of the bag which has been observed in a past study. High volumes of rainfall, in which soil moisture will be altered, contains high presence of large-bodies detritivores (Wise & Lensing, 2019).

My litterbag study conducted at a coastal plain wetland long-term fertilization experiment contributed to our understanding of nutrient enrichment history and plant functional group effects on root litter decomposition rates. Factors such as litterbag mesh size and local soil nutrient concentrations play a role in mass loss in root litter. Future studies could resolve the relative importance of the local environmental conditions that include temperature, moisture, and nutrient concentrations and plant composition on decomposition rates. For example, my study could be expanded to evaluate temperature-dependent effects by burying and collecting litterbags over multiple timepoints within one year could resolve how seasonality influences decomposition rates. This study could help address limitations proposed such as length of experiment and different weather conditions by allowing an observation of both short-term and long-term changes of decomposition (Canessa et al., 2020).

In conclusion, this study showed an increased relative abundance of woody species (*Rhus copallinum*) with higher mass loss plant roots. These results provide evidence that gains from increased plant biomass could be negated by increased root turnover of native plant species, resulting in subsequent CO_2 release from soils. Understanding the relationship between long-term fertilization and different plant functional groups could help with the evaluation of plant root composition (differing C:N ratios) and decomposition rates (due to microbial activity), improving wetland carbon predictions.

REFERENCES

Adamczyk, B., Sietiö, O., Straková, P., Prommer, J., Wild, B., Hagner, M., Pihlatie, M., Fritze, H., Richter, A., & Heinonsalo, J. (2019). Plant roots increase both decomposition and stable organic matter formation in boreal forest soil. *Nature Communications*, *10*(1), 1-9. https://doi.org/10.1038/s41467-019-11993-1

Allison, S. D., Lu, Y., Weihe, C., Goulden, M. L., Martiny, A. C., Treseder, K. K., & H. Martiny, J. B. (2013). Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology*, *94*(3), 714-725. https://doi.org/10.1890/12-1243.1

Bai, X., Li, J., & Chang, S. (2023). Effects of different carbon and nitrogen ratios on nitrogen removal efficiency and microbial communities in constructed wetlands. *Water*, *15*(24), 4272. https://doi.org/10.3390/w15244272

Bodker, J. E., Turner, R. E., Tweel, A., Schulz, C., & Swarzenski, C. (2015). Nutrient-enhanced decomposition of plant biomass in a freshwater wetland. *Aquatic Botany*, *127*, 44-52. https://doi.org/10.1016/j.aquabot.2015.08.001

Bradford, M. A., Berg, B., Maynard, D. S., Wieder, W. R., & Wood, S. A. (2016). Understanding the dominant controls on litter decomposition. *Journal of Ecology*, *104*(1), 229-238. https://doi.org/10.1111/1365-2745.12507

Bradford, M. A., Tordoff, G. M., Eggers, T., Jones, T. H., & Newington, J. E. (2002). Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos*, *99*(2), 317-323. https://doi.org/10.1034/j.1600-0706.2002.990212.x

Canessa, R., Saldaña, A., Rios, R. S., Hättenschwiler, S., Mueller, C. W., Prater, I., Tielbörger, K., & Bader, M. Y. (2021). Relative effects of climate and litter traits on decomposition change with time, climate and trait variability. *Journal of Ecology*, *109*(1), 447-458. https://doi.org/10.1111/1365-2745.13516

Carlesso, L., Beadle, A., Cook, S. M., Evans, J., Hartwell, G., Ritz, K., Sparkes, D., Wu, L., & Murray, P. J. (2019). Soil compaction effects on litter decomposition in an arable field: Implications for management of crop residues and headlands. *Applied Soil Ecology*, *134*, 31-37. https://doi.org/10.1016/j.apsoil.2018.10.004

Coutinho, D. N., Chaves, C. S., Dos Anjos, A. J., Jefferson, A., Alves, W. S., Da Silveira, T. C., Pereira, O. G., Fernandes, R. B., Dubeux, J. C., & Ribeiro, K. G. (2025). Root decomposition in Urochloa decumbens pastures fertilized with increasing nitrogen doses or intercropped with Calopogonium mucunoides. *Scientific Reports*, *15*(1), 1-12. https://doi.org/10.1038/s41598-025-91796-1

Fantozzi, D., Montagnoli, A., Trupiano, D., Di Martino, P., Scippa, G. S., Agosto, G., Chiatante, D., & Sferra, G. (2024). A systematic review of studies on fine and coarse root traits measurement: Towards the enhancement of urban forests monitoring and management. *Frontiers in Forests and Global Change*, *7*, 1322087. https://doi.org/10.3389/ffgc.2024.1322087

Fennessy, M.S., Rokosch, A. & Mack, J.J. (2008). Patterns of plant decomposition and nutrient cycling in natural and created wetlands. *Wetlands, 28*, 300–310. https://doi.org/10.1672/06-97.1

Fornara, D. A., Flynn, D., & Caruso, T. (2020). Effects of nutrient fertilization on root decomposition and carbon accumulation in intensively managed grassland soils. *Ecosphere*, *11*(4), e03103. https://doi.org/10.1002/ecs2.3103

Fu, X., Dai, Y., Cui, J., Deng, P., Fan, W., & Xu, X. (2024). Soil bacterial and fungal communities resilience to long-term nitrogen addition in subtropical forests in China. *Journal of Forest Research, 35,* 17. https://doi.org/10.1007/s11676-023-01675-6

Goodwillie, C., McCoy, M. W., & Peralta, A. L. (2020). Long-term nutrient enrichment, mowing, and ditch drainage interact in the dynamics of a wetland plant community. *Ecosphere*, *11*(10), e03252. https://doi.org/10.1002/ecs2.3252

Gougoulias, C., Clark, J. M., & Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, *94*(12), 2362-2371. https://doi.org/10.1002/jsfa.6577

Guignard, M. S., Leitch, A. R., Acquisti, C., Eizaguirre, C., Elser, J. J., Hessen, D. O., Jeyasingh, P. D., Neiman, M., Richardson, A. E., Soltis, P. S., Soltis, D. E., Stevens, C. J., Trimmer, M., Weider, L. J., Woodward, G., & Leitch, I. J. (2017). Impacts of nitrogen and phosphorus: From genomes to natural ecosystems and agriculture. *Frontiers in Ecology and Evolution*, *5*, 258706. https://doi.org/10.3389/fevo.2017.00070

Harpole, W. S., Sullivan, L. L., Lind, E. M., Firn, J., Adler, P. B., Borer, E. T., Chase, J., Fay, P. A., Hautier, Y., Hillebrand, H., MacDougall, A. S., Seabloom, E. W., Williams, R., Bakker, J. D., Cadotte, M. W., Chaneton, E. J., Chu, C., Cleland, E. E., Davies, K. F., . . . Wragg, P. D. (2016). Addition of multiple limiting resources reduces grassland diversity. *Nature*, *537*(7618), 93-96. https://doi.org/10.1038/nature19324

Hobbie, S. E., & Vitousek, P. M. (2000). Nutrient limitation of decomposition in Hawaiian Forests. *Ecology*, *81*(7), 1867-1877. https://doi.org/10.1890/0012-9658(2000)081[1867:NLODIH]2.0.CO;2

Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G., & Pineiro, G. (2017). The Ecology of soil carbon: Pools, vulnerabilities, and biotic and abiotic controls. *Annual Review of Ecology, Evolution, and Systematics*, *48*, 419-445. https://doi.org/10.1146/annurev-ecolsys-112414-054234

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., & Kopriva, S. (2017). The Role of Soil Microorganisms in plant mineral nutrition—Current knowledge and future directions. *Frontiers in Plant Science*, *8*, 292271. https://doi.org/10.3389/fpls.2017.01617

Jing, H., Wang, J., Wang, G., Liu, G., & Cheng, Y. (2024). Understory vegetation had important impact on soil microbial characteristics than canopy tree under N addition in a Pinus tabuliformis plantation. *Agriculture, Ecosystems & Environment*, *360*, 108763. https://doi.org/10.1016/j.agee.2023.108763

Kassambara A (2023). *ggpubr: 'ggplot2' Based Publication Ready Plots*. R package version 0.6.0, https://rpkgs.datanovia.com/ggpubr/

Knapp, B.D., & Kerwyn C.H. (2022). The effects of temperature on cellular physiology. *Annual Review of Biophysics 51*, 499–526. https://doi.org/10.1146/annurev-biophys-112221-074832

Koceja, M. E., Bledsoe, R. B., Goodwillie, C., & Peralta, A. L. (2021). Distinct microbial communities alter litter decomposition rates in a fertilized coastal plain wetland. *Ecosphere*, *12*(6), e03619. https://doi.org/10.1002/ecs2.3619

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, *47*, 583–621.

Kumar, A., Shahbaz, M., Blagodatskaya, E., Kuzyakov, Y., & Pausch, J. (2018). Maize phenology alters the distribution of enzyme activities in soil: Field estimates. *Applied Soil Ecology*, *125*, 233-239. https://doi.org/10.1016/j.apsoil.2018.02.001

Lecerf, A. (2017). Methods for estimating the effect of litterbag mesh size on decomposition. *Ecological Modelling*, *362*, 65-68. https://doi.org/10.1016/j.ecolmodel.2017.08.011

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K. S., Knops, J. M., McCulley, R. L., La Pierre, K., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J., & Fierer, N. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences*, *112*(35), 10967-10972. https://doi.org/10.1073/pnas.1508382112

Ma, A., Liu, H., Song, C., Tian, E., & Wang, X. (2023). Home-field advantage in litter decomposition: A critical review from a microbial perspective. *Journal of Basic Microbiology*, *63*(7), 709-721. https://doi.org/10.1002/jobm.202200657

McLaren, J. R., & Turkington, R. (2011). Plant identity influences decomposition through more than one mechanism. *PLOS ONE*, *6*(8), e23702. https://doi.org/10.1371/journal.pone.0023702

Morrison, E. W., Pringle, A., Van Diepen, L. T., & Frey, S. D. (2018). Simulated nitrogen deposition favors stress-tolerant fungi with low potential for decomposition. *Soil Biology and Biochemistry*, *125*, 75-85. https://doi.org/10.1016/j.soilbio.2018.06.027

Nie, H., You, C., & Gao, J. (2024). Effects of fertilization on litter decomposition dynamics and nutrient release in orchard systems. *Frontiers in Plant Science*, *15*, 1467689. https://doi.org/10.3389/fpls.2024.1467689

Ontl, T. A. & Schulte, L. A. (2012) Soil carbon storage. *Nature Education Knowledge, 3*(10):35

Porre, R. J., Van der Werf, W., De Deyn, G. B., Stomph, T. J., & Hoffland, E. (2020). Is litter decomposition enhanced in species mixtures? A meta-analysis. *Soil Biology and Biochemistry*, *145*, 107791. https://doi.org/10.1016/j.soilbio.2020.107791

Prescott, C. E., & Vesterdal, L. (2021). Decomposition and transformations along the continuum from litter to soil organic matter in forest soils. *Forest Ecology and Management*, *498*, 119522. https://doi.org/10.1016/j.foreco.2021.119522

R Core Team (2024). _R: A language and environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rejmánková, E., & Houdková, K. (2006). Wetland plant decomposition under different nutrient conditions: what is more important, litter quality or site quality?. *Biogeochemistry*, *80*, 245–262. https://doi.org/10.1007/s10533-006-9021-y

Salamanca, E. F., Kaneko, N., & Katagiri, S. (2003). Rainfall manipulation effects on litter decomposition and the microbial biomass of the forest floor. *Applied Soil Ecology*, *22*(3), 271-281. https://doi.org/10.1016/S0929-1393(02)00153-1

Silver, W.L., & Miya, R.K. (2001). Global patterns in root decomposition: comparisons of climate and litter quality effects. *Oecologia*, *129*, 407–419. https://doi.org/10.1007/s004420100740

Torsekar, V.R., Sagi, N., Daniel, J.A., Hawlena, Y., Gavish-Regev, E., & Hawlena, D. (2024). Contrasting responses to aridity by different-sized decomposers cause similar decomposition rates across a precipitation gradient *eLife 13*:RP93656. https://doi.org/10.7554/eLife.93656.3

Von Haden, A. C., & Dornbush, M. E. (2014). Patterns of root decomposition in response to soil moisture best explain high soil organic carbon heterogeneity within a mesic, restored prairie.

Agriculture, Ecosystems & Environment, *185*, 188-196. https://doi.org/10.1016/j.agee.2013.12.027

Wickham H (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Wickham H (2007). Reshaping data with the reshape package. *Journal of Statistical Software*, **21**(12). https://www.jstatsoft.org/v21/i12/.

Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). Welcome to the tidyverse. *Journal of Open Source Software*, 4(43), 1686. doi:10.21105/joss.01686.

Wickham H, François R, Henry L, Müller K, Vaughan D (2023). *dplyr: A grammar of data manipulation*. R package version 1.1.4, https://github.com/tidyverse/dplyr, https://dplyr.tidyverse.org.

Wise, D. H., & Lensing, J. R. (2019). Impacts of rainfall extremes predicted by climate-change models on major trophic groups in the leaf litter arthropod community. *Journal of Animal Ecology*, *88*(10), 1486-1497. https://doi.org/10.1111/1365-2656.13046

Wu, H., Cui, H., Fu, C., Li, R., Qi, F., Liu, Z., Yang, G., Xiao, K., & Qiao, M. (2024). Unveiling the crucial role of soil microorganisms in carbon cycling: A review. *Science of The Total Environment*, *909*, 168627. https://doi.org/10.1016/j.scitotenv.2023.168627

Xie, Y. (2020). A meta-analysis of critique of litterbag method used in examining decomposition of leaf litters. *J Soils Sediments*, *20*, 1881–1886. https://doi.org/10.1007/s11368-020-02572-9

Yang, K., Zhu, J., Zhang, W., Zhang, Q., Lu, D., Zhang, Y., Zheng, X., Xu, S., & Wang, G. G. (2022). Litter decomposition and nutrient release from monospecific and mixed litters: Comparisons of litter quality, fauna and decomposition site effects. *Journal of Ecology*, *110*(7), 1673-1686. https://doi.org/10.1111/1365-2745.13902

Zhang, D., Hui, D., Luo, Y., & Zhou, G. (2008). Rates of litter decomposition in terrestrial ecosystems: Global patterns and controlling factors. *Journal of Plant Ecology*, *1*(2), 85-93. https://doi.org/10.1093/jpe/rtn002

Zhang, J., He, N., Liu, C., Xu, L., Chen, Z., Li, Y., Wang, R., Yu, G., Sun, W., Xiao, C., H. Chen, H. Y., & Reich, P. B. (2020). Variation and evolution of C:N ratio among different organs enable plants to adapt to N-limited environments. *Global Change Biology*, *26*(4), 2534-2543. https://doi.org/10.1111/gcb.14973

Zhang, L., Wang, H., Zou, J., Rogers, W. E., & Siemann, E. (2014). Non-native plant litter enhances soil carbon dioxide emissions in an invaded annual grassland. *PLOS ONE*, *9*(3), e92301. https://doi.org/10.1371/journal.pone.0092301

Zhang, X., & Wang, W. (2015). The decomposition of fine and coarse roots: Their global patterns and controlling factors. *Scientific Reports*, *5*(1), 1-10. https://doi.org/10.1038/srep09940

Zhou, X., Dong, K., Tang, Y., Huang, H., Peng, G., & Wang, D. (2023). Research progress on the decomposition process of plant litter in wetlands: A Review. *Water*, *15*(18), 3246. https://doi.org/10.3390/w15183246

APPENDIX

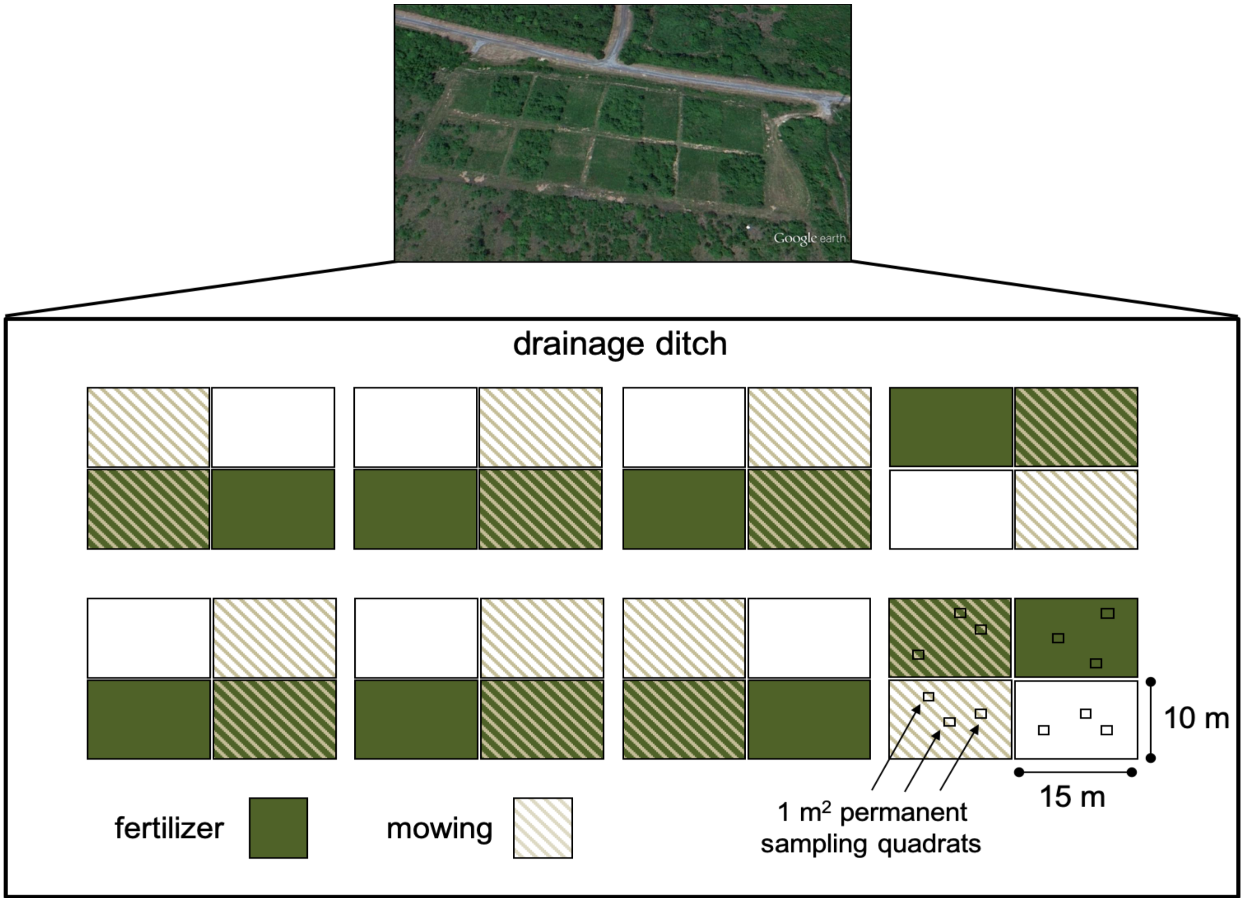

Figure 1. Diagram of long-term ecology experiment located at East Carolina University's West Research Campus (Greenville, North Carolina). The factorial design included two levels of fertilization (fertilized in green, unfertilized in white) and disturbance by mowing (unmowed in solid, mowed in dashed lines). There are four replicate blocks adjacent to the drainage ditch which represent dry plots and four replicate blocks away from the drainage ditch which represent wet plots.

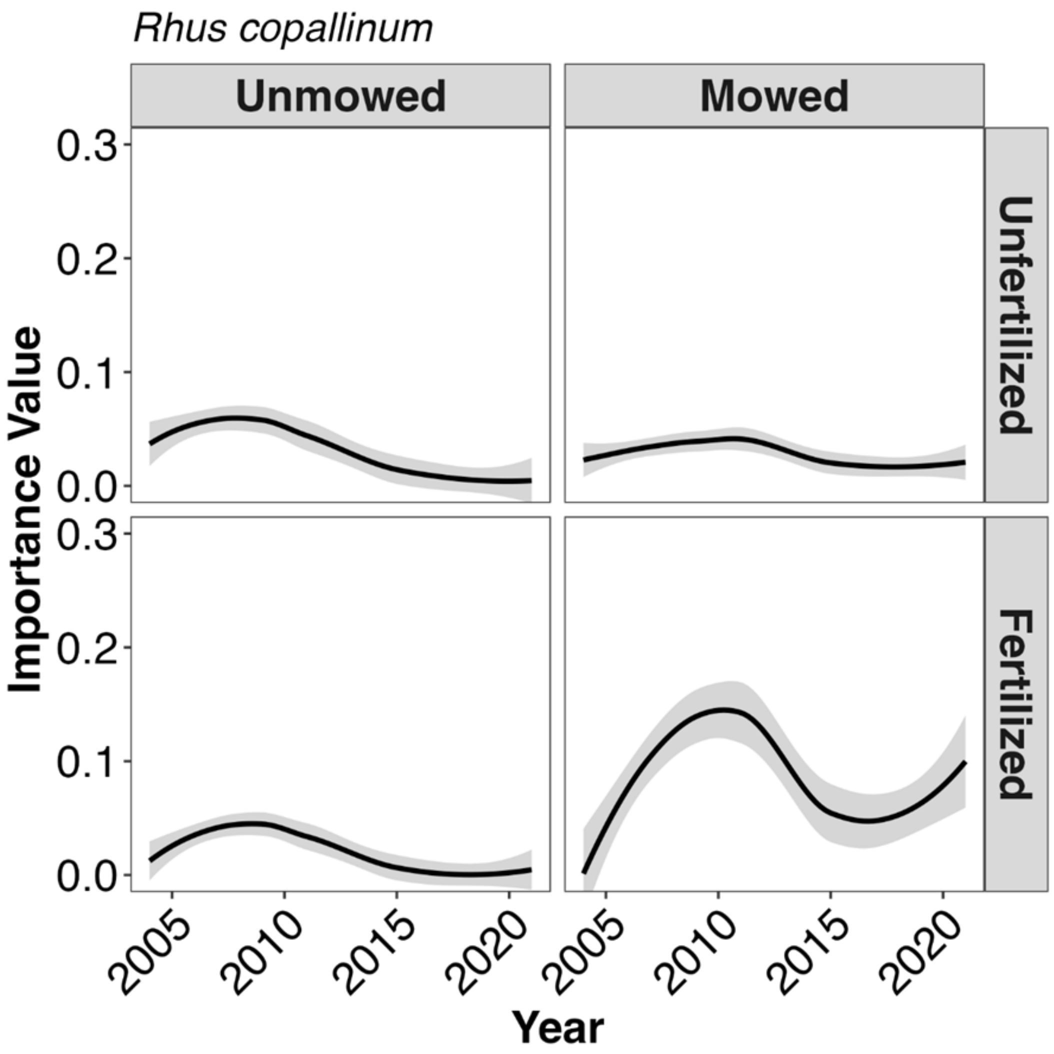

Figure 2. Graph representing importance value of *Rhus copallinum* based on year and levels of treatment. The columns represent two levels of disturbance (mowed, unmowed). The rows represent two levels of nutrient enrichment (fertilized, unfertilized).

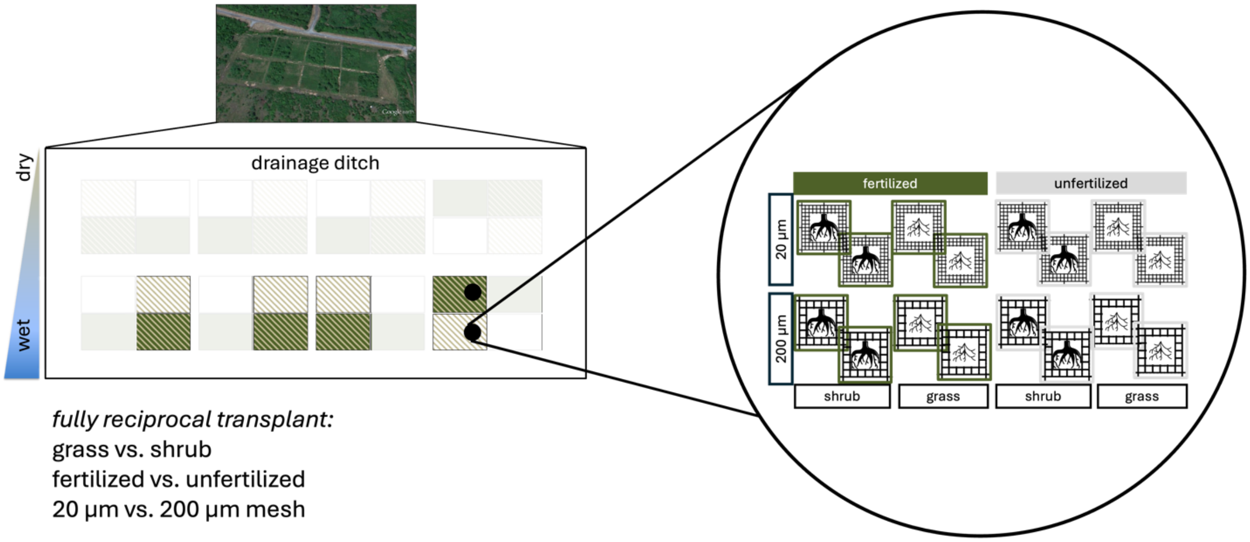

Figure 3. Diagram of experimental design depicting litterbag experiment. The litterbag experiment was deployed in the mowed (hashed pattern) and wet plots (away from the drainage ditch). A factorial design of litter type x mesh size x source of litter was conducted: two litter types (shrub *Rhus copallinum* roots in thicker black outline vs. grass *Chasmanthium laxum* roots in thin black outline) sourced from fertilized (green) or unfertilized (gray) plots were buried in two different nylon mesh bags with bigger spaces represent 200 µm mesh size and smaller spaces represent the 20 µm mesh size (smaller mesh size allows microbial access only).

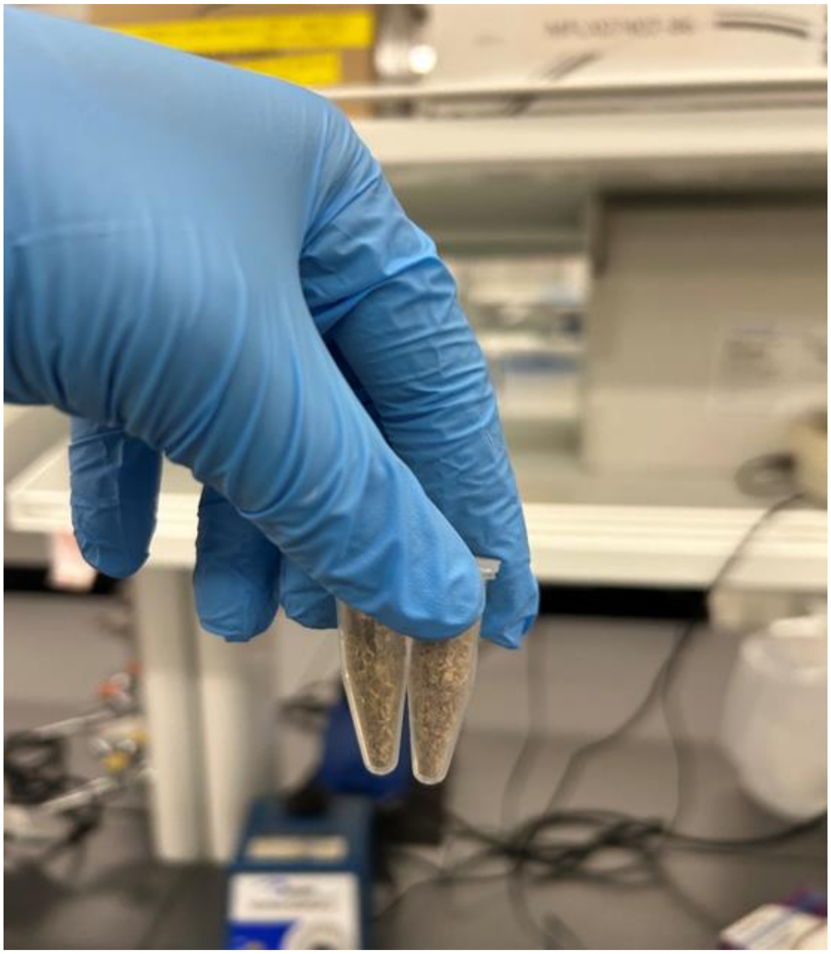

Figure 4. Photo of root samples that were ground using mortar and pestle in preparation for elemental analysis to measure carbon to nitrogen (C:N) ratio.

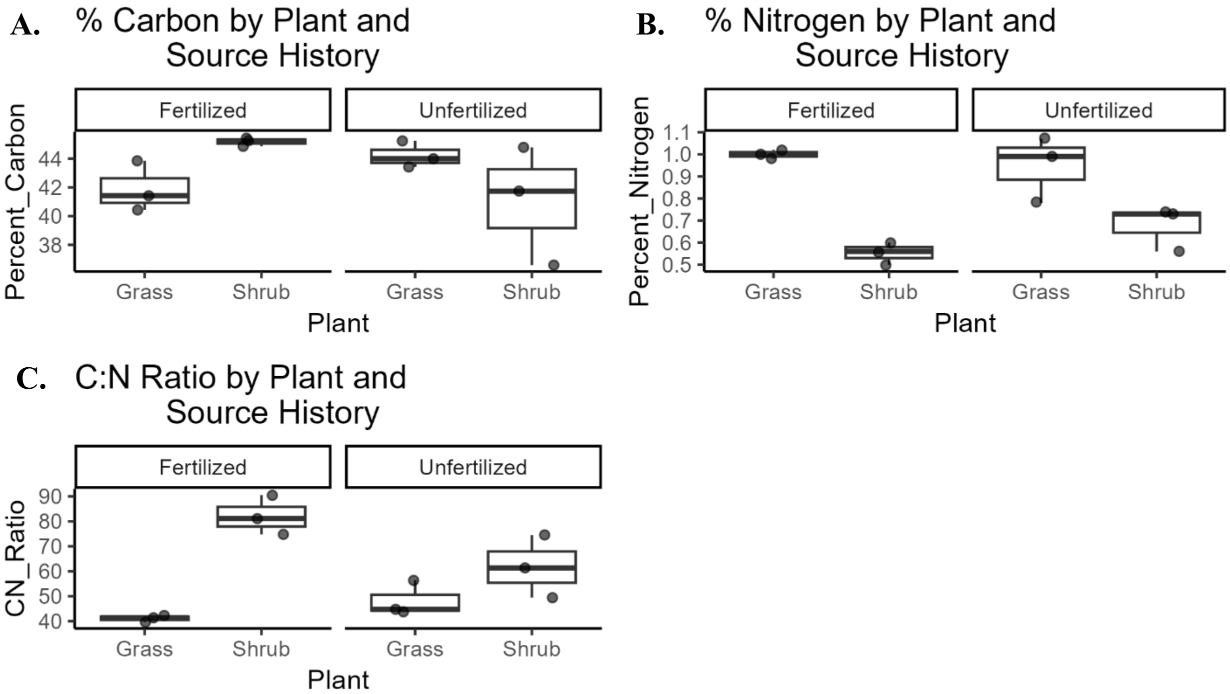

Figure 5. Boxplots summarizing plant root litter percent carbon (A), percent nitrogen (B), and the carbon:nitrogen (C:N) ratio (wt:wt) (C) according to plant type (grass or shrub) and source nutrient history (fertilized or unfertilized wetland plots detailed in Fig. 2). The boxplot is a visual representation of five key summary statistics: the median, the 25% and 75% percentiles, and the whiskers which represent the feasible range of data as determined by 1.5 x the interquartile range. Symbols represent individual raw data points from four replicate samples.

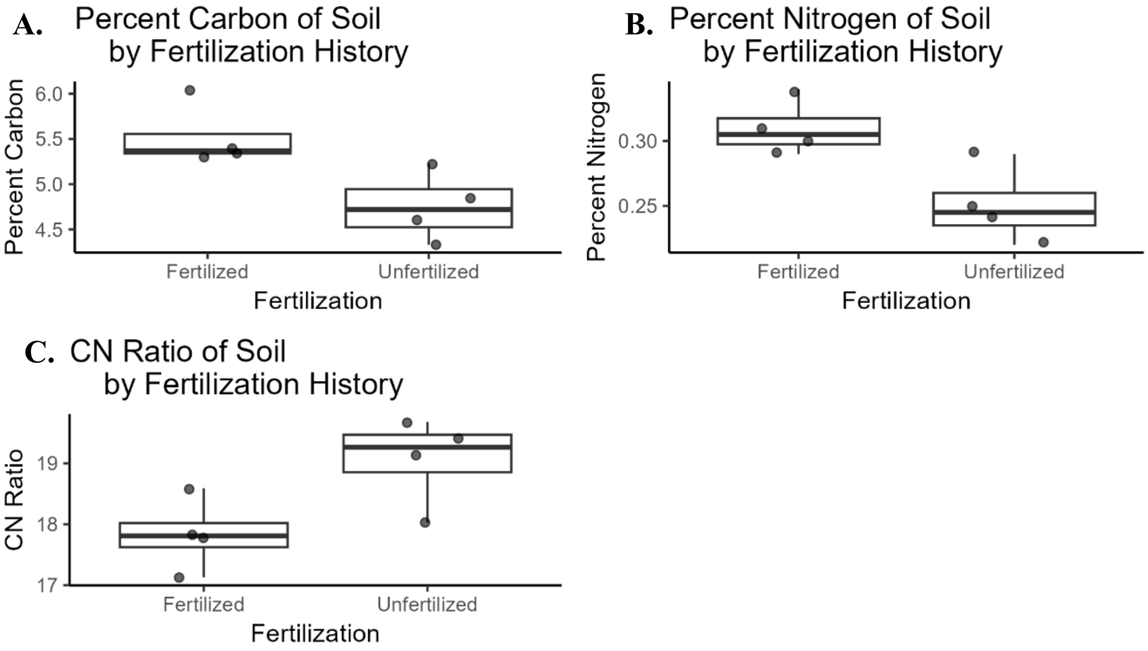

Figure 6. Boxplots summarizing soil percent carbon (A), percent nitrogen (B), and the carbon:nitrogen (C:N) ratio (wt:wt) (C) according to source nutrient history (fertilized or unfertilized). The boxplot is a visual representation of five key summary statistics: the median, the 25% and 75% percentiles, and the whiskers which represent the feasible range of data as determined by 1.5 x the interquartile range. Symbols represent individual raw data points from four replicate samples at the plot-scale.

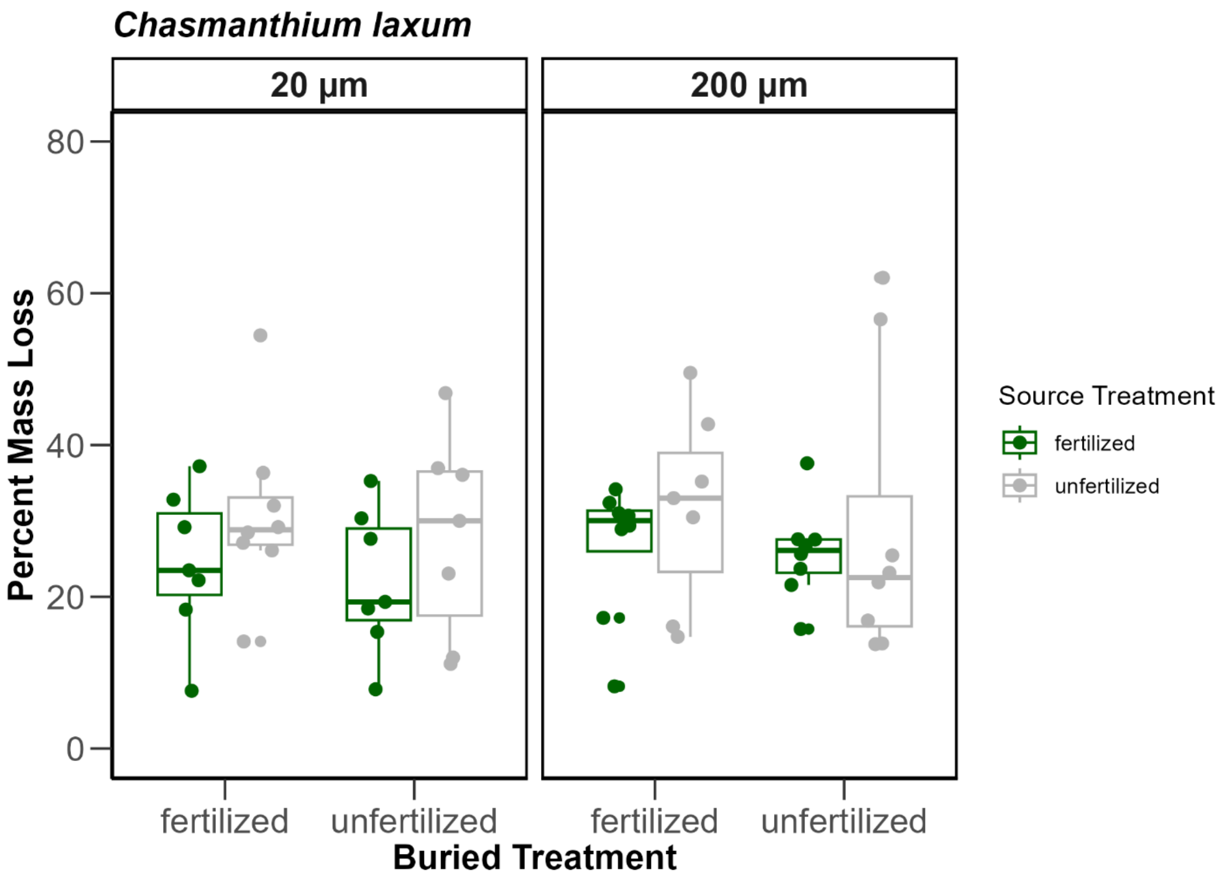

Figure 7. Boxplots showing percent mass loss for *Chasmanthium laxum* after 8 weeks of burial. Source treatment represents plant nutrient history fertilized (gray) or unfertilized (green). Litter was buried in different soil treatments of fertilized and unfertilized. The columns represent two different mesh sizes: 20 µm (microbial access only) and 200 µm (allows soil invertebrate < 200 µm access). The boxplot is a visual representation of five key summary statistics: the median, the 25% and 75% percentiles, and the whiskers which represent the feasible range of data as determined by 1.5 x the interquartile range. Symbols represent individual raw data points from four replicate samples.

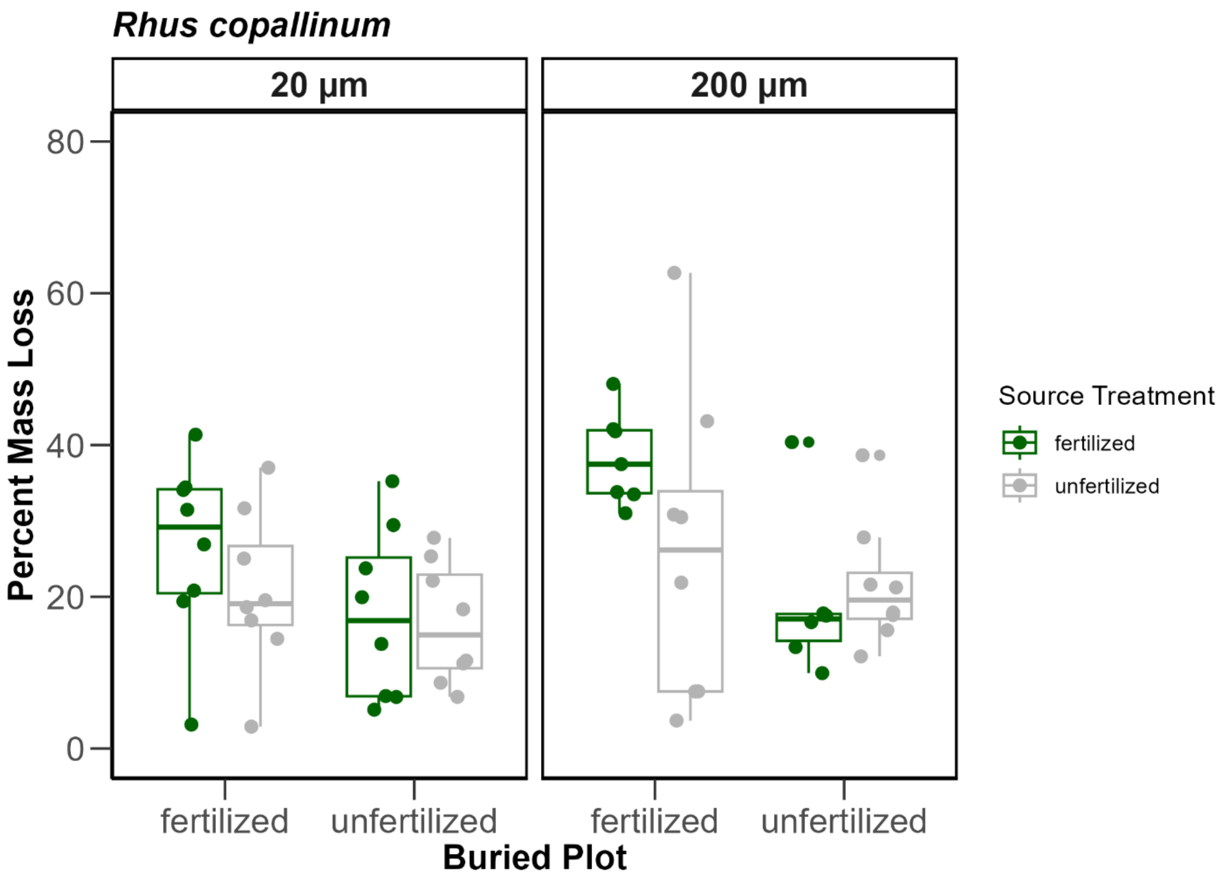

Figure 8. Boxplots showing percent mass loss of *Rhus copallinum* after 8 weeks of burial. Source treatment represents plant nutrient history: fertilized (gray) or unfertilized (green). Litter was buried in different soil treatments of fertilized and unfertilized. The columns represent two different mesh sizes: 20 µm (microbial access only) and 200 µm (allows soil invertebrate < 200 µm access). Letters represent statistical significance between groups where groups sharing the same letter are statistically similar. The boxplot is a visual representation of five key summary statistics: the median, the 25% and 75% percentiles, and the whiskers which represent the feasible range of data as determined by 1.5 x the interquartile range. Symbols represent individual raw data points from four replicate samples. Different letters above bars are considered significantly different at $P < 0.05$.

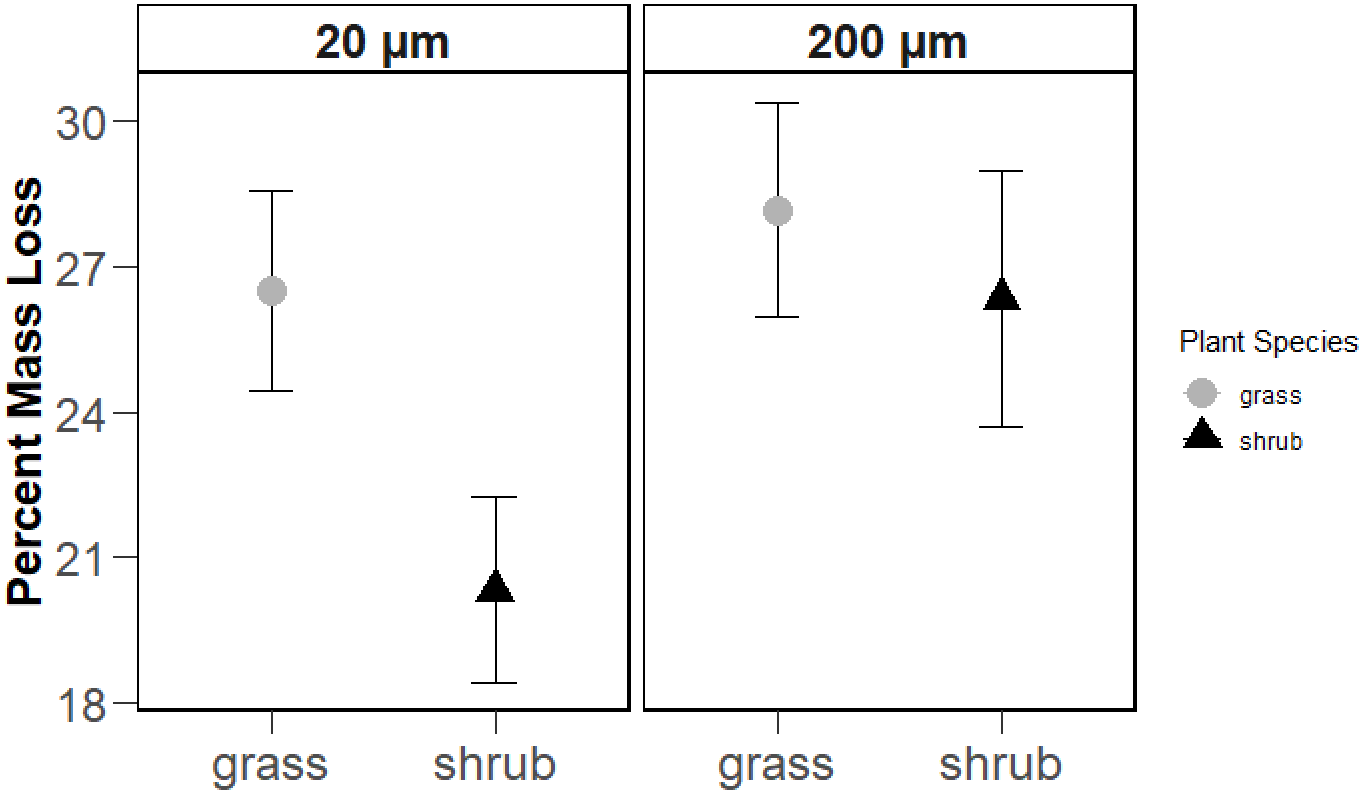

Figure 9. Mean and standard error of the percent mass loss for grass and shrub species based on mesh size. The columns represent the two different mesh sizes: 20 µm (microbial access only) and 200 µm (allows soil invertebrate < 200 µm access). The different plant functional types are grass *Chasmanthium laxum* (grey circles) and shrub *Rhus copallinum* (black triangles). Error bars represent the standard error of the mean.

Table 1. Nonparametric (Dunn's test) measuring plant litter percent carbon by pairwise comparison of plant type and soil nutrient history (fertilization).

Comparison	**Z**	**P.unadj**	**P.adj**
Grass.Fertilized – Grass.Unfertilized	-1.2455	0.2130	1.0000
Grass.Fertilized – Shrub.Fertilized	-2.3778	0.0174	0.1045
Grass.Unfertilized – Shrub.Fertilized	-1.1323	0.2575	1.0000
Grass.Fertilized – Shrub.Unfertilized	0.2265	0.8208	1.0000
Grass.Unfertilized – Shrub.Unfertilized	1.0190	0.3082	1.0000
Shrub.Fertilized – Shrub.Unfertilized	-2.1513	0.0315	0.1887

Abbreviations: Z = Z score, P.unadj= unadjusted p-value, P.adj = adjusted p-value

Table 2. Nonparametric (Dunn's test) measuring plant litter percent nitrogen by pairwise comparison of plant type and soil nutrient history (fertilization).

Comparison	Z	P.unadj	P.adj
Grass.Fertilized – Grass.Unfertilized	0.1134	0.9097	1.0000
Grass.Fertilized – Shrub.Fertilized	2.4387	0.0147	0.0885
Grass.Unfertilized – Shrub.Fertilized	2.3252	0.0201	0.1204
Grass.Fertilized – Shrub.Unfertilized	1.7581	0.0787	0.4724
Grass.Unfertilized – Shrub.Unfertilized	1.6447	0.1000	0.6002
Shrub.Fertilized – Shrub.Unfertilized	-0.6806	0.4962	1.0000

Abbreviations: Z = Z score, P.unadj= unadjusted p-value, P.adj = adjusted p-value

Table 3. Nonparametric (Dunn's test) measuring plant carbon:nitrogen ratio by pairwise comparison of plant type and soil nutrient history (fertilization).

Comparison	**Z**	**P.unadj**	**P.adj**
Grass.Fertilized – Grass.Unfertilized	-0.6794	0.4969	1.0000
Grass.Fertilized – Shrub.Fertilized	-2.8307	0.0046	0.0279
Grass.Unfertilized – Shrub.Fertilized	-2.1513	0.0315	0.1887
Grass.Fertilized – Shrub.Unfertilized	-1.6984	0.0894	0.5366
Grass.Unfertilized – Shrub.Unfertilized	-1.0190	0.3082	1.0000
Shrub.Fertilized – Shrub.Unfertilized	1.1323	0.2575	1.0000

Abbreviations: Z = Z score, P.unadj= unadjusted p-value, P.adj = adjusted p-value

Table 4. Nonparametric (Dunn's test) for *Rhus copallinum* based on mesh size (µm) and treatment based on plant nutrient history and soil nutrient history.

Comparison	Z	P.unadj	P.adj
Fertilized (20 µm) – Fertilized (200 µm)	-1.4073	0.1593	0.9560
Fertilized (20 µm) – Unfertilized (20 µm)	1.5832	0.1134	0.6802
Fertilized (200 µm**)** – Unfertilized (20 µm)	2.9648	0.0030	0.0182
Fertilized (20 µm**)** – Unfertilized (200 µm)	0.8644	0.3874	1.0000
Fertilized (200 µm**)** – Unfertilized (200 µm)	2.2123	0.0269	0.1617
Unfertilized (20 µm**)** – Unfertilized (200 µm)	-0.6652	0.5060	1.0000

Abbreviations: Z = Z score, P.unadj= unadjusted p-value, P.adj = adjusted p-value

Table 5. Summary table measuring mass percent loss by comparison of mesh size (μm) with plant species.

Plant species	Mesh Size (μm)	N	Percent Mass Loss	SD	SE	CI
Grass	20	29	26.5103	11.1048	2.0621	4.2240
Grass	200	31	28.1668	12.3057	2.2102	4.5138
Shrub	20	32	20.3297	10.7687	1.9036	3.8825
Shrub	200	29	26.3348	14.1892	2.6349	5.3972

Abbreviations: SD= standard deviation, SE= standard error, CI = confidence interval