Exploring carbon dynamics in remnant and restored open ecosystems of the southeastern United States

by

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Abstract

Open ecosystems (i.e., prairies, savannas, and open woodlands) are often considered global biodiversity hotspots and terrestrial carbon (C) sinks. In the southeastern U.S., these fire-maintained landscapes have declined significantly since European settlement, largely due to conversion to agriculture and pasture, fire exclusion, and woody encroachment, with only a few remnant areas remaining. While open ecosystem restoration is increasing in the region to enhance biodiversity and associated ecosystem services, C storage potential remains under-researched, particularly regarding belowground pools. This thesis explores these dynamics across ecosystem pools (trees and snags, groundlayer vegetation, leaf litter, soil organic layer, mineral soil (0–30 cm depth), and fine and coarse roots) through two complementary studies. The first study quantified C stocks across an 80-year fire-managed mosaic in the Black Belt Prairie, Southeastern Plains, comparing remnant prairies, savannas, and woodlands to recently restored areas as well as closed, degraded woodlands. Remnant prairies contained the greatest total ecosystem C (287.76 Mg C ha^{-1}), significantly outperforming closed woodlands (220.83 Mg C ha^{-1}) and savannas (132.48 Mg C ha^{-1}). Notably, prairies stored the majority of C within mineral soil (279.39 Mg C ha^{-1}), highlighting that while closed systems sequester C primarily in tree biomass, some prairies store considerable belowground C. The second study evaluated restoration trajectories in the Interior Plateau across a time-since-restoration gradient ranging from active cropland and grazing fields to nine years since prairie restoration, using remnants as benchmarks. Total ecosystem C increased from 50.80 Mg C ha^{-1} (early stages) to 71.74 Mg C ha^{-1} at five years, with advanced stages (9 years: 59.66 Mg C ha^{-1}) approaching remnant values (62.81 Mg C ha^{-1}). Aboveground C was dynamic. Groundlayer vegetation C increased from 0.82 to 3.26 Mg C ha^{-1} by 9 years. Conversely, mineral soil C remained stable, suggesting more time or additional factors impact this pool. Collectively, this research highlights that open

ecosystems in the southeastern U.S. can support substantial C stocks, thus providing ecosystem service co-benefits related to biodiversity conservation and C storage.

Artificial Intelligence (AI) Use Disclosure Statement

In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Microsoft 365 Copilot and Grammarly. These tools were used primarily to refine the code used in R and to improve grammar and spelling throughout the text. The author accepts full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

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In the preparation of this thesis, the following digital accessibility tools were used to ensure this document complies with federal requirements: Microsoft Word Accessibility Checker. The author acknowledges full responsibility for the intellectual content of this work and has made a good faith effort to comply with digital accessibility requirements in publishing, wherein the nature of the content does not significantly change in order to do so. Furthermore, all content has been reviewed and revised to meet these requirements prior to final publication.

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List of Abbreviations

%C	Carbon concentration
ANOVA	Analysis of variance
BD	Bulk density
C	Carbon
cm	Centimeters
CR	Coarse roots
CWD	Coarse woody debris
DBH	Diameter at breast height
FR	Fine roots
FWD	Fine woody debris
GAMs	Generalized additive models
GLV	Groundlayer vegetation
h	Hour
LL	Leaf litter
m	Meters
Mg	Megagram
mm	Millimeters
MS	Mineral soil
NMDS	Non-metric multidimensional scaling
PERMANOVA	Permutational multivariate analysis of variance
PFT	Plant functional type
PKP	Pennyroyal Karst Plains

SD	Standard deviation
SGI	Southeastern Grasslands Institute
SOL	Soil organic layer
TN	Tennessee
U.S.	United States
WHR	Western Highland Rim

Chapter 1: Shifts in carbon allocation across fire-maintained ecosystems reveal belowground dominance in remnant prairie systems: evidence from a prairie-woodland mosaic in the Southeastern Plains, United States

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Highlights

- Total ecosystem carbon varied widely but did not increase with canopy cover
- Prairie systems stored the highest carbon despite minimal aboveground biomass
- Mineral soil dominated carbon storage across all ecosystem types
- Soil carbon depth patterns differed, increasing with depth only in prairies
- Soil carbon variation was driven primarily by carbon concentration rather than bulk density

Abstract

Open ecosystems, including prairies, savannas, and woodlands, play a critical role in terrestrial carbon (C) storage, yet their contribution is often underrepresented due to a focus on aboveground biomass in forest-dominated systems. In fire-maintained landscapes, C storage may depend more on allocation among ecosystem pools than on total biomass accumulation. Here, C storage across above- and belowground pools was characterized in a southeastern United States landscape spanning a mosaic of prairie, savanna, and woodland systems under long-term prescribed fire management, as well as degraded woodlands lacking recent fire. We

also evaluated depth-wise distributions of mineral soil (0–30 cm) and fine root C to determine whether variation in soil C was more strongly associated with carbon concentration (%C) or bulk density. Total ecosystem C differed significantly among ecosystem types ($p < 0.001$) but did not increase consistently with canopy cover. Prairie systems contained the greatest total C stocks (287.76 Mg C ha^{-1}), exceeding those of woodlands (156.37 Mg C ha^{-1}) and savannas (132.48 Mg C ha^{-1}), despite minimal aboveground biomass. In contrast, closed woodlands accumulated substantially more aboveground C (142.58 Mg C ha^{-1}) but lower total C (220.83 Mg C ha^{-1}) relative to prairies. Recently restored systems contained much lower total C (~50.64 Mg C ha^{-1}), indicating limited C accumulation and early-stage ecosystem development. Mineral soil C was the dominant C pool across all ecosystem types, with prairie soils containing substantially greater C (279.39 Mg C ha^{-1}) than all other systems (<75 Mg C ha^{-1}). Depth patterns varied significantly among ecosystem types ($p < 0.001$), with mineral soil C increasing with depth in prairies but declining in more closed systems. Fine root C was concentrated on surface soils across all ecosystem types and showed limited differentiation among systems. Variation in mineral soil C was more strongly associated with differences in carbon concentration (%C) than with bulk density, indicating that organic matter inputs and stabilization processes play a larger role than soil physical structure in determining C storage. These findings demonstrate that C storage in fire-maintained open ecosystems is driven by shifts in allocation among pools rather than increases in woody biomass. Open ecosystems can support substantial and persistent C stocks through belowground pathways, highlighting their importance in regional carbon budgets and the need to incorporate grasslands and savannas into carbon management and restoration strategies.

Keywords: Black Belt, Carbon, grasslands, open ecosystems, prescribed fire, Southeastern Plains

1. Introduction

Open ecosystems such as prairies, savannas, and open woodlands (also known as grasslands, Parr et al., 2014; Veldman et al., 2015) dominate ~40% of terrestrial landscapes and remain critical for biodiversity (Scholtz & Twidwell, 2022; Squires & Feng, 2018; J. B. Wilson et al., 2012) and cultural heritage (Fischer et al., 2008; Lamarque et al., 2011) conservation. Additionally, grassland ecosystems collectively store one-third of total terrestrial carbon (Bai & Cotrufo, 2022; Bengtsson et al., 2019; Tariq et al., 2024), with much of this carbon residing belowground in soils and roots rather than in standing biomass (Bardgett et al., 2021a; Jobbágy & Jackson, 2000). In contrast, forests typically store a greater proportion of carbon (C) in aboveground woody biomass, reflecting fundamentally different C allocation strategies among biomes (Pan et al., 2011; Poorter et al., 2012). These distinctions suggest that total ecosystem carbon cannot be inferred solely from aboveground biomass, particularly in open ecosystems where belowground pools may play a dominant role.

Comparative studies across global biomes indicate that variation in carbon storage among ecosystem types is often associated with differences in allocation among pools rather than simply total biomass accumulation. Open ecosystems are generally characterized by greater investment in soil and root pools, whereas forests tend to accumulate carbon in aboveground biomass (Guo & Gifford, 2002; Jackson et al., 2017). However, the extent to which these allocation patterns consistently translate into differences in total ecosystem carbon remains uncertain, particularly across landscapes experiencing varying disturbance regimes. For instance, land-use transitions from grasslands to forests can increase aboveground carbon while producing variable responses in soil C depending on depth, climate, and soil properties (Deng et al., 2016; Guo & Gifford, 2002; Nave et al., 2013). This decoupling between above- and belowground responses highlights that increases in woody biomass do not necessarily translate into proportional gains in total ecosystem carbon.

Disturbance regimes, particularly fire, are central in regulating vegetation structure in grassy biomes and may influence how carbon is partitioned among ecosystem pools. Frequent, low-intensity prescribed fires are commonly associated with the maintenance of open canopy conditions and herbaceous dominance (Bond & Keeley, 2005; Miller et al., 2017; Simpson et al., 2016), whereas reduced fire frequency can promote woody encroachment and canopy closure (Archer et al., 2017; Ratajczak et al., 2014; Twidwell et al., 2016). These structural shifts are expected to alter C allocation pathways, yet the direction and magnitude of these changes are not always predictable. Long-term fire can reduce aboveground biomass while simultaneously affecting soil C through complex interactions among plant inputs, decomposition, and soil organic matter stabilization (Certini, 2005; Pellegrini et al., 2018; Xu et al., 2022). In some cases, fire-induced changes in soil processes, including the formation of pyrogenic carbon, may partially offset losses from biomass combustion, leading to context-dependent outcomes (Santín et al., 2016). As a result, the net effects of fire on total ecosystem C and its distribution among pools remain an active area of investigation.

In the southeastern United States, these dynamics are particularly relevant in mixed oak-pine systems and associated prairies, which historically covered extensive areas but have been substantially reduced due to land-use change and altered fire regimes (Frost, 2007; Noss, 2012; Noss et al., 2015). Fire exclusion has been linked to woody encroachment, canopy closure, and shifts in understory composition (Hanberry, 2021; Kane et al., 2008; Robertson & Hmielowski, 2014; Young & Koerner, 2022), with likely consequences for ecosystem carbon storage and distribution. Although these structural changes are expected to influence C dynamics, relatively few studies have quantified carbon across multiple pools in landscapes that retain a mosaic of remnants, degraded, and restored ecosystems under long-term fire management.

Restoration efforts further highlight these dynamics in this geographical region, where extensive losses of open ecosystems have occurred due to fire exclusion, woody encroachment, and

land-use conversion (Borowy, 2025; Dixon et al., 2021; Hanberry et al., 2014; J. Jones et al., 2007; Southeastern Grasslands Institute, 2025). In response, restoration initiatives have increasingly focused on reestablishing prairie and savanna conditions, particularly within longleaf pine (*Pinus palustris)* ecosystems, through a combination of mechanical tree removal, herbicide application, and the reintroduction of frequent prescribed fire (Brockway et al., 2004; Dixon et al., 2022; Harrington et al., 2013; Robertson et al., 2019; Varner et al., 2005). These management approaches have been shown to recover herbaceous-dominated vegetation structure and biodiversity, including increases in native grasses and forbs diversity following restoration treatments (Dixon et al., 2021; Fill et al., 2021; Hanberry et al., 2018). Despite these advances, relatively little is known about how carbon storage responds across different phases of restoration, particularly belowground. Soil C and root biomass may respond more slowly than aboveground vegetation due to time lags in organic matter inputs, root system development, and soil stabilization processes (Baer et al., 2002; Post & Kwon, 2000). As a result, recently restored systems may differ substantially from remnant ecosystems in both total C storage and its distribution among pools. Understanding how C storage evolves along restoration trajectories is especially important in fire-managed landscapes, where mosaics of remnant, degraded, and recently restored ecosystems coexist, yet C dynamics across these states remain poorly quantified.

Given these differences across ecosystem types and restoration stages, the vertical distribution of soil carbon represents a critical but less well-constrained component of carbon dynamics. Mineral soil is the largest terrestrial carbon pool, yet its distribution with depth varies widely across ecosystems and is often underrepresented (Jackson et al., 2017; Jobbágy & Jackson, 2000; Rumpel & Kögel-Knabner, 2011). In grass-dominated systems, deep rooting by perennial species has been proposed as an important mechanism for transferring C into subsoil layers, potentially enhancing long-term carbon storage (Conant et al., 2017; Liebig et al., 2005;

Sprunger et al., 2017). However, the extent to which depth-related C patterns differ among ecosystem types and how they are influenced by disturbance history remains unclear (Slessarev et al., 2026).

Uncertainty also persists regarding the controls over soil C stocks, particularly the relative influence of carbon concentration (%C) and bulk density (BD). Although both parameters are essential components of soil C calculations, several studies suggest that variation in %C may explain a large proportion of differences in soil C across ecosystems (Périé & Ouimet, 2008; Yang et al., 2016). Additionally, soil texture, mineralogy, and aggregation processes can interact with these factors to regulate carbon stabilization and persistence (Ruehlmann & Körschens, 2020; Six et al., 2000). Disentangling these controls is necessary for improving predictions of soil carbon dynamics across heterogeneous landscapes. Despite advances in understanding carbon cycling across ecosystems, there remains a need for integrative studies that quantify carbon across multiple pools while explicitly accounting for variation in ecosystem structure and disturbance history. Fire-maintained landscapes that encompass a gradient from open prairies to closed woodlands and degraded systems provide a unique opportunity to examine how C storage and allocation respond to these factors within a shared environmental context.

Here, C storage across aboveground and belowground pools was quantified in a fire-managed landscape at Sumter Farm in the Southeastern Plains, United States, characterized by a mosaic of prairie, savanna, woodland, and degraded ecosystems. The following research questions were addressed in this research: (1) how does carbon storage (total, above- and belowground, and among ecosystem pools) differ between ecosystem types?, (2) does the depth-wise distribution of mineral soil and fine root C vary across ecosystem types?, and (3) how do patterns of mineral soil C vary in relation to %C and BD across ecosystem types and soil depth?

Based on existing theory and empirical evidence, the following hypotheses were tested: (1) total ecosystem C will vary among ecosystem types primarily through shifts in allocation among pools rather than a consistent increase with canopy cover, with open systems supporting greater relative contributions from belowground pools; (2) mineral soil C will exhibit ecosystem-specific depth distributions, with prairie systems supporting greater C accumulation to more closed systems; and (3) variation in mineral soil C will more closely reflect patterns in carbon concentration than bulk density across ecosystem types and soil depths. The integration of pool-specific and depth-explicit measurements aimed to advance understanding of C storage dynamics in fire-maintained open ecosystems and their role in broader carbon stocks.

2. Methods

2.1. Study area description

The study was conducted within the Southeastern Plains ecoregion of the southeastern United States, which extends from the Atlantic Coastal Plain to the Mississippi River Valley and is characterized by rolling topography underlain by sandy, silty, and clay-rich sediments (Shaw et al., 2010; Sohl, 2016). Soils are predominantly Ultisols with localized occurrences of Entisols and Alfisols (Shaw et al., 2010; Soil Survey Staff, 2022; Wiken et al., 2011) and generally low to moderate fertility. Moreover, the region experiences a humid subtropical climate with a mean annual temperature of ~19 °C and precipitation distributed throughout the year (NOAA, 2026). This landscape was historically maintained by frequent, low-intensity fire regimes that supported open pine savannas, oak–hickory woodlands, bottomland hardwood clusters, and several dry prairie patches (Sohl, 2016). However, extensive agricultural and silvicultural conversion post-European settlement has resulted in a highly fragmented landscape dominated by croplands, grazing pasture, and managed pine plantations (Brown et al., 2005; Napton et al., 2010).

Within this region, the Black Belt subregion is a distinct physiographic and ecological zone defined by calcareous chalk and marl substrates (Harper, 1943; Swenson et al., 1941) that form

dark, clay-rich soils with high fertility and water-holding capacity (Campbell & Seymour, 2011). These edaphic conditions historically supported prairie, savanna, and oak woodland ecosystems dominated by warm-season grasses (*A. gerardii*, *S. scoparium*), along with diverse forb communities (*S. laciniatum*, *S.integrifolium*, *R. columnifera*), and several trees species (*C. ovata*, *L. styraciflua*, *P. echinata, Q. falcata, Q.marilandica*, *Q. muehlenbergii*,, *Q. stellata*, *Q. velutina,*) [Barone & Hill, 2007; Campbell & Seymour, 2011; DeSelm & Murdock, 1993; Hill et al., 2009]. Despite their historical extent, these ecosystems have undergone extensive conversion to cropland and livestock activities due to the high agricultural value of Black Belt soils (Napton et al., 2010; Purifoy, 2022), and remaining natural communities are now rare and fragmented, persisting primarily as remnants or managed systems.

The study site, Sumter Farm & Co. (SF; 32°52′7.48″N, 88°18′9.19″W), is an approximately 4,450 ha (11,000-acre) property located near Geiger, Alabama, within the Black Belt subregion (Fig. 1). The property contains a mosaic of remnant prairies, savannas, and woodlands that have been actively managed with frequent prescribed fire for several decades (~80 years). These systems have never been plowed and retain high-quality native ground-layer vegetation, previously assessed by the Southeastern Grasslands Institute, indicating strong ecological continuity with pre-settlement conditions. The long-term low-intensity surface fire regime has maintained open-canopy structure, limited woody encroachment, and promoted herbaceous-dominated ground layers across much of the site. These remnant systems occur alongside areas that experienced historical timber production, including pine planting (*P. taeda*) and periods of fire exclusion, resulting in a gradient of conditions from relatively intact ecosystems to degraded woodlands. Degraded areas are generally characterized by younger loblolly pine-dominated stands (~25–40 years old), simplified vertical structure, and reduced ground-layer diversity, although some recovery is currently occurring under renewed fire management. This combination of long-term prescribed fire, remnant ecosystem persistence, and variable land-use

history provides a unique framework for evaluating carbon storage across a gradient of ecosystem structure and condition within a fire-maintained landscape.

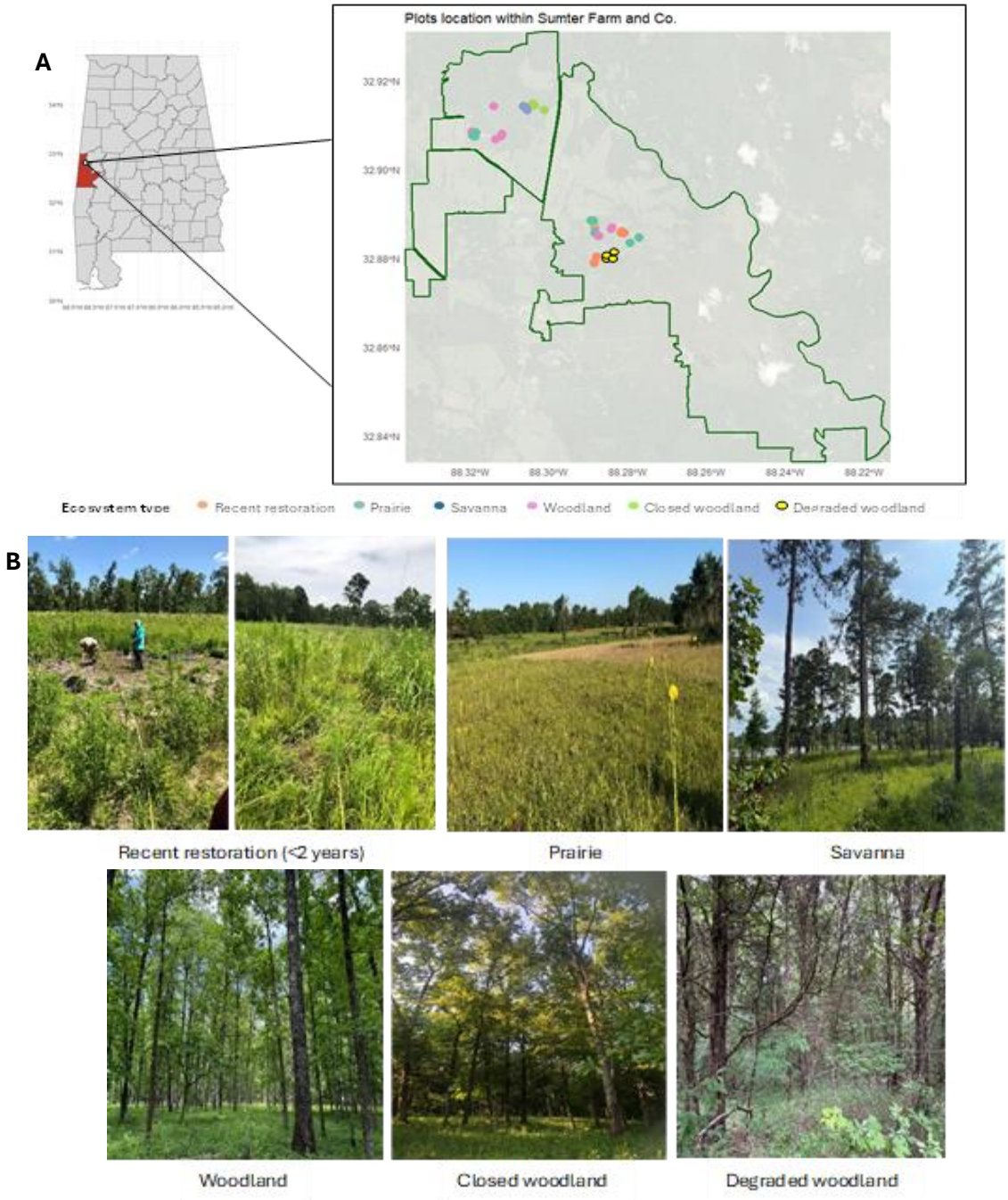

Figure 1. **Study area, sampling design, and ecosystem types at Sumter Farm, Alabama, USA**. (A) Location of Sumter Farm within Sumter County, Alabama, with sampling plot locations within the property, with points colored by ecosystem type: recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland. (B) Representative photographs of each ecosystem type, including recent restoration (<2 years), prairie, savanna, woodland, closed woodland, and degraded woodland. Plot locations were derived from GPS coordinates and overlaid on high-resolution imagery. Background imagery was adjusted for visual clarity.

2.2. Study design

Plot locations at Sumter Farm were determined using a combination of preliminary field exploration and satellite imagery in Google Earth Pro. In the field, a total of 38 plots were established during the peak growing season in 2024, representing a gradient of ecosystem states from prairie to woodland. Plots were located at least 50 meters apart when within the same sampling area and consisted of three 15 m transects extending from the plot center at fixed directions (0° N, 135° SE, and 225° SW), which served as the basis for all subsequent measurements and sampling. Minor adjustments to plot locations were made in the field relative to their initial placement based on satellite imagery when local conditions (e.g., polygon size, vegetation structure, overstory composition) differed from expectations. Average canopy cover was used to assign plots to ecosystem structural categories based on canopy closure thresholds (Dey et al., 2017; Nelson, 2010): prairie (<10%), savanna (10–30%), woodland (30–80%), and closed woodland (>80%). A degraded woodland category was also included to represent systems with canopy cover >30% that exhibited altered structure and composition, such as dominance by loblolly pine, relatively uniform age structure, or evidence of past management, distinguishing them from remnant woodland conditions. Additionally, recent restoration plots were taken into consideration due to the recent management interventions (tree removal and prescribed fire reintroduction) in areas intended to transition previously closed systems toward open ecosystem conditions but had not yet developed the structural or compositional characteristics of either prairies or savannas.

2.3. Carbon sampling

To examine how carbon dynamics respond in open remnant ecosystems characterized by a long history of prescribed fire, several ecosystem pools were measured to quantify C at above- and belowground levels (Lal, 2005), including live trees and snags, downed woody debris, ground-layer vegetation (GLV), leaf litter (LL), soil organic layer (SOL), mineral soil (MS), and fine and coarse roots. To estimate C stored in trees and snags, if present, stand inventories

were conducted to characterize tree composition and structure within each plot (Lund & Thomas, 1989). Diameter at breast height (DBH; 1.37 m aboveground) was measured for live and dead overstory trees (≥ 20 cm DBH) within a 10 m radius, midstory trees (10.1–19.9 cm DBH) within a 5 m radius, and saplings (0.1–10 cm DBH) within a 2 m radius using a DBH tape or calipers. Trees were measured systematically across plot sections defined by transect orientation to ensure full coverage and avoid double-counting. Distances from the plot center were measured when necessary to confirm inclusion within the specified sampling radius. Snags, or remaining dead standing tree material, were assessed by estimating the percentage of remaining biomass using nearby trees of comparable size as reference. Species identities were recorded, and when it was not possible to do so, classification was made at the genus level or by wood type (hardwood or softwood).

Woody debris (fine and coarse; FWD+CWD) was quantified along each transect using the planar intercept method (Brown, 1974), where each piece intersecting the transect line was recorded once. Fine woody debris (FWD) measurements began at the 2 m mark using a standardized diameter gauge and were categorized into fuel time-lag classes based on diameter (1-hour: 0.00–0.64 cm; 10-hour: 0.65–2.54 cm; 100-hour: 2.55–7.62 cm). The 1- and 10-hour classes were tallied between the 2–4 m marks, and the 100-hour class between the 2–7 m marks. Coarse woody debris (CWD; 1000-hour: >7.62 cm diameter) was measured from the 2 m point to the end of each transect (15 m). Diameter and decay class (1–5; Lutes et al., 2006) were recorded for each piece, along with identification when possible, and notes were taken regarding origin (i.e., natural vs. anthropogenic).

Ground-layer vegetation (GLV) sampling was conducted within 0.25-m² quadrats (50 cm × 50 cm) located 1 m from the 9 m point along each transect. When the designated location was obstructed due to the presence of a large tree or woody debris, quadrats were repositioned to the nearest representative location. All live and standing dead vegetation (<1.37 m height) was

clipped at the soil surface and collected, excluding detached litter to isolate actively rooted biomass. Three GLV samples were collected per plot and transported for laboratory processing. Later, depth measurements for accumulated leaf litter and the soil organic layer (loose top horizon material at varying stages of decomposition) were taken using 0.0625 m² quadrats (25 cm × 25 cm) located at 5, 10, and 15 m along each transect. LL depth was measured first by inserting a ruler vertically until reaching the SOL boundary. The SOL was then measured at the same point by inserting the ruler through organic material to the mineral soil surface.

Belowground pools were quantified across Mineral soil horizons and root size classes. MS and FR (< 2mm diameter; McCormack et al., 2015) sampling was conducted at the 9 m point along each transect following removal of LL and SOL. A metal soil corer (6.35 cm diameter) was used to extract cores to a target depth of 30 cm. Each core was subdivided into three depth intervals (0–10, 10–20, and 20–30 cm), resulting in nine samples per plot. When obstacles prevented full coring depth, an alternative nearby sampling attempt was made; if the problem persists, the maximum depth achieved through coring was recorded and considered in next C calculation steps. Coarse root biomass was quantified using soil monoliths (30 cm × 30 cm × 30 cm) dug along each transect following GLV sampling. Monolith depth was recorded at each corner to account for variability and bulk density (BD) estimation. CR were treated as roots exceeding 2 mm in diameter (Arunachalam et al., 1996; Ling et al., 2025) including rhizomes, stolons, and geoxyles (Milton et al., 2026). Samples were transported to the laboratory and stored at −20 °C until processing.

2.4. Laboratory sampling processing

Following field collection, samples were transported to the laboratory and processed using workflows designed to translate structurally diverse materials into comparable C estimates across ecosystem pools. Ground-layer vegetation samples were processed by first partitioning biomass into five plant functional type groups: woody vegetation, graminoids, forbs, vines, and

mosses. Woody vegetation included tree seedlings and woody shrubs, graminoids comprised grasses, sedges, and rushes, and forbs encompassed non-woody herbaceous broadleaf species. Vines consisted of climbing or trailing stem plants, while mosses included non-vascular bryophytes occurring at the soil surface. Separating GLV biomass into functional groups allowed carbon contributions from structurally and ecologically distinct components of the ground layer to be quantified independently. Once sorted, material from each functional group was transferred into newly labeled paper bags that retained original sample identifiers while adding functional group information. Samples were then oven-dried at 60 °C for 48 hours to constant mass. After drying, samples were briefly equilibrated to room temperature to minimize moisture reabsorption prior to weighing. Dry biomass was determined using tared aluminum tins, and weights were recorded immediately. Following weighing, samples were reorganized by plot and stored for archival purposes.

Mineral soil samples and monolith-derived materials were processed following standardized laboratory procedures. Upon return from the field, samples were thawed and air-dried (~48 h) prior to further handling. MS was subsequently passed through 2 mm and 1 mm metal sieves to separate the fine soil fraction from rock fragments, while simultaneously allowing for the manual recovery of FR. The processed soil fraction was then transferred to aluminum containers and dried at 105 °C for a minimum of 48 hours to determine dry mass. For C analysis, a representative subsample (~3–5 g) was obtained from each dried soil sample and homogenized to a fine powder using a SPEX SamplePrep 8000D Mixer/Mill. Ground samples were placed into labeled vials and submitted to the University of Georgia Agricultural and Environmental Services Laboratories for determination of total organic carbon (% of dry mass) via high-temperature combustion (Elementar Vario Max, Langenselbold, Germany).

Fine roots recovered during sieving were rinsed thoroughly with water to remove adhered soil particles and oven-dried at 60 °C for approximately 48 h prior to biomass determination. Coarse

roots were separated from soil monolith samples, washed to remove residual soil, and dried under similar conditions (60 °C, 48 h) before dry mass was recorded. Coarse root material included all belowground structural components such as rhizomes and other persistent woody tissues. Rock fragments isolated during sieving were used to estimate coarse fraction volume via water displacement, with volume determined from the change in water level following submersion in a graduated cylinder. All belowground processed materials were retained and stored in sealed bags.

2.5. C calculations in above- and below-ground pools

Aboveground carbon stocks for live trees and snags were estimated by first converting field-based diameter measurements into biomass using species- and location-specific allometric equations. Diameter at breast height (DBH) values for sapling, midstory, and overstory individuals were processed using the *allodb* package in R (version 4.4.3), which applies geographically appropriate models to estimate aboveground biomass (Gonzalez-Akre et al., 2022). Resulting biomass estimates were subsequently converted to C units (Mg C ha^{-1}) using species-specific C concentration factors derived from the Forest Inventory and Analysis (FIA) Database, following the approach of Doraisami et al. (2022).

The downed woody debris C pool was estimated using a combination of approaches depending on fuel class. FWD biomass was calculated from transect-based counts using the planar intercept method and standardized equations developed by Brown (1974). These calculations incorporated fuel-class-specific average diameters by fuel classes (inches; 1-hr: 0.0151, 10-hr: 0.289, 100-hr: 2.76), specific gravity suggested by Anderson (1978): 0.70 for 1-hr and 10-hr fuels, and 0.58 for 100-hr fuels, and correction factors accounting for transect angle and slope, set to 1 because the set plots were mostly fat. Biomass estimates were converted from imperial to metric units and subsequently transformed to carbon using a 0.5 biomass-to-carbon conversion factor (Domke et al., 2012; Smith et al., 2006). Coarse woody debris (1000-hr fuels)

required additional adjustments to incorporate structural and compositional variability. Carbon estimates for CWD were derived using species-specific wood density values, decay class modifiers, and C concentration parameters obtained from FIA DataMart (Miles & Smith, 2009; Woodall & Monleon, 2008). In cases where species identification was not possible due to advanced decay or fire damage, plot-level weighted averages of overstory species characteristics (wood density, C content, and decay factors) were applied to estimate C stocks (Milton et al., 2026). Carbon estimates from FWD and CWD were combined to represent a single woody debris pool.

Ground-layer vegetation carbon derived from biomass estimates standardized to unit area. Dry mass measured within each quadrat was expressed on an area basis (g m^{-2}) by dividing by the quadrat surface area (0.25 m^2) and subsequently converted to Mg ha^{-1}. Carbon content was then estimated using a fixed biomass-to-carbon conversion factor of 0.5 (Johnson et al., 2017) . Carbon stored in surface pools (LL and SOL) was estimated using depth-based empirical models (Shrestha et al., unpublished data), where carbon storage is directly predicted as a function of average layer depth (AD):

$$\mathrm{C}_{LL} = 1.6 \times (AD_{LL})^{0.8}$$

$$\mathrm{C}_{SOL} = 4.5 \times (AD_{SOL})^{1.1}$$

These models implicitly incorporate bulk density and carbon concentration; therefore, no additional carbon conversion factors were applied. Carbon estimates for LL and SOL were summed to represent a combined surface carbon pool (LL + SOL).

Mineral soil C storage was estimated by integrating bulk density, carbon concentration (%C), and sampling depth. BD was calculated as the ratio of oven-dry mass to sample volume and converted to kg m^{-3}. These values were combined with measured %C and depth increments (0.10 m) to estimate C stocks on an area basis (kg ha^{-1}), which were subsequently converted to

Mg ha^{-1}. If BD values exceed typical mineral soil particle densities (>2.5 g cm^{-3}; Ruehlmann & Körschens, 2020), they were replaced (1% total samples) with mean values from the corresponding depth interval within each plot. When sampling depth was incomplete due to subsurface constraints, C values were scaled proportionally to the target depth in each soil horizon (0.10 m). Missing depth increments due to compaction or sampling limitations were estimated using mean BD and C concentration values from the same depth interval on other transects.

Fine root C was estimated using pool-specific bulk density values and a fixed sampling depth of 0.10 m. Carbon content was derived using a conversion factor of 45.6% (Milton et al., 2026). Estimates were standardized to Mg ha^{-1}. When soil cores did not reach the target depth, C stocks were scaled proportionally, and missing depth increments were estimated using the mean value for the corresponding depth interval. Coarse root C was estimated using two complementary approaches to capture different structural components of belowground biomass. Coarse root biomass associated with understory vegetation was quantified from soil monoliths using measured BD and a fixed sampling depth of 0.30 m, with carbon derived using the same conversion factor applied to fine roots. To account for larger structural roots associated with trees in forested systems (canopy cover > 10%), CR biomass was additionally estimated using the allometric relationship proposed by Mokany et al. (2006), which relates aboveground biomass to belowground root biomass:

$$y = 0.489\, x^{0.890}$$

where X represents aboveground tree biomass. This approach captures coarse root biomass not effectively sampled by soil monoliths. Final coarse root C estimates represent the integration of both measured (monolith-based) and modeled (allometric) components. All values were converted to Mg C ha^{-1} for consistency across pools.

2.6. Data analysis

All statistical analyses were conducted in R version 4.4.3 (R Core Team, 2025). To evaluate differences in carbon storage across ecosystem types, linear models with ecosystem type as a fixed factor were used for total C, aboveground and belowground C, and individual ecosystem pools (trees + snags, woody debris, ground-layer vegetation, leaf litter + soil organic layer, mineral soil, fine roots, and coarse roots). For these analyses, carbon pools were aggregated to the plot level, such that each plot represented a single independent observational unit. Because no repeated or hierarchical structure remained in these aggregated data, random effects were not included in these models. Response variables were log-transformed when necessary to meet assumptions of normality and homoscedasticity. Model assumptions were assessed using visual inspection of residuals and diagnostic tests implemented in the *performance* package (Lüdecke et al., 2021). Post-hoc pairwise comparisons among ecosystem types were conducted using estimated marginal means with Tukey-adjusted p-values for multiple comparisons, implemented in the *emmeans* package (Lenth & Piaskowski, 2017).

To assess depth-wise variation in carbon pools, linear mixed-effects models were used with ecosystem type, soil depth (0–10, 10–20, 20–30 cm), and their interaction as fixed effects. In these analyses, plot was included as a random effect to account for the hierarchical structure of the data, as multiple soil horizon samples were collected within the same plot along transects. This random effect accounts for the non-independence of depth-specific observations within plots. Separate models were fitted for mineral soil and fine root C. Additionally, to examine variation in mineral soil properties associated with C patterns, we analyzed carbon concentration and bulk density as response variables using similar mixed-effects modeling approaches, including ecosystem type, depth, and their interaction as fixed effects and plot as a random effect. For all models, the significance of fixed effects was assessed using analysis of

variance (ANOVA), and model fit was evaluated using marginal and conditional R^2 values. Statistical significance was determined at $\alpha = 0.05$.

3. Results

3.1. Carbon storage across ecosystem types and pools

Carbon storage differed significantly among ecosystem types across all measured pools (Table 1; Figures 2–3). Total ecosystem C varied widely among ecosystem types ($p < 0.001$), spanning more than a fivefold range. Prairie systems exhibited the greatest total C stocks (287.76 Mg C ha^{-1}), exceeding all other ecosystem types. Closed woodlands (220.83 Mg C ha^{-1}) also supported relatively high total carbon, whereas woodlands (156.37 Mg C ha^{-1}) and savannas (132.48 Mg C ha^{-1}) exhibited intermediate and broadly similar values. Degraded woodlands (117.82 Mg C ha^{-1}) and recent restoration sites (50.64 Mg C ha^{-1}) stored the least total carbon. Differences in total carbon were primarily driven by variation in belowground pools (Figure 2b).

Aboveground C differed among ecosystem types ($p < 0.001$; Figure 2a). Closed woodlands stored the greatest aboveground C (142.58 Mg C ha^{-1}), followed by woodlands (99.61 Mg C ha^{-1}), savannas (77.24 Mg C ha^{-1}), and degraded woodlands (66.38 Mg C ha^{-1}), which showed broadly similar values. In contrast, prairies (1.90 Mg C ha^{-1}) and recent restoration sites (3.56 Mg C ha^{-1}) contained minimal aboveground C stored relative to all other ecosystem types. Variation in aboveground C was largely driven by tree and snag biomass ($p < 0.001$; Figure 3a), which increased from near-zero values in prairies and recent restoration sites (0.00–0.68 Mg C ha^{-1}) to substantially greater values in woodlands and closed woodlands (87.56–137.98 Mg C ha^{-1} respectively). FWD + CWD also varied among ecosystem types ($p < 0.001$; Figure 3b), with the greatest loads observed in woodlands (10.08 Mg C ha^{-1}), while prairies (0.02 Mg C ha^{-1}) and degraded woodlands (0.21 Mg C ha^{-1}) contained comparatively

little C in this pool. Closed woodlands (1.58 Mg C ha^{-1}) and recent restoration systems (1.27 Mg C ha^{-1}) also exhibited relatively low woody debris compared to woodlands. Similarly, C stored in LL + SOL differed among ecosystem types ($p < 0.001$; Figure 3d). Degraded woodlands (5.24 Mg C ha^{-1}) stored the greatest amounts, followed by closed woodlands (2.49 Mg C ha^{-1}). Savannas (1.22 Mg C ha^{-1}), woodlands (1.06 Mg C ha^{-1}), and recent restoration systems (1.01 Mg C ha^{-1}) exhibited similar values, whereas prairie systems contained no measurable carbon in this pool (0.00 Mg C ha^{-1}).

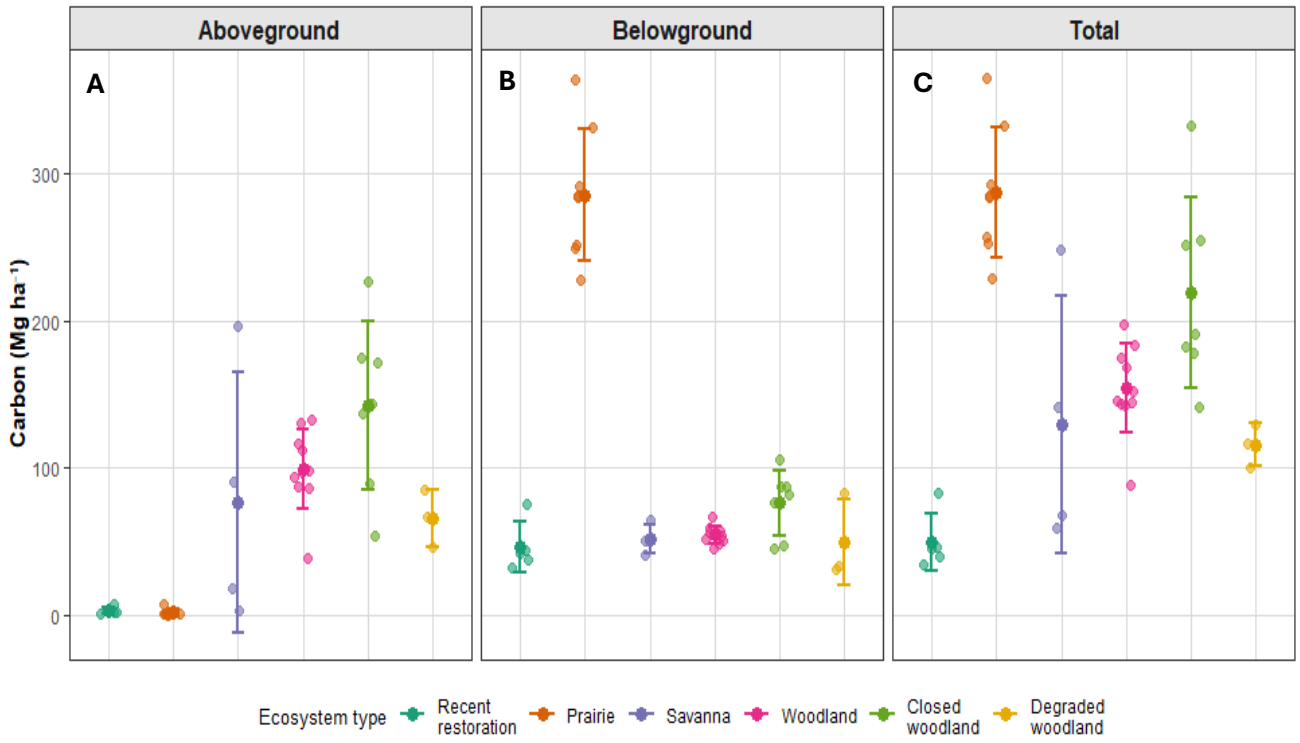

Figure 2. **Aboveground (A), belowground (B), and total (C) carbon stored (Mg C ha^{-1}) across ecosystem types at Sumter Farm, Alabama.** Points represent individual plot observations, while larger points with error bars indicate mean ± standard deviation for each ecosystem type. Ecosystem categories include recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland.

Additionally, ground-layer vegetation C varied among ecosystem types ($p = 0.002$; Figure 3c), although differences were smaller in magnitude than other pools. GLV carbon was highest in savannas (1.57 Mg C ha^{-1}), prairies (1.20 Mg C ha^{-1}), and degraded woodlands (1.45 Mg C ha^{-1}),

while closed woodlands had the lowest values (0.53 Mg C ha^{-1}). Woodland (0.91 Mg C ha^{-1}) and recent restoration systems (1.28 Mg C ha^{-1}) fall within an intermediate range. Differences in GLV carbon were associated with variation in functional group composition (Table S1, Figure S1). Graminoids dominated GLV carbon in prairies, contributing 68.63% of total GLV C (0.825 Mg C ha^{-1}), whereas their relative contribution declined in more wooded systems, including closed woodlands (32.95%, 0.174 Mg C ha^{-1}) and woodlands (33.68%; 0.308 Mg C ha^{-1}). In contrast, woody vegetation contributed substantially greater proportions of GLV carbon in savannas (40.59%; 0.637 Mg C ha^{-1}) and degraded woodlands (31.11%; 0.451 Mg C ha^{-1}), while remaining a minor component in prairies (3.94%; 0.047 Mg C ha^{-1}). Forbs were the dominant component in recent restoration systems, accounting for 49.53% of GLV carbon (0.635 Mg C ha^{-1}), and remained an important contributor across all ecosystem types, although their relative contribution declined in savannas (15.36%, 0.241 Mg C ha^{-1}) and degraded woodlands (11.58%; 0.168 Mg C ha^{-1}). In closed woodlands, forbs comprised a relatively large proportion of GLV C (39.68%; 0.210 Mg C ha^{-1}) despite low total GLV biomass.

Moreover, vines exhibited a clear increase in relative contribution with increasing woody structure, contributing minimally in open systems such as prairies (0.81%; 0.01 Mg C ha^{-1}) and recent restoration (1.48%; 0.019 Mg C ha^{-1}), but representing a larger proportion of GLV carbon in woodlands (15.63%; 0.143 Mg C ha^{-1}) and degraded woodlands (16.97%; 0.246 Mg C ha^{-1}). Moderate C contributions from this functional group were also observed in closed woodlands (13.28%; 0.07 Mg C ha^{-1}). Last, moss contributions to GLV carbon were negligible across all ecosystem types, with values near zero in both absolute and proportional terms. Across all ecosystem types, total GLV C reflected these shifts in functional group composition, with prairies and savannas characterized by greater contributions from graminoids and woody species, whereas forbs dominated recent restoration systems.

Belowground C differed strongly among ecosystem types ($p < 0.001$; Figure 2b). Prairie systems supported substantially greater belowground C (285.86 Mg C ha^{-1}) than all other ecosystems. In contrast, savannas (52.24 Mg C ha^{-1}), woodlands (56.76 Mg C ha^{-1}), degraded woodlands (51.44 Mg C ha^{-1}), and closed woodlands (78.25 Mg C ha^{-1}) exhibited lower and broadly comparable values. Mineral soil C was the dominant contributor to belowground C ($p < 0.001$; Figure 3e). Prairies contained markedly greater mineral soil C (279.39 Mg C ha^{-1}) than all other ecosystems. Closed woodlands (73.08 Mg C ha^{-1}) showed intermediate values. In contrast, recent restoration (44.22 Mg C ha^{-1}), savanna (49.48 Mg C ha^{-1}), woodland (52.55 Mg C ha^{-1}), and degraded woodland (47.76 Mg C ha^{-1}) systems were relatively similar. Fine root carbon also differed among ecosystem types ($p < 0.001$; Figure 3f), with prairies (6.07 Mg C ha^{-1}) exceeding all other systems, which clustered within a narrower range (1.84–3.18 Mg C ha^{-1}). Coarse root C showed moderate variation among ecosystem types ($p = 0.005$; Figure 3g), with higher values in savannas (3.18 Mg C ha^{-1}) and woodlands (2.08 Mg C ha^{-1}) relative to prairies (0.40 Mg C ha^{-1}), while the remaining ecosystem types fell within an intermediate range.

Across ecosystem types, the proportional contribution of C pools differed substantially. Belowground C dominated total ecosystem C in prairies and recent restoration systems, whereas aboveground pools represented a progressively larger proportion of total C in savannas, woodlands, and closed woodlands (Figures 2–3).

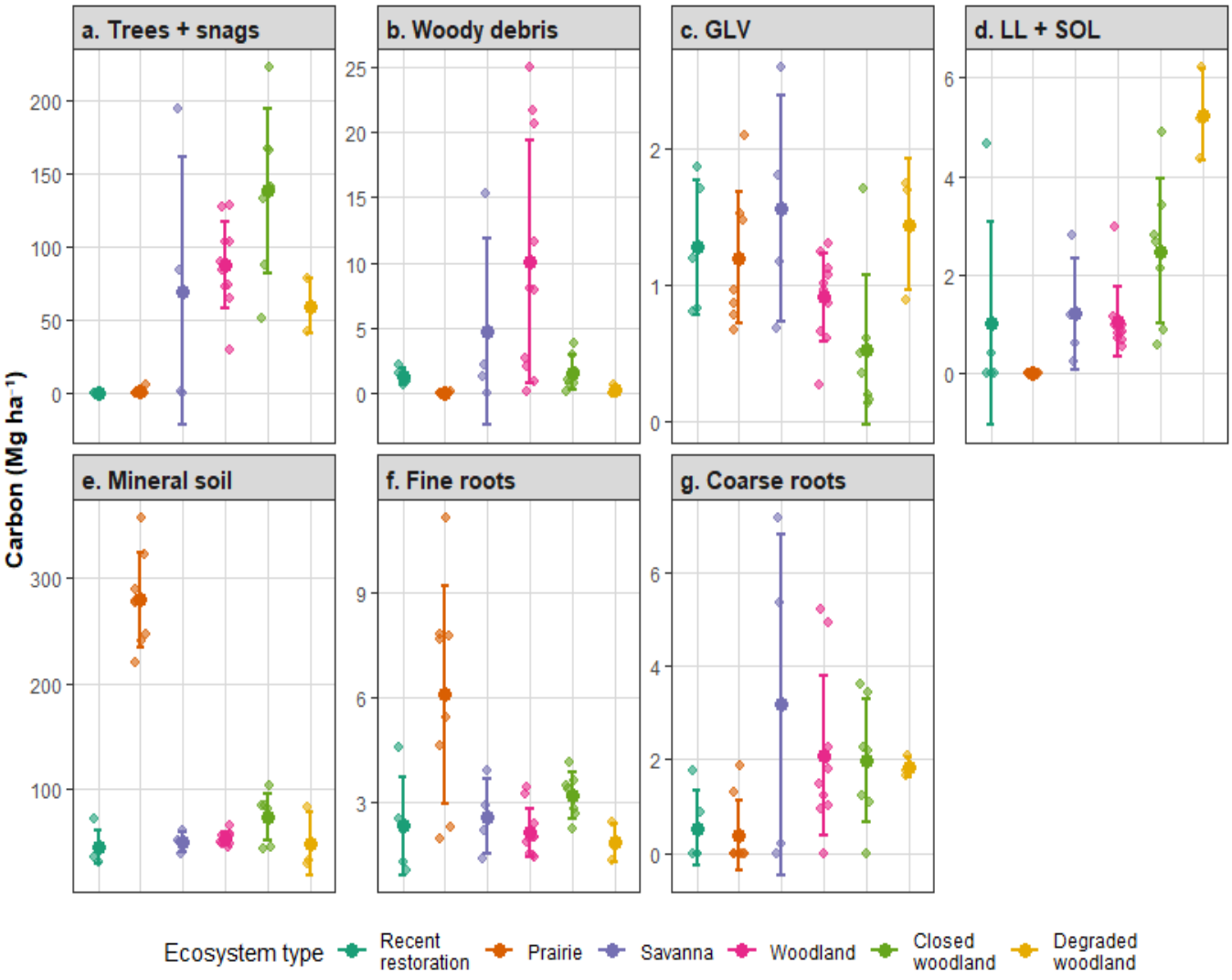

Figure 3. **Carbon stored (Mg C ha⁻¹) across ecosystem types partitioned into ecosystem pools:** (a) trees and snags, (b) woody debris, (c) ground-layer vegetation [GLV], (d) leaf litter and soil organic layer [LL + SOL], (e) mineral soil (0–30 cm), (f) fine roots, and (g) coarse roots. Points represent individual plot values; arger symbols and error bars denote means ± SD.

Table 1. **Carbon stored (mean ± SD; Mg C ha^{-1}) across ecosystem types at Sumter Farm, Alabama, partitioned into aboveground and belowground carbon pools**. Aboveground pools include trees and snags, woody debris (coarse, CWD; fine, FWD), ground-layer vegetation (GLV), and leaf litter plus the soil organic layer (LL + SOL), whereas belowground pools include mineral soil (0–30 cm) and coarse and fine roots. Total C represents the sum of all ecosystem pools. Ecosystem types include recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland. Different letters indicate significant differences among ecosystem types within each pool based on Tukey's HSD post hoc comparisons ($p < 0.05$). F- and p-values are from one-way ANOVA testing the effect of ecosystem type on each carbon pool. Bold p-values indicate $p < 0.05$.

	Recent restoration	Prairies	Savannas	Woodlands	Closed woodlands	Degraded woodlands	F-value	p-value
Trees + snags	0.00 (0.00)	0.68^{a} (1.92)	69.77^{b} (91.56)	87.56^{bc} (29.91)	137.98^{c} (56.17)	59.49^{bc} (18.64)	36.78	**< 0.001**
Woody debris (FWD + CWD)	1.27 (0.64)	0.02^{a} (0.06)	4.69^{bc} (7.12)	10.08^{c} (9.31)	1.58^{c} (1.30)	0.21^{ab} (0.36)	15.27	**< 0.001**
GLV	1.28 (0.49)	1.20^{b} (0.48)	1.57^{b} (0.83)	0.91^{ab} (0.32)	0.53^{a} (0.55)	1.45^{b} (0.48)	5.04	**0.002**
LL + SOL	1.01 (2.05)	0.00^{a} (0.00)	1.22^{b} (1.12)	1.06^{b} (0.70)	2.49^{b} (1.47)	5.24^{b} (0.93)	26.00	**< 0.001**
Mineral soil	44.22 (15.87)	279.39^{c} (45.28)	49.48^{ab} (9.21)	52.55^{ab} (6.04)	73.08^{b} (22.15)	47.76^{ab} (29.73)	52.58	**< 0.001**
Fine roots	2.32 (1.40)	6.07^{b} (3.11)	2.58^{a} (1.07)	2.12^{a} (0.70)	3.18^{a} (0.65)	1.84^{a} (0.55)	6.33	**< 0.001**
Coarse roots	0.53 (0.79)	0.40^{a} (0.75)	3.18^{ab} (3.63)	2.08^{b} (1.69)	1.98^{b} (1.30)	1.84^{ab} (0.22)	4.26	**0.005**
Aboveground	3.56 (2.39)	1.90^{a} (2.36)	77.24^{b} (88.51)	99.61^{b} (27.07)	142.58^{b} (57.09)	66.38^{b} (19.44)	44.48	**< 0.001**
Belowground	47.08 (17.67)	285.86^{b} (44.60)	52.24^{a} (8.04)	56.76^{a} (6.28)	78.25^{a} (22.96)	51.44^{a} (29.21)	97.79	**< 0.001**
Total	50.64 (20.03)	287.76^{d} (43.95)	132.48^{abc} (86.41)	156.37^{bc} (29.72)	220.83^{cd} (64.60)	117.82^{ab} (14.58)	18.71	**< 0.001**

3.2. Depth-wise distribution of mineral soil and fire root carbon

The vertical distribution of C within mineral soil and fine root pools varied with both ecosystem type and soil depth, although the magnitude and consistency of these effects differed between pools (Table S2; Figure 4). For MS carbon, both ecosystem type ($p < 0.001$) and soil depth ($p < 0.001$) showed strong effects in C stocks, and a significant ecosystem type × depth interaction ($p < 0.001$) indicated that depth-related patterns differed among ecosystem types (Table S3). Across most ecosystem types, mineral soil C generally declined with depth. However, prairie systems showed a contrasting pattern, with carbon values increasing with depth (Figure 4).

At the 0–10 cm interval, mineral soil C differed among ecosystem types (Table S2). Prairie systems contained significantly greater C stocks (74.82 Mg C ha^{-1}) than all other ecosystem types, which were statistically similar and ranged from 23.08 Mg C ha^{-1} in degraded woodlands to 32.33 Mg C ha^{-1} in closed woodlands. Recent restoration, savanna, and woodland systems exhibited relatively narrow and overlapping ranges between ~27 and 30 Mg C ha^{-1}. Variation within ecosystem types was relatively low compared to deeper layers, as indicated by smaller standard deviations (Figure 4, top left panel).

Differences among ecosystem types at the 10–20 cm MS depth became more pronounced. Prairie systems maintained the greatest C stored (89.93 Mg C ha^{-1}) in this soil layer and were significantly different from all other ecosystem types. Closed woodlands showed intermediate values (22.16 Mg C ha^{-1}) and were significantly greater than recent restoration, savanna, and woodland systems, which remained comparatively low (8.79–12.77 Mg C ha^{-1}) and did not differ among themselves. Degraded woodlands exhibited values (13.72 Mg C ha^{-1}) that overlapped with both lower and intermediate groups (Table S2; Figure 4, middle left panel).

This pattern was further amplified at the 20–30 cm interval. Prairie systems continued to exhibit markedly higher MS carbon (114.64 Mg C ha^{-1}) relative to all other ecosystem types. Closed

woodlands (18.70 Mg C ha^{-1}) remained intermediate, whereas recent restoration, savanna, woodland, and degraded woodland systems (6.46–10.96 Mg C ha^{-1}) exhibited lower and largely overlapping values (Figure 4, bottom left panel). Within these latter ecosystem types, divergences increased slightly relative to surface layers, as reflected by broader distributions of plot-level values in Figure 4 (bottom left panel).

Fine root C displayed more consistent patterns across ecosystem types and a weaker dependence on ecosystem × depth interactions. Both ecosystem types ($p < 0.001$) and depth ($p < 0.001$) significantly influenced carbon storage, whereas the interaction term was not significant ($p = 0.734$), indicating similar vertical trends across ecosystem types (Table S3). This C pool was concentrated in the upper soil layer (0–10 cm) across all ecosystem types, with values ranging from 1.32 Mg C ha^{-1} in degraded woodlands to 3.90 Mg C ha^{-1} in prairies (Table S2; Figure 4). Despite this range, no significant differences among ecosystem types were detected at this depth.

A pronounced decline in fine root C occurred between the 0–10 cm and 10–20 cm layers across all ecosystem types. At 10-20 cm, values ranged from 0.28 Mg C ha^{-1} in recent restoration systems to 0.98 Mg C ha^{-1} in prairies, with most ecosystem types showing overlapping values (Figure 4, middle right panel). Fine root C within the 20-30 cm horizon remained low across all ecosystem types (0.15-1.19 Mg C ha^{-1}). Minimal differentiation was observed at this depth, with most plot-level values tightly clustered near zero (Figure 4, bottom right panel).

Overall, mineral soil C exhibited strong variation with both depth and ecosystem type, with differences among ecosystem types becoming more pronounced at deeper soil layers. In contrast, fine root C showed a consistent depth-dependent decline across all ecosystem types, with limited differentiation among systems and no detectable variation in vertical distribution patterns (Figure 4).

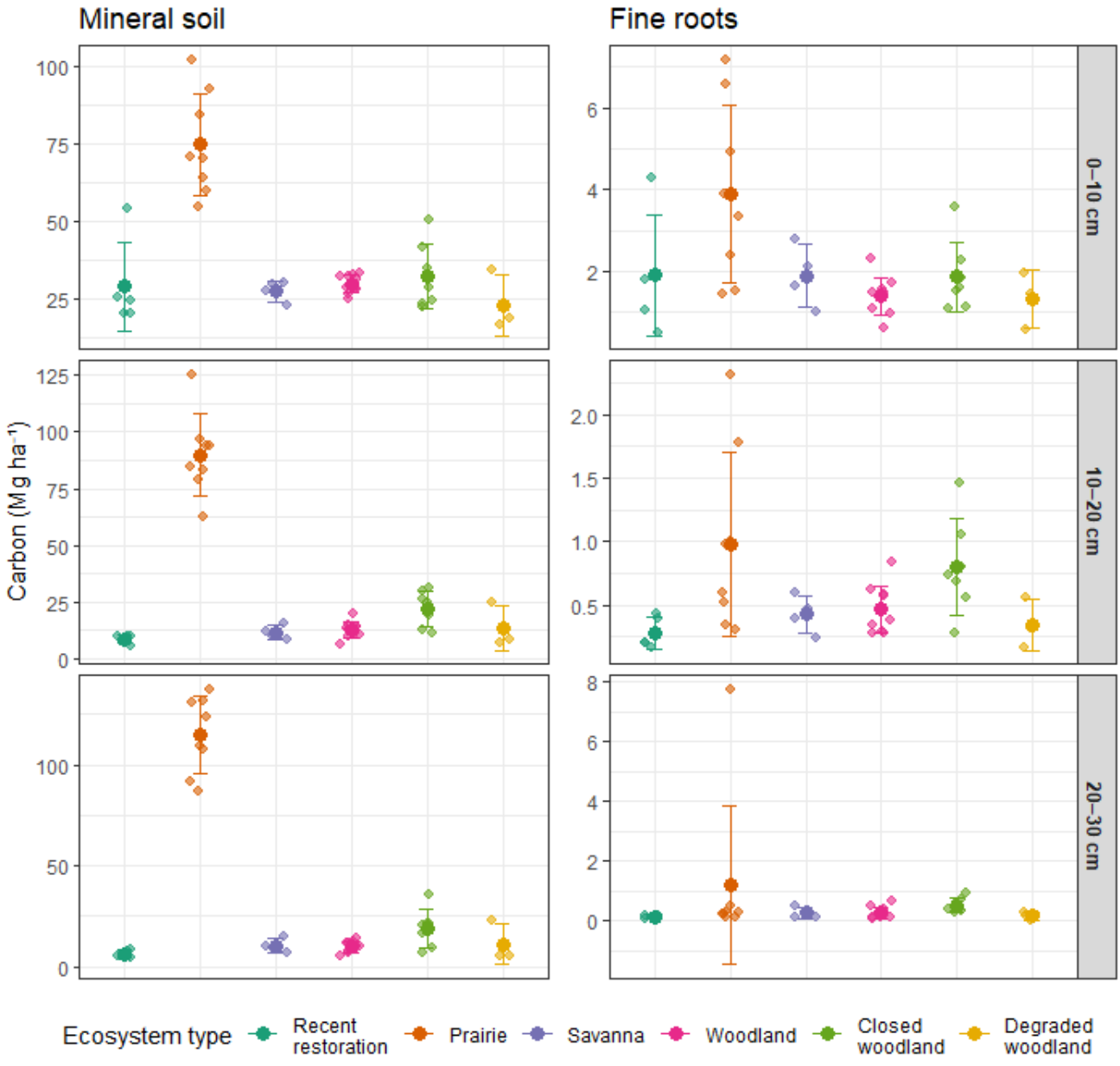

Figure 4. **Depth-specific carbon stored (Mg C ha^{-1}) in mineral soil (left column) and fine roots (right column) across ecosystem types.** Rows represent soil depth intervals (0–10 cm, 10–20 cm, and 20–30 cm). Points indicate individual plot-level observations, while larger symbols and vertical bars represent mean values ±1 standard deviation for each ecosystem type. Ecosystem types include recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland.

3.3. Drivers of mineral soil carbon

Soil carbon concentration and bulk density showed distinct and contrasting patterns across ecosystem types and soil depth intervals (Fig. 5; Tables S4–S5). Soil C concentration differed strongly among ecosystem types at all depths (Table S4), with prairies consistently exhibiting the greatest values throughout the soil profile (0–10 cm: 6.48 %; 10–20 cm: 6.68 %; 20–30 cm: 7.64 %). In contrast, all other ecosystem types (recent restoration, savannas, woodlands, closed woodlands, and degraded woodlands) remained substantially lower and presented broadly similar values within each depth interval. These differences were statistically significant at each depth ($p < 0.001$; Table S4), with prairie consistently distinct from all other systems. The magnitude of separation among ecosystem types increased with depth, as %C declined in non-prairie systems while remaining comparatively high in prairies (Fig. 5; Table S4).

Depth had a strong influence on soil C concentration across ecosystem types (Fig. 5; Table S4). All non-prairie systems exhibited pronounced declines in %C with increasing depth, with restoration (0–10 cm: 2.34; 10–20 cm: 0.50; 20–30 cm: 0.35) and savannas (0–10 cm: 1.92; 10–20 cm: 0.74; 20–30 cm: 0.64) decreased across the mineral soil depths. Woodlands and degraded woodlands followed similar patterns, with values at 20–30 cm consistently below 1% (0.69 and 0.73, respectively) across these systems. Closed woodland showed a comparable but less steep decline (0–10 cm: 2.70, 10-20 cm: 1.50, 20–30 cm: 1.31). However, prairies maintained high %C across all depths, with no comparable decline in magnitude relative to other ecosystem types, resulting in increasing divergence among systems with depth (Fig. 5; Table S4).

Bulk density exhibited lower variability and less consistent differentiation among ecosystem types compared to %C (Table S4; Fig. 5). At 0–10 cm, BD values ranged narrowly from 1.19 to 1.51 g cm^{-3} across all ecosystem types, with weak but statistically significant differences ($p = 0.047$; Table S3), and substantial overlap among systems. Moderate differentiation was

observed in the 10-20 cm interval, with higher BD in recent restoration (1.78 g cm^{-3}) and degraded woodlands (1.83 g cm^{-3}) relative to prairies (1.37 g cm^{-3}), unlike savannas, woodlands, and closed woodlands, which exhibited intermediate values that overlapped broadly. Differences in the 20–30 cm depth interval among ecosystem types were not significant ($p = 0.074$), with BD values converging within a narrow range (1.45–1.88 g cm^{-3}) across all systems (Table S4; Fig. 5).

Depth-related patterns in bulk density were consistent across ecosystem types (Fig. 5; Table S4). BD increased with depth in all systems and to a similar extent across ecosystem types. Prairies increased from 1.19 g cm^{-3} at 0–10 cm to 1.53 g cm^{-3} at 20–30 cm, while recent restoration increased from 1.37 to 1.88 g cm^{-3} over the same interval. Comparable increases were observed in savannas, woodlands, closed woodlands, and degraded woodlands, indicating parallel vertical trends across ecosystem types. As a result, differences among ecosystem types remained relatively small across depth intervals, with similar rates of increase contributing to convergence in bulk density at greater depths.

The magnitude and structure of variation differed markedly between %C and BD (Fig. 5; Table S4). Across depths, %C values spanned more than a sixfold range among ecosystem types (1.53–7.64%), whereas bulk density varied within a much narrower interval (1.19–1.88 g cm^{-3}; Table S4). These differences were consistent across all depth intervals, with %C exhibiting pronounced separation among ecosystem types within each depth, particularly between prairie and all other systems. Conversely, BD showed limited differentiation among ecosystem types within depths, with substantial overlap in values. This contrast is clearly reflected in the panel separation (Fig. 5), with mineral soil concentration showing strong stratification among ecosystem types across depths, whereas bulk density exhibits constrained variation and limited separation within comparable depth intervals.

Results from the linear mixed-effects models were consistent with these patterns (Table S5). For %C, both ecosystem type and depth had strong effects ($p < 0.001$), and their interaction was also significant ($p < 0.001$), indicating that depth-related changes in %C varied among ecosystem types. In contrast, although ecosystem type and depth significantly influenced bulk density ($p < 0.001$), their interaction was not significant ($p = 0.311$), indicating consistent depth-related patterns across ecosystem types and reinforcing that variation in %C was greater and more strongly structured than variation in bulk density (Tables S5).

4.

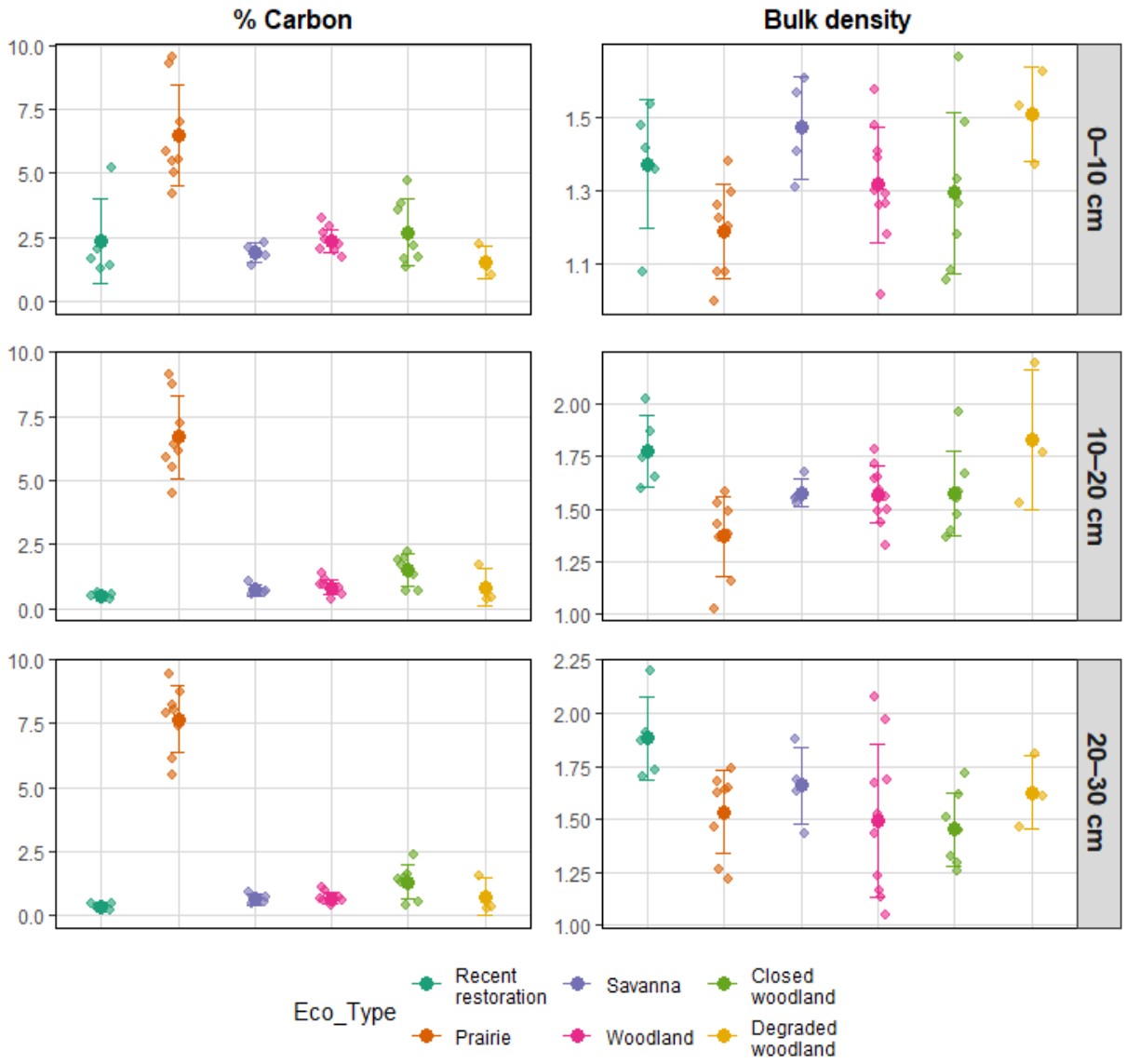

Figure 5. **Soil carbon concentration (% Carbon; left column) and bulk density (g cm⁻³; right column) across ecosystem types and soil depth intervals (0–10, 10–20, and 20–30 cm).** Points represent individual plot observations, while larger points with error bars indicate mean ± standard deviation for each ecosystem type. Ecosystem categories include recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland. Soil depth is shown along the right side of the figure, and bulk density panels are displayed with independent y-axis scales to improve visualization of variation across depths.

5. Discussion

Carbon storage across ecosystem types reflects a shift in how carbon is partitioned among pools, rather than a simple increase or decrease in the total C stored at Sumter Farm. This variation appears to be primarily driven by belowground pools, particularly MS carbon, rather than aboveground biomass. This pattern supports foundational work demonstrating that soil carbon stocks dominate total ecosystem carbon and often vary independently of aboveground biomass (Georgiou et al., 2022; Jackson et al., 2017; Jobbágy & Jackson, 2000). Although forest systems traditionally emphasize aboveground carbon storage, recent studies have shown that grasslands may store comparable or greater total C when soil pools are considered (Dass et al., 2018). Differences among ecosystem types therefore arise from contrasting carbon allocation strategies, where open systems concentrate carbon belowground while closed systems accumulate C aboveground.

Carbon pools vary distinctly across ecosystem types, indicating that ecosystem structure governs how C is distributed among components of the system (Sun et al., 2025). Closed woodlands and woodlands concentrate C in trees and snags, consistent with global syntheses showing that forests store a major proportion of carbon in aboveground biomass due to the increase in canopy cover (Pan et al., 2011; Solomon et al., 2024). Woody debris pools follow similar patterns, reflecting dependence on tree biomass turnover and mortality (Harmon et al., 1986; Iwashita et al., 2013; Russell et al., 2015), yet it represents a relatively transient C pool due to decomposition processes (Chao et al., 2017; Harmon et al., 2020). In contrast, prairies and open systems exhibit minimal tree biomass and reduced woody debris accumulation, reflecting a shift toward belowground carbon storage and indicating that increases in woody biomass do not necessarily correspond to greater total ecosystem carbon when soil and root contributions are considered (Guo & Gifford, 2002; Mensah et al., 2016; Poorter et al., 2012).

Additionally, variation in ground-layer vegetation and other surface pools (LL+SOL) across ecosystem types reflects differences in vegetation structure, productivity, and disturbance regimes. Greater relative importance of GLV in open ecosystems is consistent with the dominance of herbaceous vegetation, where rapid turnover of aboveground biomass contributes continuous carbon inputs to the soil through both litter deposition and root exudation (Bai & Cotrufo, 2022; Cotrufo et al., 2013) supported by the efficient microbial communities that can dissolve different organic compounds (Cotrufo et al., 2013; Fossum et al., 2022). Conversely, reduced GLV contribution in more closed ecosystems likely reflects shading and competitive suppression of understory vegetation (Barrioz et al., 2013; Feltrin et al., 2016; Nowacki & Abrams, 2008; Vander Yacht et al., 2020), which limits herbaceous productivity and its role in C cycling. The accumulation of litter and organic layers, and C contribution, observed in closed and degraded woodlands, is consistent with increased aboveground biomass inputs and reduced prescribed-fire regimes (Dawson-Glass et al., 2023; Hopkins et al., 2012; Pile Knapp et al., 2024), allowing surface organic matter to build up over time. In contrast, lower litter and SOL accumulation in open, fire-maintained systems likely reflects repeated disturbance that limits surface organic matter buildup and redistributes C through combustion and rapid cycling pathways. Prescribed fires carried out in the site reduced these pools and may have altered the quality of carbon inputs and influenced decomposition processes (Alcañiz et al., 2018; Certini, 2005; Pellegrini et al., 2021).

Belowground carbon dominates ecosystem C storage in prairies, particularly in the mineral soil pool. Exceptionally high soil carbon obtained in these systems aligns with global patterns, indicating that grass-like biomes store most of their C belowground (Dass et al., 2018), often exceeding 90% of total ecosystem carbon (Bardgett et al., 2021a; J. M. Briggs & Knapp, 1995; Y. Wang et al., 2018). Greater carbon representation at depth in these systems has been linked to the development of extensive fine root systems associated with perennial grasses (Liebig et

al., 2005; Poeplau et al., 2021), which extend into deeper soil layers (>30–120 cm) in response to water and nutrient availability following disturbance. These deep-rooting patterns provide a key pathway for transferring carbon into MS profiles. However, not all prairies exhibit similar depth-related patterns, as some systems show lower C storage at depth and reduced fine root inputs in deeper layers (Dietz et al., 2024; Matamala et al., 2008), suggesting that high belowground C in prairie ecosystems reflects the combined influence of soil properties, plant community composition, climate, and long-term land management.

The high mineral soil C observed in prairie systems at Sumter Farm may not only reflect strong belowground inputs but also the potential long-term influence of prescribed fire regimes characteristic of the research site. Annual, low-intensity surface burns maintained herbaceous dominance and may have promoted continuous root-derived carbon inputs, which may have favored the transfer of carbon into mineral soil layers and may contribute to the persistence of carbon with depth. Experimental evidence suggests that frequent fire can shift C allocation toward belowground pools by limiting accumulation in transient aboveground components and altering decomposition dynamics (Pellegrini et al., 2018, 2020), thereby increasing the relative importance of soil carbon in frequently burned systems. In addition, repeated burning may contribute directly to MS carbon through the formation of pyrogenic carbon, a relatively recalcitrant form of organic matter that can persist in soils over long timescales and represent a substantial fraction of total soil organic C (Glaser & Amelung, 2003; Santín et al., 2016; Singh et al., 2012). Pyrogenic C can be fragmented into smaller particles and transported to deeper soil layers, where it becomes associated with mineral fractions and contributes to long-term carbon stabilization (Bird et al., 2015; Pignatello et al., 2015; Santín et al., 2016). These processes could help explain the persistence of carbon in mineral-soil pools and its depth distribution in prairie systems.

However, fire alone cannot solely explain the high magnitude of MS carbon observed in prairie systems, as savannas and woodlands at the site are also subject to long-term prescribed fire yet do not exhibit similarly elevated soil C stocks. This suggests that differences in ecosystem structure, particularly vegetation composition, may regulate how organic matter and carbon are allocated and transferred into soil across ecosystem types (Angst et al., 2025). Although frequent fire can influence C cycling, its effects on soil carbon are highly variable, as indicated in global syntheses where fire frequency often reduces soil %C, particularly in savanna and broadleaf systems (Pellegrini et al., 2018). Alternatively, other studies report neutral or context-dependent C responses driven by changes in vegetation and nutrient cycling (Pellegrini et al., 2015), noting that fire is not a consistent driver of soil C accumulation but may instead act indirectly by shaping vegetation structure and associated carbon inputs.

The dominance of graminoids in prairie systems, where they contributed the majority of GLV carbon, highlights a key functional difference among ecosystem types that may influence belowground C dynamics. This vegetation structure could enhance C allocation pathways, as grass-dominated systems are characterized by dense, fibrous networks and high rates of fine-root turnover (An et al., 2026a; Craine et al., 1999). These traits promote continuous inputs of carbon to the soil and enhance its incorporation into mineral-associated pools, with root-derived C contributing disproportionately to stable soil organic matter compared to aboveground inputs (Feng et al., 2024; Lynch & Wojciechowski, 2015; Panchal et al., 2022). Together, these relationships provide a mechanistic explanation linking vegetation composition to the elevated mineral soil C observed in prairie systems. Likewise, variation in plant functional composition may further influence these dynamics. Grass-dominated systems, particularly those with abundant C_4 species, are often associated with greater belowground productivity and enhanced soil C accumulation (D. A. Fornara & Tilman, 2008; O'Brien et al., 2010). However, these relationships are not universal due to reported inconsistent effects depending on species

composition, diversity, and environmental context (Anacker et al., 2021). This variability suggests that C accumulation is directed not only by functional group identity but also by interactions among plant traits, community structure, and ecosystem processes.

Beyond biomass inputs, rhizosphere (soil area influenced by plant roots) processes might further contribute to soil C stabilization. Root exudates represent a continuous flux of labile C that fuels microbial activity and promotes the formation of microbial-derived carbon, which is more likely to persist in mineral-associated fractions (Chen et al., 2025; Panchal et al., 2022; Rumpel & Kögel-Knabner, 2011; Yang et al., 2023). Plant community composition and diversity may influence these processes by altering root traits, exudation rates, and dissolved organic matter production that can facilitate C transport to deeper soil horizons through root systems and preferential flow paths (An et al., 2026a; Angst et al., 2025). Although plant diversity was not directly quantified in this study, the strong dominance of graminoids suggests that functional composition may capture key processes driving belowground carbon inputs and stabilization.

In contrast, savannas and woodlands (all types) exhibited greater contributions of woody vegetation, which allocate a larger proportion of C to aboveground biomass and coarse roots (Jackson et al., 1996; Poorter et al., 2012). These inputs are processed primarily through litter-based pathways, which are more susceptible to decomposition and less efficiently incorporated into stable mineral-associated soil C compared to root-derived inputs (Cotrufo et al., 2013; Kögel-Knabner, 2002; Rasse et al., 2005). Consequently, a greater proportion of C in woody systems may remain in aboveground biomass or surface organic layers rather than being transferred to mineral soil pools (Jobbágy & Jackson, 2000; Wiesmeier et al., 2019a). In addition, frequent fire can further limit soil carbon storage through combustion losses and volatilization, particularly in savanna ecosystems, where repeated burning can reduce soil C stocks and alter nutrient dynamics (Certini, 2005; Pellegrini et al., 2018). Collectively, these

processes constrain the transfer and stabilization of C in mineral soils, contributing to the lower MS carbon observed in savannas and woodlands relative to prairie systems.

These vegetation- and process-driven differences in belowground carbon inputs and stabilization are reflected in patterns of soil carbon concentration and bulk density across ecosystem types. Differences in %C relative to BD further support that C storage is more closely associated with variation in carbon content than with differences in soil density. Limited variation in bulk density across ecosystem types indicates that differences in carbon stocks are not primarily driven by soil mass or compaction. Instead, variation in %C reflects differences in organic inputs and in soil processes that regulate C retention. Similar conclusions have been reported in global analyses showing that soil C variation is more strongly associated with carbon concentration than with bulk density (Kučera et al., 2025; Périé & Ouimet, 2008; Yang et al., 2016). Nevertheless, other studies have shown that soil carbon concentration and its vertical distribution are also influenced by soil mineralogy and texture, which affect C stabilization and storage capacity (Jobbágy & Jackson, 2000; Mathieu et al., 2015; Yang et al., 2022). Because soil texture was not assessed in the mineral soil samples analyzed in this study, the extent to which these factors contributed to the observed %C patterns cannot be determined. Future research should explicitly consider soil physical properties to evaluate better their role in shaping soil C distribution across ecosystems.

Broadly, prairie ecosystems at Sumter Farm represent a distinct carbon storage strategy characterized by low aboveground biomass but substantial and persistent C stored in mineral soil. These findings demonstrate that variation in total ecosystem C is driven by differences in carbon allocation and retention in soil pools rather than increases in woody biomass, challenging forest-centric perspectives on carbon storage. The strong representation of soil C, including at depth, suggests that sustained belowground inputs, combined with stabilization processes and long-term fire regimes, play a key role in shaping C storage in these systems.

Further research is needed to quantify these mechanisms, particularly the roles of microbial processing, mineral stabilization, and pyrogenic carbon dynamics, to better understand the persistence of soil carbon in open ecosystems. Recognizing the importance of belowground C pathways is essential for improving C accounting and ensuring that grasslands and other fire-maintained systems are accurately represented in global carbon mitigation strategies.

CRediT authorship contribution statement

Josue Chevez-Sahona: Writing – review & editing, Writing – original draft, conceptualization, data curation, formal analysis, investigation, software, visualization. **Heather Alexander:** Writing – review & editing, conceptualization, methodology, supervision, visualization, resources, project administration, funding acquisition. **Dwayne Estes:** Writing – review & editing, supervision, resources, project administration, funding acquisition. **Tamara Milton:** Writing – review & editing, data curation, software, visualization. **Timothy Shearman:** Writing – review & editing, visualization.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that could have influenced the work reported in this article.

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Appendix A. Supplementary material

Table S1. **Mean carbon stored (Mg C ha^{-1}) and proportional contribution (%) from observed data of ground-layer functional groups across ecosystem types at Sumter Farm, Alabama,** including recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland**.** Functional groups include graminoids, forbs, woody plants, vines, and mosses. Percent values represent the relative contribution of each functional group to total ground-layer vegetation carbon within each restoration stage. Total C values represent the sum of all functional groups (100%).

Mean (Mg C ha^{-1}) (%)	**Recent restoration**	**Prairie**	**Savanna**	**Woodland**	**Closed woodland**	**Degraded woodland**
Graminoid	0.357 (27.84)	0.825 (68.63)	0.650 (41.37)	0.308 (33.68)	0.174 (32.95)	0.584 (40.33)
Forb	0.635 (49.53)	0.319 (26.61)	0.241 (15.36)	0.181 (19.84)	0.210 (39.68)	0.168 (11.58)
Woody	0.271 (21.15)	0.047 (3.94)	0.637 (40.59)	0.281 (30.84)	0.074 (13.90)	0.451 (31.11)
Vine	0.019 (1.48)	0.010 (0.81)	0.042 (2.69)	0.143 (15.63)	0.070 (13.28)	0.246 (16.97)
Moss	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.0001 (0.01)	0.001 (0.18)	0.000 (0.00)
Total C	1.28 (100)	1.20 (100)	1.57 (100)	0.91 (100)	0.53 (100)	1.45 (100)

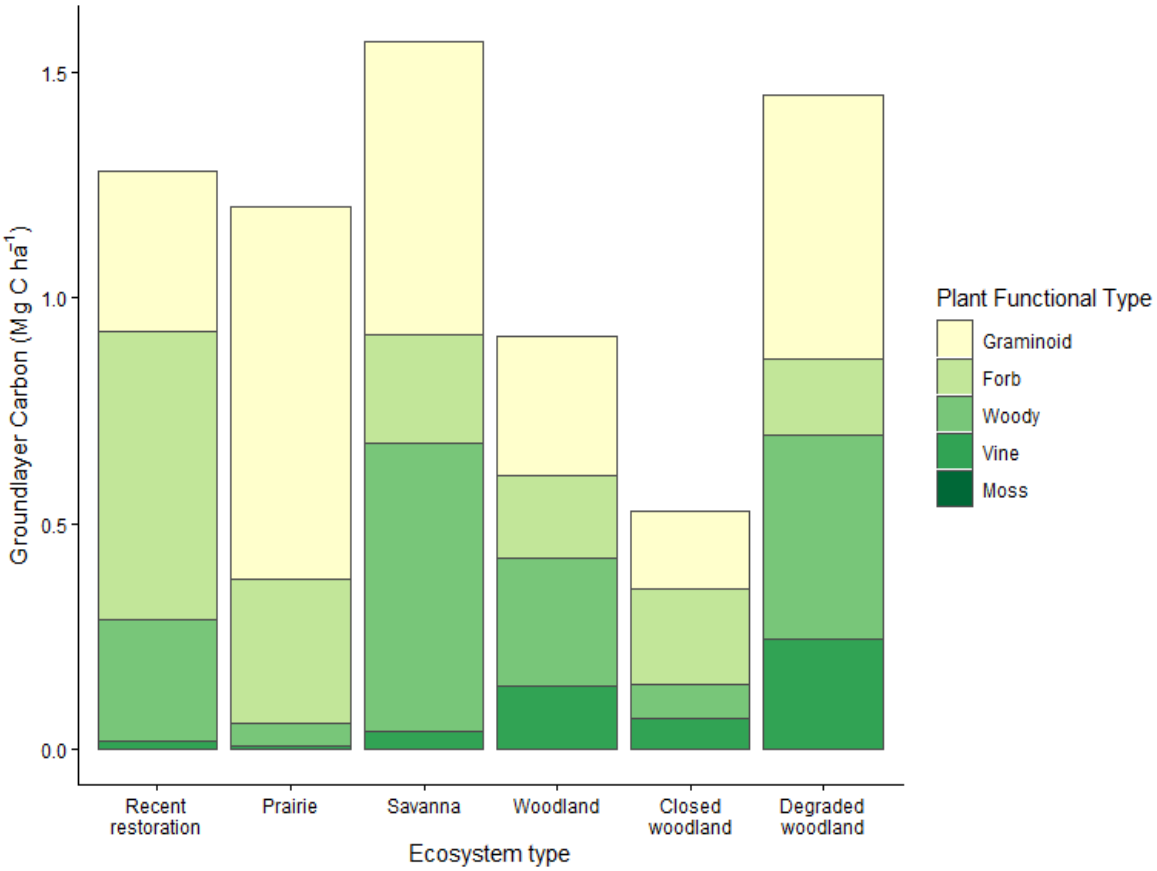

Figure S1. **Groundlayer carbon stocks (Mg C ha^{-1}) partitioned by plant functional type across ecosystem types at Sumter Farm, Alabama**. Stacked bars represent the cumulative contribution of graminoids, forbs, woody plants, vines, and moss to total groundlayer C within each ecosystem type, including recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland. Values represent mean C stored per functional group averaged across plots within each ecosystem type.

Table S2. **Depth-specific carbon stocks (mean ± SD; Mg C ha⁻¹) in mineral soil and fine root pools across ecosystem types at Sumter Farm, Alabama**. Values are reported for soil depth intervals of 0–10, 10–20, and 20–30 cm. Ecosystem types include recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland. Different lowercase letters indicate significant differences among ecosystem types within each depth interval based on Tukey's HSD post hoc comparisons ($p < 0.05$). Statistical tests were derived from linear mixed-effects models evaluating the effects of ecosystem type, depth, and their interaction. Bold values represent significant p-values ($p < 0.05$).

Mean (Mg C ha⁻¹) (%)	**Recent restoration**	**Prairies**	**Savannas**	**Woodlands**	**Closed woodlands**	**Degraded woodlands**
Mineral soil						
0-10 cm	28.97[a] (14.27)	74.82[b] (16.66)	27.36[a] (3.30)	29.76[a] (2.88)	32.33[a] (10.62)	23.08[a] (9.93)
10-20 cm	8.79[a] (1.70)	89.93[c] (17.64)	11.66[a] (3.19)	12.77[a] (3.57)	22.16[b] (7.91)	13.72[ab] (9.77)
20-30 cm	6.46[a] (1.65)	114.64[c] (19.04)	10.46[ab] (3.32)	10.02[a] (2.76)	18.70[b] (9.58)	10.96[a] (10.05)
Fine roots						
0-10 cm	1.90[a] (1.46)	3.90[a] (2.18)	1.89[a] (0.76)	1.40[a] (0.46)	1..87[a] (0.86)	1.32[a] (0.70)
10-20 cm	0.28[a] (0.13)	0.98b[b] (0.72)	0.42[ab] (0.15)	0.46[ab] (0.19)	0.80[ab] (0.38)	0.35[ab] (0.20)
20-30 cm	0.15[a] (0.06)	1.19[ab] (2.65)	0.27[ab] (0.17)	0.26[ab] (0.20)	0.35[ab] (0.20)	0.17[a] (0.13)

Table S3. **Results from linear mixed-effects models evaluating the effects of ecosystem type, soil depth (0–10, 10–20, and 20–30 cm), and their interaction on mineral soil and fine root carbon storage**. F- and p-values correspond to Type III ANOVA tests with Satterthwaite's approximation for degrees of freedom. Significant main effects of ecosystem type and depth indicate differences in carbon stocks among ecosystem types and across soil depths, whereas the ecosystem type × depth interaction term reflects variation in vertical carbon distribution among ecosystem types. Bold values represent significant p-values ($p < 0.05$).

	Mineral soil		**Fine roots**	
	F-value	**p-value**	**F-value**	**p-value**
Ecosystem type	112.68	**< 0.001**	6.93	**< 0.001**
Depth	48.63	**< 0.001**	74.90	**< 0.001**
Ecosystem × Depth	7.54	**< 0.001**	0.69	0.734

Table S4. **Soil carbon concentration (%C) and bulk density (g cm^{-3}) across ecosystem types and depth intervals at Sumter Farm, Alabama.** Values represent mean ± standard deviation for each ecosystem type at 0–10, 10–20, and 20–30 cm soil depths. Ecosystem types include recent restoration, prairie, savanna, woodland, closed woodland, and degraded woodland. Different lowercase letters indicate significant differences among ecosystem types within each depth and soil property based on Tukey's HSD post hoc comparisons ($p < 0.05$). F- and p-values correspond to one-way ANOVA tests evaluating the effect of ecosystem type within each depth interval. Bold values represent significant p-values ($p < 0.05$).

Mean (Mg C ha^{-1}) (%)	Recent restoration	Prairies	Savannas	Woodlands	Closed woodlands	Degraded woodlands	F-value	p-value
% Carbon								
0-10 cm	2.34^{a} (1.64)	6.48^{b} (1.97)	1.92^{a} (0.38)	2.38^{a} (0.45)	2.70^{a} (1.30)	1.53^{a} (0.64)	13.8	**< 0.001**
10-20 cm	0.50^{a} (0.09)	6.68^{b} (1.60)	0.74^{a} (0.21)	0.84^{a} (0.28)	1.50^{a} (0.63)	0.84^{a} (0.74)	59.2	**< 0.001**
20-30 cm	0.35^{a} (0.09)	7.64^{b} (1.30)	0.64^{a} (0.21)	0.69^{a} (0.21)	1.31^{a} (0.67)	0.73^{a} (0.73)	114	**< 0.001**
Bulk density								
0-10 cm	1.37^{a} (0.18)	1.19^{a} (0.13)	1.47^{a} (0.14)	1.32^{a} (0.16)	1.29^{a} (0.22)	1.51^{a} (0.13)	2.56	**0.047**
10-20 cm	1.78^{b} (0.17)	1.37^{a} (0.19)	1.58^{ab} (0.07)	1.57^{ab} (0.14)	1.57^{ab} (0.20)	1.83^{b} (0.33)	4.46	**0.004**
20-30 cm	1.88^{a} (0.20)	1.53^{a} (0.20)	1.66^{a} (0.18)	1.49^{a} (0.36)	1.45^{a} (0.17)	1.63^{a} (0.17)	2.25	0.074

Table S5. **Type III analysis of variance (ANOVA) results for soil carbon concentration (% Carbon) and bulk density (g cm^{-3}) across ecosystem types and soil depth intervals (0–10, 10–20, and 20–30 cm).** Values are derived from linear mixed-effects models including ecosystem type, depth, and their interaction as fixed effects, and plot as a random effect. Soil C concentration was log-transformed before analysis to meet model assumptions. F- and p-values were calculated using Satterthwaite's approximation for degrees of freedom. Bold values represent significant p-values ($p < 0.05$).

	% Carbon		Bulk density	
	F-value	p-value	F-value	p-value
Ecosystem type	92.09	**< 0.001**	6.52	**< 0.001**
Depth	52.52	**< 0.001**	16.48	**< 0.001**
Ecosystem × Depth	5.24	**< 0.001**	1.19	0.311

Chapter 2: Time since restoration shapes carbon accumulation trajectories on prairies in the Interior Plateau, southeastern United States

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Author contributions: HDA conceived and designed the research; JCS led fieldwork, sample collection, and processing, and data curation; JCS, TFM performed data analysis; JCS, TFM, TMS performed visualization; JCS wrote the original manuscript; HDA, LDE provided supervision, resources, and funding; JCS, HDA, LDE, TFM, TMS reviewed and edited the manuscript.

Abstract

Introduction: Prairie ecosystems provide critical ecosystem services, including carbon (C) storage, yet have experienced extensive decline, particularly in the southeastern U.S. Restoration efforts aim to recover ecosystem structure and function, but the trajectory of C recovery in prairies remains poorly understood across diverse ecosystem pools and environmental contexts.

Objectives: This study evaluated how C storage varies among ecosystem pools across a gradient of time since restoration and moisture conditions, and how groundlayer vegetation (GLV) carbon is distributed among plant functional types.

Methods: Carbon stocks were quantified across ecosystem pools (GLV, litter/organic layers, mineral soil [0–30 cm], roots, and total ecosystem C) in 62 plots across 7 sites within the Interior Plateau. The gradient ranged from active cropland (pre-restoration) and early (<1 year) to advanced restoration stages (9 years), with remnant prairies serving as benchmarks. Effects of time since restoration and moisture on C storage were assessed using linear and generalized additive models.

Results: Total ecosystem C increased with time since restoration ($p = 0.009$), ranging from 50.80 Mg C ha^{-1} in early stages (<1 year) to 71.74 Mg C ha^{-1} at 5 years. Total C after 9-year restoration (59.66 Mg C ha^{-1}) was comparable to remnant prairie values (62.81 Mg C ha^{-1}), indicating substantial gains relative to initial active agricultural states. Aboveground and root pools responded rapidly; GLV carbon significantly increased from 0.82 Mg C ha^{-1} (early stages) to 3.26 Mg C ha^{-1} (9 years; $p = 0.007$). Conversely, 0–30 cm mineral soil C showed substantial variability (48–68 Mg C ha^{-1}) but no significant directional trend over time ($p = 0.49$). While moisture condition significantly influenced GLV carbon ($p = 0.02$), with higher values in mesic systems, this effect did not propagate to total ecosystem C ($p = 0.17$).

Conclusions: Findings suggest that C recovery in restored prairies is driven by contrasting responses among ecosystem pools. Vegetation and root biomass recover rapidly, whereas mineral soil C shows limited sensitivity to time since restoration and remains stable within the first decade. Restored systems can approximate remnant conditions for aboveground biomass within nine years, though soil C trajectories appear more stable and less responsive to immediate restoration actions.

Implications for practice: Prairie restoration supports C storage, but outcomes are contingent on how carbon is distributed among ecosystem pools rather than total C aggregates alone. Management should prioritize practices that enhance groundlayer productivity and belowground allocation, such as prescribed fire, to sustain graminoid dominance and consistent root inputs. Because mineral soil C exhibits limited temporal sensitivity within the first decade, long-term, sustained management is essential to support the development and stability of this significant C pool. Finally, as site-specific moisture conditions significantly influence groundlayer productivity and C allocation, restoration objectives should be calibrated to these underlying environmental constraints to effectively guide recovery trajectories.

1. Introduction

Prairie ecosystems are a major component of temperate grass-dominated landscapes and play a significant role in supporting biodiversity, ecosystem function, and nutrient cycling (Baghel et al., 2026; Blair et al., 2013). These systems are characterized by dominant herbaceous vegetation, limited woody cover, and strong dependence on disturbance regimes such as fire and grazing (Gleason, 1909; Noss, 2012). Although large portions of North America were historically occupied by prairies, it has been estimated that more than 90% of historical prairie ecosystems in the United States have been converted or degraded since Euro-American settlement (Noss, 2012), due to fire exclusion, agricultural expansion, urban development, and land-use intensification (Bardgett et al., 2021b; Hanberry et al., 2020; Noss et al., 2015; Veldman, Overbeck, et al., 2015).

In the southeastern United States, the loss and fragmentation of prairie ecosystems are considered particularly critical. This has resulted in substantial declines in native plant and animal diversity and the loss of regionally unique grassland communities (Estes et al., 2016; Noss et al., 2021). The Interior Plateau ecoregion is thought to have originally supported unique prairie communities maintained by frequent, low-intensity surface fires that limited tree recruitment and favored the persistence of diverse, fire-adapted herbaceous flora (Estes et al., 2016). However, the progressive reduction of historical fire regimes following Euro-American settlement, and later organized fire suppression efforts with other land-use changes, promoted landscape mesophication by allowing fire-sensitive, shade-tolerant woody species to encroach upon historically open habitats (Alexander et al., 2021; Nowacki & Abrams, 2008). This structural shift toward closed-canopy forests has been linked to the loss of regionally unique prairie communities and the disruption of ecosystem services that historically sustained these open landscapes (Noss, 2012).

Ecological restoration is used to reverse this degradation potentially; however, documented outcomes of such efforts within the Interior Plateau are rarely reported. This scarcity of evidence may be partially attributed to a prevailing bias, where restoration attention has been disproportionately focused on the perception of forests as the primary agents of climate mitigation. Therefore, the role of restoration in services such as carbon (C) storage has remained poorly understood. Additionally, the challenge of quantifying C recovery is further compounded by the inherent heterogeneity of the study sites. Restoration is often commenced on lands with distinct "legacy" conditions (i.e., former row-crop fields, abandoned pastures, or closed forests), which are known to influence the trajectory of ecological recovery (Briggs & Knapp, 1995; Coffey & Otfinowski, 2018; Veldman et al., 2015). Consequently, a comprehensive regional framework is currently required to determine how different restoration stages and starting conditions influence the accumulation and stabilization of carbon across diverse ecosystem pools.

While prairies have been hypothesized as the most resilient ecosystems to sequester and store C belowground despite climate change effects and land-use disturbance (Dass et al., 2018), it remains unclear whether these carbon accumulation projections are universally applicable. In the U.S. context, previous studies conducted in the Great Plains (Derner et al., 2006; Euliss et al., 2006; X. Wang et al., 2016; Woller-Skar et al., 2024) have observed that prairies store considerable C belowground. Nevertheless, these results cannot be inferred for the Interior Plateau because the literature on prairie carbon dynamics is limited, due to the different soil characteristics and other abiotic factors present in this ecoregion.

To evaluate these outcomes, it is necessary to examine the mechanisms that govern carbon recovery, particularly vegetation functional composition and soil moisture. Groundlayer vegetation represents the primary source of carbon inputs in prairie ecosystems (Bai & Cotrufo, 2022; Jones & Donnelly, 2004), and shifts in functional group dominance can alter the chemical

quality of litter and root exudates entering the soil (Cotrufo et al., 2013; Panchal et al., 2022; Shen et al., 2020). These functional characteristics may influence both the rate at which carbon enters the soil profile and the pathways through which it is stabilized as mineral-associated organic matter (Cotrufo et al., 2013; Fornara & Tilman, 2008). Examining changes in vegetation composition may therefore provide insight into the mechanisms underlying carbon accumulation in restored prairie systems beyond simple estimates of carbon stocks.

Simultaneously, soil moisture also represents an important abiotic control on carbon ecosystem dynamics in the Interior Plateau. The region's complex karst topography creates a mosaic of environmental conditions ranging from dry, well-drained uplands to mesic lowlands (Fenneman, 1938; Griffin et al., 1998). These moisture gradients can influence both plant productivity and microbial decomposition, thereby affecting the balance between carbon inputs and losses (Bai & Cotrufo, 2022; Cotrufo et al., 2013). Although other abiotic factors such as soil chemistry and pH conditions may also contribute to variation among sites, moisture is expected to be a primary driver of restoration trajectories in this landscape. Understanding how moisture conditions interact with restoration age and vegetation composition may therefore improve our understanding of variation in carbon recovery across the Interior Plateau.

To address these knowledge gaps, carbon storage was examined across multiple ecosystem pools (i.e., groundlayer vegetation, leaf litter, the soil organic layer, mineral soil, and root biomass) within prairie systems under ongoing restoration in the Interior Plateau. By comparing prairies across a gradient of restoration duration and moisture conditions, the changes in C allocation were evaluated through successional time. Specifically, two primary questions were addressed: (1) how does carbon storage vary among ecosystem pools in prairies under different durations of restoration and moisture regimes?, and (2) how does the relative proportion of groundlayer carbon stored in different functional groups change with time since restoration?

It was hypothesized that total carbon storage would increase with restoration duration, but that moisture regimes would dictate the rate of this accumulation; specifically, mesic sites are expected to support higher groundlayer vegetation productivity and lead to greater C storage. Furthermore, given that restoration may represent a transition from early-phase forbs to late-phase graminoids, it is anticipated that this functional shift will correlate with changes in C storage; specifically, as communities converge toward graminoid dominance, a corresponding increase in belowground biomass and total ecosystem C is expected. Through this multi-pool and functional approach, this study aims to improve understanding of C recovery pathways in restored prairie systems of the southeastern U.S. and to provide a regional framework for evaluating their potential as long-term carbon sinks.

2. Methods

2.1. Study area and site descriptions

The study was conducted within the Interior Plateau ecoregion, specifically the Pennyroyal Karst Plains (PKP) and the Western Highland Rim (WHR) subregions of Kentucky and Tennessee. This physiographic region is defined by flat-lying plateaus shaped by Paleozoic continental sediments, which have been eroded into a moderately to deeply dissected surface with elevations ranging from 200 to 300 m (DeSelm et al., 1999; Fenneman, 1938). Soils are predominantly Alfisols and Ultisols, characterized by deep, clay-rich subsoils that facilitate moisture retention during the growing season (Dickson & Sheffield, 2003). The climate is temperate and humid, with a mean (2019–2025) annual temperature of 15°C and 1,422 mm of precipitation (NOAA, 2026). Historically, these landscapes supported open oak woodlands, savannas, and grasslands maintained by frequent, low-intensity fires, with dendroecological evidence indicating fire-return intervals commonly ranging from approximately 3 to 5 years and annual fires occurring in some locations (Stambaugh et al., 2016). Following Euro-American

settlement, reductions in fire frequency, together with agricultural expansion, urbanization, and other land-use changes, contributed to substantial declines in open ecosystems across the Interior Plateau (Estes et al., 2016; Noss, 2012; Noss et al., 2021). Many prairies were converted to row-crop agriculture or Eurasian grass pastures, while others experienced woody encroachment and mesophication resulting in more closed-canopy vegetation conditions. Ongoing restoration efforts in the PKP and WHR, led by state agencies (TN Department of Environment and Conservation), research institutions (Southeastern Grasslands Institute; SGI), and private landowners, aim to recover these ecosystems by reintroducing disturbance regimes and native vegetation.

Carbon dynamics were quantified across a network of prairie ecosystems distributed throughout this region, using a restoration-time design to assess C pools across restoration states ranging from active croplands and recent reconstructions to advanced restoration stages (Fig. 1; Table S6). Remnant prairies, characterized by long-term persistence of native conservative-heliophilic vegetation and the absence of mechanical soil disturbance, served as reference benchmarks (Larson et al., 2020; Lord et al., 2024). Within the Interior Plateau, Barnett Woods State Natural Area (36°31’33.74’’ N, 87°33’41.87’’ W) in Montgomery County, Tennessee, includes approximately 141.6 ha (350 acres) targeted for the restoration of historically open ecosystems; management here focuses on recovering dry remnant prairies through native seeding and fire, while adjacent crop fields serve as pre-restoration benchmarks. Additionally, Dunbar Cave State Park (36°33’36.43’’ N, 87°18’13.52’’ W) in Clarksville, Tennessee, encompasses a 5.5-ha (13.5-acre) prairie restoration program initiated in 2015, featuring both dry and mesic plots that provide a reference for intermediate stages of development. Montgomery Bell State Park (36°03’45.63’’ N, 87°17’35.33’’ W) in Burns, Tennessee, contains recently established prairie restorations following tornado damage, representing early stages of successional recovery through clearing and seeding.

Fort Campbell Army Base (36°39′13″ N, 87°27′35″ W), located on the Kentucky–Tennessee border, contains high-quality dry prairie remnants and ongoing restoration projects managed by the U.S. Army through prescribed fire and mowing, with specific restorations initiated in 2021 representing mid-stage development. Baker Natural Area (36°51′17.9″ N, 86°55′00.4″ W), an urban conservation park in Russellville, Kentucky, contains dry prairie remnants that serve as critical reference conditions. Google Prairie (36°37’01.26’’N, 87°16’09.00’’W) is an SGI-managed project with the support of the Google Data Center Montgomery County, on former row-crop fields with restoration phases initiated in 2020 and 2024, while the nearby Guthrie Prairie (36°38’29.67’’ N, 87°09’26.26’’ W) is a small mesic remnant that has undergone management for invasive species control without mechanical soil disturbance.

In addition, this study included privately owned properties that offered unique opportunities to examine restoration across diverse land-use legacies. Wessyngton Plantation (36°31’8.48’’N, 87° 0’32.81’’W), a historic farm located near Cedar Hill in Robertson County, Tennessee, possesses a land-use history characterized by nineteenth and twentieth-century dark-fired tobacco cultivation and livestock production. Eurasian grass species currently dominate its 37.4-ha (92.4-acre) grazing fields and also serve as a pre-restoration reference for future prairie reintroduction efforts led by the SGI, representing conditions prior to active management. Similarly, Best Hope Farm (36°04’49.41’’ N, 87°15’27.20’’ W), located near White Bluff, Tennessee, contains both a dry prairie remnant and reconstructed dry prairies, serving as a critical location for comparing long-persisting prairie systems with those under active restoration. Management activities at Best Hope Farm are led by the landowners and involve targeted herbicide applications, mechanical removal of overstory invasive vegetation, native seeding, and prescribed fire.

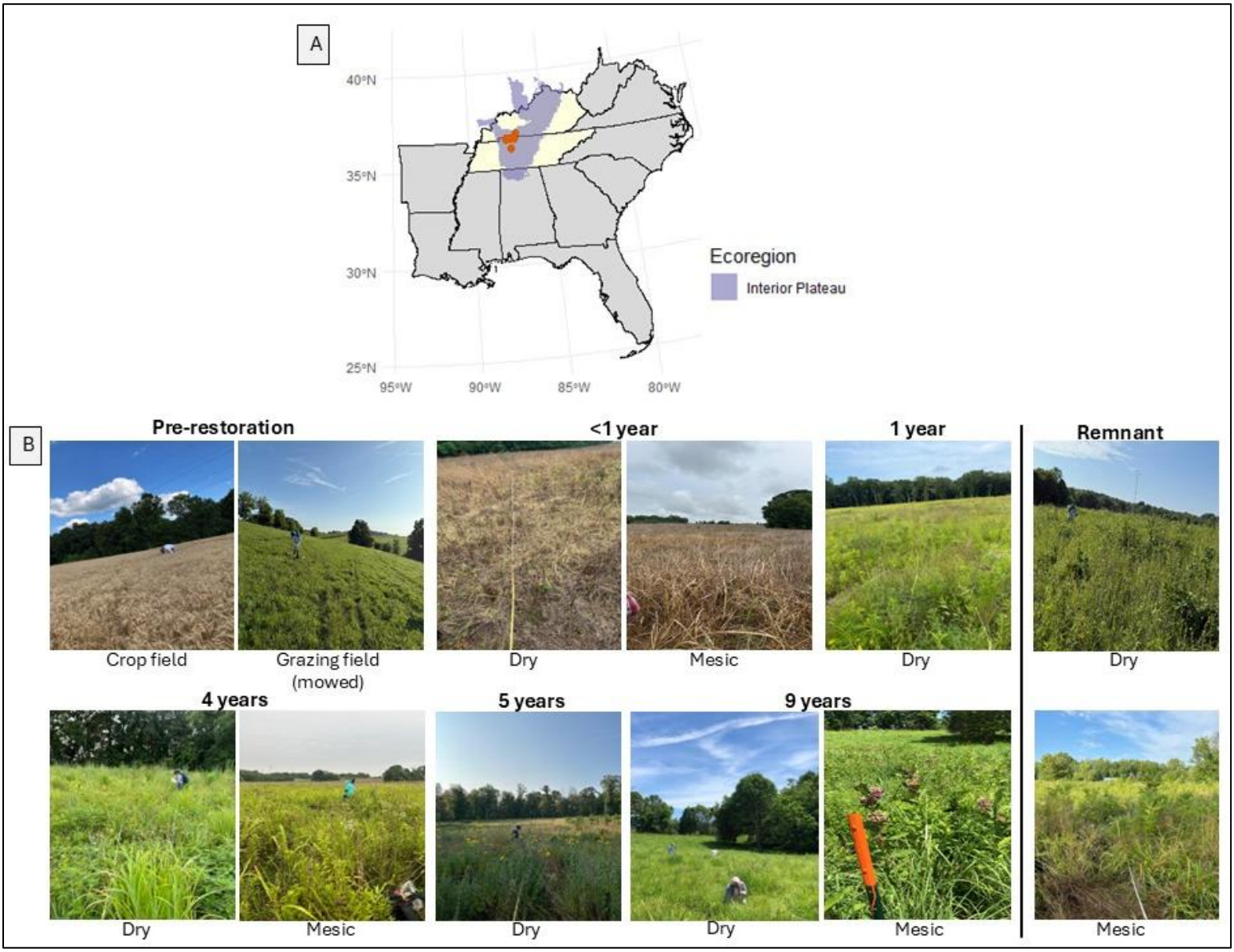

Figure 6. (A) Location of study sites within the Interior Plateau ecoregion of the southeastern U.S. Colored areas indicate the spatial extent of the Interior Plateau, with points representing sampled prairie sites. (B) Representative pictures of prairie sites across the restoration gradient and moisture regimes. Images show vegetation composition from pre-restoration conditions (crop field and grazed pasture), early restoration (<1 year and 1 year; dry and mesic), intermediate (4–5 years), late restoration (9 years) stages, and remnant prairies.

2.2. Study design

This study evaluated carbon pools across a gradient of restoration ages to capture a gradient of successional states, ranging from active croplands (pre-restoration) and recent reconstructions (≤1 year) to intermediate (4–5 years) and advanced restoration stages (9 years). Sites were selected to represent a range of restoration histories and ownership frameworks, with remnant prairies serving as primary reference benchmarks. Within each site, plots were established to capture variation in prairie moisture regimes (dry vs. mesic); dry prairies were characterized by well-drained, sandy-gravelly soils, while mesic prairies occupied lowland positions with higher drainage constraints and periodic flooding. To facilitate this assessment, 62 plots were established using a stratified design, with locations distributed across sites to capture within-site heterogeneity in topographical position, soil moisture regime, and local management practices. Plots were spaced at least 50 m apart and adjusted in the field based on site size and vegetation structure. Within each plot, three 15 m transects were established from the plot center at fixed directions (0°N, 135°SE, and 225°SW) as the sampling framework for all C pools. All field sampling was conducted during peak growing conditions from June to September 2024–2025.

2.3. Carbon sampling

Carbon was quantified across major ecosystem pools, including groundlayer vegetation (GLV), leaf litter (LL), soil organic layer (SOL), mineral soil (MS), and fine (FR) and coarse root (CR) biomass (Lal, 2005). Groundlayer vegetation, defined as all herbaceous vegetation <1.37 m in height, was sampled using a 0.25-m² quadrat placed at the 9-m mark along each transect (~ 1m apart from the transect line). All live and standing dead biomass within the quadrat was clipped at the soil surface, excluding litter material. When sampling locations were obstructed (e.g., by large trees), quadrats were relocated slightly to maintain representative sampling conditions. Samples were placed in labeled paper bags for laboratory processing. Moreover, LL and SOL depths were measured at the 10-m position along each transect using a 0.0625 m² (25 × 25 cm)

quadrat. Leaf litter was defined as recognizable plant material undergoing decomposition at the soil surface, while the soil organic layer consisted of partially to fully decomposed organic material directly beneath the litter layer (Briggs, 2004). Depth measurements were taken sequentially, first recording LL depth and then SOL depth within the same quadrat while minimizing disturbance.

Mineral soil was sampled at 9 meters along each transect, following GLV harvesting, using a 6.35 cm diameter metal soil corer to a target depth of 30 cm. Cores were divided into three depth increments (0–10, 10–20, and 20–30 cm), generating three samples per transect (n=9 per plot). The corer was inserted and rotated to the desired depth, then carefully removed to maintain sample integrity. When obstructions such as rocks or other hard materials prevented reaching 30 cm, an additional attempt was made nearby, or the maximum attainable depth was recorded. MS was defined as soil below the organic horizon composed primarily of inorganic material (< 20% organic matter; Finch et al., 2014; Wilson, 2019).

Root biomass C was quantified from both soil cores and monoliths. Fine roots (≤ 2 mm diameter), including rhizomes and other small belowground structures, were collected from soil cores. Coarse roots (> 2 mm diameter) were sampled using soil monoliths (30 × 30 × 30 cm) dug adjacent to each transect at the 10-m mark, after LL and SOL were measured. Monolith depths were recorded at each monolith corner, and when full depth was not achievable due to obstructions, the actual depth was noted. CR samples included all belowground woody and structural plant material (rhizomes and geoxiles) encountered within the digging volume (Milton et al., 2026). All mineral soil and monolith samples were transported to the laboratory and stored at −20 °C before processing.

2.4. Samples processing and C estimation

2.4.1. Laboratory processing

Every material collected in the field was transported to the lab, where multiple processing protocols were used to generate the data needed for carbon estimates in each ecosystem pool. Groundlayer vegetation samples were sorted into five plant functional groups: woody (seedlings and woody shrubs), graminoids (grasses, sedges, and rushes), forbs (non-woody herbaceous/broadleaf plants), vines (climbing or trailing stems), and “other” (plants that did not fit into the previous categories, including non-vascular species). After sorting, each functional group was placed into a new labeled paper bag and then placed in a drying oven at 60 °C for 48 hours. Once removed, samples were allowed to cool (~ 10 minutes) and transferred into tared aluminum tins and weighed. GLV samples were returned to their respective paper bags afterwards.

MS and CR samples were thawed and air-dried (~ 48 hours) before processing. Mineral soil samples were double-sieved using 2- and 1-mm metal circular sieves, which helped separate fine roots and rocks from the soil. The sieved soil was placed in small aluminum tins and oven-dried at 105 °C for a minimum of 48 hours. Sample weights were recorded, and the soil was then stored in the original bags. A subsample (3–5 grams) was taken from each sample and ground into a fine powder using a SPEX SamplePrep 8000D Mixer/Mill grinder. Once ground, the subsamples were placed into uniquely labeled small plastic vials and later sent to the Agricultural and Environmental Services Laboratories at the University of Georgia (Athens, GA) for total organic C analysis using a high-temperature elemental combustion analyzer (Elementar Vario Max; Langenselbold, Germany). Carbon concentrations were reported as a percentage of dry mass.

In addition, fine roots were manually extracted from mineral soil samples, rinsed with water to remove adhering soil particles, and placed into labeled coin envelopes. Samples were

oven-dried at 60 °C for 48 hours, after which dry weights were recorded. Rock volume was determined using the water displacement method by submerging extracted rock fragments in a graduated cylinder and recording the displaced volume. CR were separated from soil monolith samples that had been placed in large aluminum trays, rinsed to remove residual soil, and oven-dried at 60 °C for 48 hours. Dry coarse-root biomass was recorded, and samples were stored in sealed plastic bags for subsequent analyses.

2.4.2. Carbon pools calculations

Groundlayer vegetation C was quantified by first expressing biomass on an area basis (g m^{-2}) using the dry mass of harvested material and the sampled quadrat area (0.25 m^2). These values were then converted to Mg ha^{-1} and multiplied by a carbon conversion factor of 0.5 (Johnson et al., 2017) to estimate GLV carbon pools. Carbon stored in LL and SOL was estimated by using equation models that were built upon bulk density (BD; calculated as dry mass per sample volume, excluding coarse fragments in SOL), and C concentration (Shrestha et al., unpublished data), which were later combined with average depths measured in the field. Because carbon values in these pools were consistently low, LL and SOL were combined into a single pool (LL + SOL), with reported values representing their summed contribution. Resulting values were expressed in Mg C ha^{-1} for analysis.

$$[1] \quad C_{LL} = 1.6 \times (AD_{LL})^{0.8}$$

$$[2] \quad C_{SOL} = 4.5 \times (AD_{SOL})^{1.1}$$

Mineral soil C storage was estimated by combining specific-pool BD, percent carbon (%C), and sampling depth (m) to quantify carbon stocks on an area basis (kg ha^{-1}; Equation 3). Bulk density was obtained as the ratio of oven-dried soil mass to sample volume (g cm^{-3}) and subsequently converted to kg m^{-3}. Carbon stored for fine and coarse roots (kg ha^{-1}; Equation 4) was derived using pool-specific bulk density estimates, standardized sampling depths (0.10 m

for fine roots and 0.30 m for coarse roots), and a carbon conversion factor of 0.456 (Milton et al., 2026). When sampling depth could not be performed due to obstructions encountered during coring or digging, carbon estimates for mineral soil and root pools were adjusted proportionally to reflect full target depths. In cases where depth intervals were missing entirely, C pools were estimated using mean percent carbon and bulk density values from equivalent depths. All roots C stocks were converted to Mg ha^{-1} prior to analysis.

$$[3] \quad C_{MS} = BD \times depth \times \%C \times 10000$$

$$[4] \quad C_{FR\ and\ CR} = BD_{each\ pool} \times depth_{each\ pool} \times 0.456 \times 10000$$

2.5. Data analysis

To evaluate variation in ecosystem carbon storage across the time since restoration gradient, ecosystem C pools were modeled using a fixed-effects linear model (*stats* package). Although data were collected from multiple sites, exploratory assessments showed that the primary patterns in carbon storage were driven by the restoration timeline and moisture regime, rather than site-specific effects. Therefore, individual plots were treated as independent replicates to evaluate the generalizable effects of time since restoration (continuous), prairie moisture regime (dry vs. mesic), and their interaction. Remnant prairies were excluded from the model fitting process to ensure the regression parameters reflected the successional trajectory of restoration; these sites were subsequently used as independent reference benchmarks in visualizations to assess the degree of convergence toward mature C-storage levels.

Model assumptions, including normality of residuals and homogeneity of variance, were verified using the *performance* package (Lüdecke et al., 2021). When necessary, response variables were log-transformed to meet these requirements. For each model, linear regressions were fit to quantify the relationship between restoration time and C storage, and subsequently, Type II

analysis of variance (ANOVA) was used via the *car* package (Fox & Weisberg, 2019) to evaluate the statistical significance of model predictors within the regression framework. Model performance was summarized using adjusted coefficients of determination (R^2), and predicted values with 95% confidence intervals were generated using *ggeffects* (Lüdecke, 2018). Differences in temporal trajectories between moisture regimes were further evaluated using estimated marginal trends of restoration time (*emtrends* from the *emmeans* package; Lenth & Piaskowski, 2017).

Pool-specific adjustments were employed to account for the unique distributional properties of each carbon pool. For fine root C, exploratory analysis suggested non-linear trends. Consequently, a second-order polynomial regression was applied to capture potential peak values in C storage over time, as this approach demonstrated improved model fit and statistical significance of the non-linear term compared to linear alternatives. Leaf litter and soil organic layer (LL + SOL) data exhibited zero-inflation and heteroscedasticity and were therefore analyzed using generalized additive models (GAMs) with a Tweedie error distribution (*mgcv* package; Wood, 2017). This modeling approach allowed the recovery trajectory of carbon to vary independently between dry and mesic sites, providing the flexibility to capture whether these moisture regimes followed distinct patterns of C accumulation over time. Finally, models for coarse roots were log-transformed with a small constant (+0.001) added to account for zero values.

To evaluate shifts in C allocation patterns among plant functional types (graminoids, forbs, woody plants, vines, and mosses), multivariate composition was analyzed using non-metric multidimensional scaling (NMDS) based on Euclidean distances (*vegan* package; Oksanen et al., 2017). Euclidean distance was applied because the data represent continuous, physical measurements of carbon mass (Mg ha^{-1}). This metric is specifically suited for ratio-scale data, where absolute magnitude is the primary focus. Because zero-values in this dataset represent

the physical absence of carbon rather than a sampling artifact, a distance metric was required that treats these zeros as meaningful quantitative values, thereby preserving the absolute structural differences between sites. Differences in functional composition along the restoration gradient were tested using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations (M. J. Anderson, 2001) with statistical significance assessed at $\alpha = 0.05$. All statistical analyses were conducted in R version 4.4.3 (R Core Team, 2025) .

3. Results

3.1. Carbon storage across ecosystem pools under continuous prairie restoration

Total ecosystem carbon increased with time since restoration ($p = 0.009$; Table S1) and ranged from approximately 50 to 72 Mg C ha^{-1} across this gradient (Fig. 2A; Table S2). Observed values suggest lower C stored at early stages (<1 year: 50.80 Mg C ha^{-1}; 1 year: 57.05 Mg C ha^{-1}) and generally higher values at intermediate and advanced stages (4 years: 57.65 Mg C ha^{-1}; 5 years: 71.74 Mg C ha^{-1}; 9 years: 59.66 Mg C ha^{-1}), although considerable variability occurred across all time points. Remnant prairies exhibited relatively high total carbon (62.81 Mg C ha^{-1}), with values falling within the range observed for intermediate and advanced restoration but generally exceeding early-stage values. Moisture regime ($p = 0.17$) and the interaction between time and moisture ($p = 0.34$) did not influence total carbon, indicating consistent temporal trajectories across moisture conditions.

Groundlayer vegetation C increased significantly with restoration time ($p = 0.007$; Table S1; Fig. 2B). Values ranged from 0.82 to 3.26 Mg C ha^{-1} (Table S2), with lower values generally observed at early restoration (<1 year: 1.83 Mg C ha^{-1}; 1 year: 0.82 Mg C ha^{-1}) stage and greater changes at intermediate (4 years: 3.04 Mg C ha^{-1}; 5 years: 2.94 Mg C ha^{-1}) and later (9 years: 3.26 Mg C ha^{-1}) stages. Remnant prairies (2.14 Mg C ha^{-1}) overlapped with the range observed in restored systems. Moisture levels significantly influenced GLV carbon ($p = 0.02$), with higher values observed in mesic systems (Fig. 2C), while the interaction between time and moisture was not significant ($p = 0.88$). Additionally, leaf litter and soil organic layer carbon (LL + SOL) exhibited strong temporal variation along the time-since-restoration gradient (Fig. 2D; Table S2). Values remained near zero during early stages (<1–5 years) and increased markedly at advanced restoration stages, reaching approximately 1.99 Mg C ha^{-1} at 9 years. Linear models detected a significant effect of time ($p = 0.0003$; Table S1); however, generalized

additive models (GAMs) better captured the non-linear pattern of change over time, particularly the increase at advanced restoration. Moisture conditions ($p = 0.66$) and the interaction between time and moisture ($p = 0.09$) were not significant. Remnant prairies exhibited relatively low LL + SOL carbon (0.39 Mg C ha^{-1}), overlapping with early-stage conditions.

Mineral soil carbon (0–30 cm) exhibited substantial variability across the restoration gradient, ranging from approximately 48 to 68 Mg C ha^{-1} (Table S2). Observed values were occasionally higher at intermediate stages, particularly at year 5 (67.80 Mg C ha^{-1}), where they exceeded values observed at early (<1 year: 48.31 Mg C ha^{-1}) and advanced restoration stages (9 years: 52.68 Mg C ha^{-1}), and in some cases were greater than those observed in remnant prairies (57.47 Mg C ha^{-1}). Despite this variability, linear models detected no significant effects of restoration time ($F = 0.48$, $p = 0.49$), moisture regime ($F = 3.06$, $p = 0.09$), or their interaction ($F = 0.85$, $p = 0.36$; Table S1). Mineral soil C values showed substantial overlaps across all restoration stages, indicating the absence of a consistent directional trend within the timeframe evaluated.

Depth-specific patterns of soil carbon concentration (%C) and bulk density (BD) provided additional context for mineral soil dynamics across the restoration gradient (Fig. S1; Table S3). %C was consistently highest in the surface (0–10 cm) layer and declined with depth, whereas BD increased with depth. Across time since restoration, surface soils showed greater variability in %C, with higher values generally observed at intermediate (4 years: 1.18, 5 years: 1.58) and remnant (1.58) stages. Bulk density tended to be higher at earlier (<1 year: 1.65 g cm^{-3}, 1 year: 1.51 g cm^{-3}) and lower at advanced (9 years: 1.36 g cm^{-3}) stages and in remnant prairies, particularly within the 0–10 cm layer (Fig. S1). Patterns at deeper soil layers (10–20 and 20–30 cm) were more constrained and exhibited less variation across time since restoration. These depth-dependent patterns, together with the absence of significant temporal trends in total

mineral soil C, indicate substantial overlap in soil conditions across restoration stages within the sampled timeframe.

Fine root carbon (0–30 cm) varied significantly with restoration time ($p = 0.007$; Table S1; Fig. 2E), with patterns consistent with a second-order polynomial response. Observed values ranged from 0.66 to 2.80 Mg C ha^{-1} across the restoration gradient (Table S2), with lower values at early (<1 year: 0.66 Mg C ha^{-1}) stages and greater variability at intermediate (4 years: 2.30 Mg C ha^{-1}, 5 years: 1 Mg C ha^{-1}) and later (9 years: 1.72 Mg C ha^{-1}) stages. Remnant prairies exhibited relatively high values (2.80 Mg C ha^{-1}). Despite these variations, neither moisture regime ($p = 0.43$) nor the interaction between time and moisture ($p = 0.15$) influenced fine root C. Coarse root carbon remained consistently low across all sites, with mean values near zero (<0.01 Mg C ha^{-1}; Table S2; Fig. 2F). Linear models indicated no significant effects of time since restoration ($p = 0.415$), moisture regime ($p = 0.47$), or their interaction ($p = 0.35$; Table S1), indicating minimal variation in this pool across the restoration gradient.

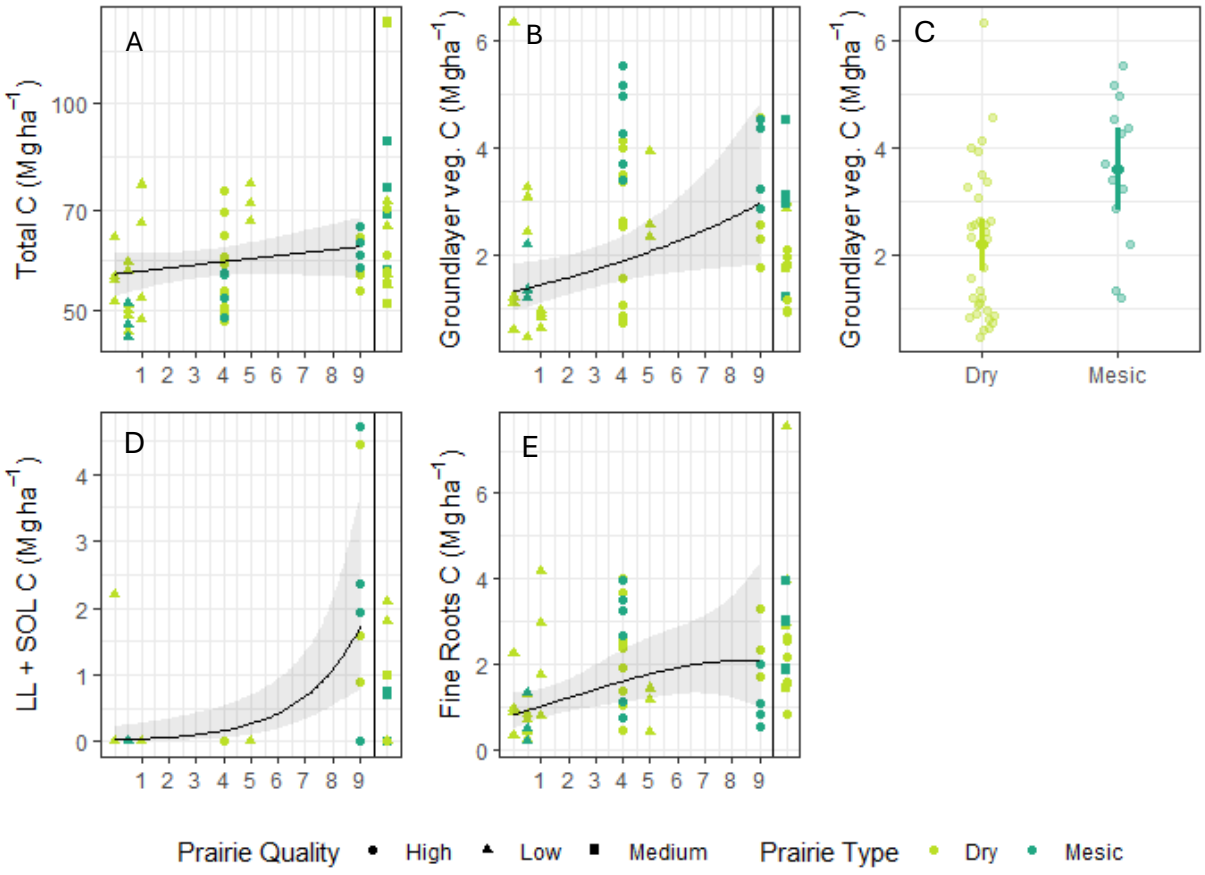

Figure 7. **Carbon storage across selected ecosystem pools along a time since restoration gradient.** Panels show relationships between time since restoration and total ecosystem C, groundlayer vegetation, leaf litter + soil organic layer (LL + SOL), mineral soil, and fine root carbon (Mg C ha^{-1}). Points represent individual plot observations, colored by ecosystem moisture and shaped by prairie quality. Solid lines indicate model-predicted trends with 95% confidence intervals. The vertical line separates restored sites from remnant prairies. Patterns reflect significant effects of time since restoration on total C (A), groundlayer vegetation (B), as well as significant effects of moisture (C), LL + SOL (D), and fine root pools (E).

3.2. Groundlayer vegetation and plant functional types C allocation

Groundlayer vegetation C differed among restoration stages and showed pronounced shifts in both magnitude and functional composition across the restoration gradient (Fig. 3; Table S3). Across all sites, GLVC ranged from <1 Mg C ha^{-1} in early restoration (1 year: 0.82 Mg C ha^{-1}) to >3 Mg C ha^{-1} in intermediate (4 years: 3.04 Mg C ha^{-1}) and advanced stages (9 years: 3.26 Mg C ha^{-1}). Immediately following restoration (t = 0), GLVC averaged 2.31 Mg C ha^{-1} and was dominated by graminoids, which accounted for 96.22% of total vegetation C (Fig. 3A; Table S3).

Contributions from forbs (0.18%), woody vegetation (0.10%), vines (3.48%), and moss (0%) were minimal, indicating low functional diversity in C allocation at this stage.

During early restoration (<1–1 year), GLVC declined and ranged from 0.82 to 1.83 Mg C ha^{-1} due to increased variability in composition (Table S3). Graminoid dominance decreased substantially, accounting for 71.68% of the total GLVC (mean value). Forb contributions increased to 10.38% average of total C, while woody vegetation contributed ~9.03% and vines became more apparent, 8.14% in average, reflecting increased compositional heterogeneity during early stages. At intermediate stages (4–5 years), GLVC increased to 2.94–3.04 Mg C ha^{-1}, coinciding with a shift in functional composition characterized by reduced graminoid dominance (62.87%; Fig. 3A) and a pronounced increase in forb contribution (36.11%–36.87% respectively; Fig. 3B). In contrast, woody vegetation declined to near-zero contribution, while vines and moss remained minor components (<1%; Fig. C-E).

By advanced restoration (9 years), GLVC reached its highest observed values (3.26 Mg C ha^{-1}) and variability among observations decreased (Table S3). Graminoids again dominated carbon storage (73.94%; Fig. 3A), while forb contributions declined relative to intermediate stages (24.74%; Fig. 3B). Woody vegetation, vines, and moss remained negligible (<1%; Fig. C-E). Remnant prairies showed slightly lower total GLVC (2.14 Mg C ha^{-1}; Table S3) than 9-year sites but exhibited comparable compositional structure. Graminoids accounted for 64.78% of total C, while forbs and woody vegetation contributed ~14% each, indicating a more balanced but still graminoid-dominated system.

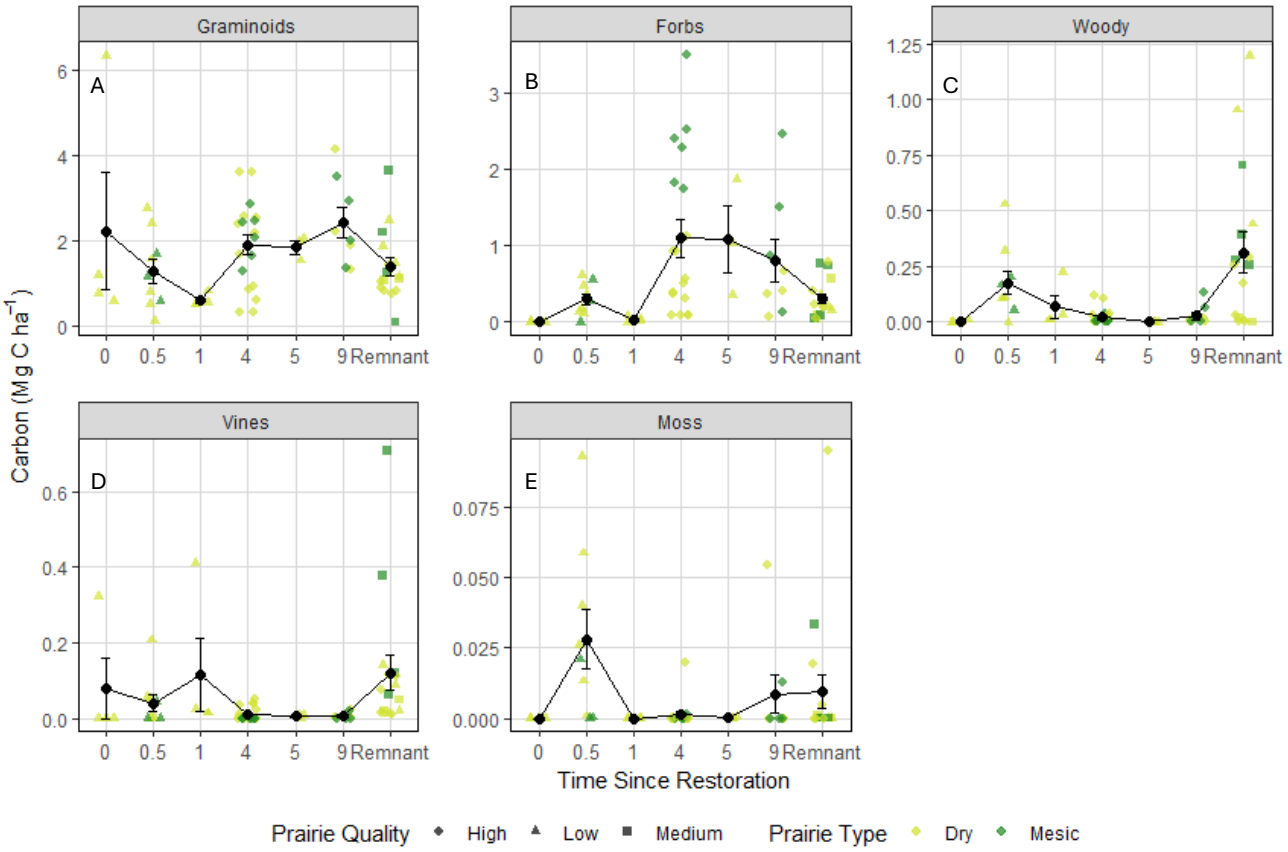

Figure 8. **Carbon distribution (Mg C ha^{-1}) by plant functional type (PFT; graminoids: A, forbs: B, woody: C, vines: D, moss: E) across a time since restoration gradient in prairies (dry and mesic)** with remnants included as the benchmark reference state. Solid black lines indicate the PFT-level mean values, with error bars denoting standard error (SE). Y-axes are scaled independently across panels to account for the varying magnitudes of C accumulation among functional groups. The overall visualization highlights a distinct temporal shift in ecosystem C contribution: an initial dominance of graminoids is followed by increased contributions from forbs and woody vegetation during intermediate restoration stages (years 4–5), culminating in a re-establishment of graminoid dominance under remnant prairie conditions.

Across the restoration gradient, graminoids consistently represented the largest C pool within functional groups, dominating immediately following restoration and re-establishing dominance in later stages. In contrast, forbs exhibited a distinct mid-successional pattern, increasing from near-absence at pre-restoration to peak contributions at intermediate stages before declining. Woody vegetation showed early but transient contributions, whereas vines were episodic and most pronounced during early restoration. Mosses contributed negligibly across all time points (<1%).

Multivariate analysis provided additional insight into functional group composition based on C contributions (Figure 4; Table S5). The NMDS ordination produced a low-stress solution (stress = 0.036), indicating a reliable representation of compositional patterns. Functional groups were distinct in ordination space, with graminoid-associated carbon aligned toward positive NMDS1 values, forbs associated with positive NMDS2 values, and woody, vine, and moss contributions aligned along negative NMDS1 values, reflecting their differing roles in compositional variation (Table S5).

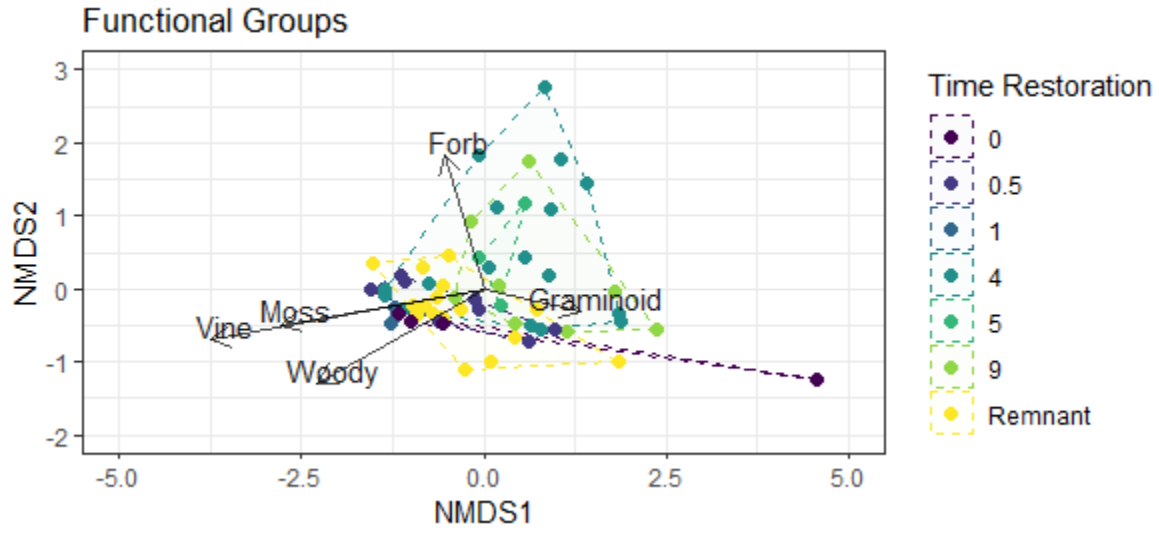

Figure 9. **Non-metric multidimensional scaling (NMDS) ordination of groundlayer vegetation composition based on C contributions of plant functional groups**. Points represent individual plots colored by time since restoration, with dashed polygons delineating variation within each time category. Vectors (arrows) indicate the direction and relative contribution of functional groups to compositional differences. The ordination illustrates a temporal shift in community composition along the time since restoration gradient, with early restoration plots exhibiting greater dispersion and later stages converging toward graminoid-dominated assemblages characteristic of remnant prairies.

Despite these differences among functional groups, plots exhibited substantial overlaps across the time-since-restoration gradient. Pre-restoration plots (t = 0) occupied a more distinct region of ordination space, consistent with strong graminoid dominance at this stage, whereas early, intermediate, and advanced restoration plots were broadly interspersed. This indicates that plots with similar functional composition occur across multiple time points rather than forming discrete, time-structured groupings. Permutational multivariate analysis (PERMANOVA) further

supported this pattern, showing no significant effect of time since restoration on functional group composition ($F = 0.36$, $p = 0.72$; Table S5). Together, these results indicate that although the relative contributions of plant functional types shift over time (Figure 3; Table S4), overall multivariate composition remains highly variable and does not follow a consistent directional trajectory along the restoration gradient.

4. Discussion

Carbon dynamics in ongoing prairie systems reflect an integrated response across ecosystem pools, vegetation composition, and soil processes, rather than a uniform trajectory of accumulation. Across the restoration gradient, C storage varied significantly among aboveground pools, whereas mineral soil C remained stable. While some pools in restored systems approached values observed in remnant prairies, these comparisons should be interpreted with caution; the remnant sites in this study represent systems of moderate condition rather than fully intact, high-quality reference standards. Main findings suggest that carbon storage was driven by contrasting responses among aboveground, surface, and belowground pools, indicating that restoration operates through multiple, temporally distinct processes. In addition, shifts in plant functional types, particularly the dominance of graminoids and the increase in forbs during intermediate restoration stages, might demonstrate that vegetation composition shifts over time, and these changes do not translate into uniform belowground C accumulation (Du et al., 2020; Jackson et al., 2017). These findings fill an important gap in southeastern U.S. prairie systems, where empirical data remain limited, and suggest that restoration trajectories in this region follow broader patterns observed in grasslands globally, where early gains in vegetation C precede slower, constrained changes in the soil carbon pool (Conant et al., 2017; Fornara & Tilman, 2008).

The contrasting responses among ecosystem C pools highlight the importance of turnover rates and stabilization processes in shaping carbon recovery. Rapid responses in groundlayer vegetation and other surface pools are consistent with findings from temperate and restored grasslands, where biomass production drives early C inputs and subsequent allocation belowground (Bai & Cotrufo, 2022; Bloom et al., 2016; Conant et al., 2017; Wu et al., 2025; Y. Yang et al., 2019). These patterns align with the significant increases observed in GLV and litter-

associated carbon pools, indicating that early stages of restoration are primarily driven by aboveground productivity and surface inputs.

In contrast to these dynamics, the limited and non-directional response of MS carbon (0-30 cm) exhibited no significant response to restoration time, reflecting its dependence on longer-term accumulation and stabilization processes (Ambaw et al., 2026; Rumpel & Kögel-Knabner, 2011). The absence of a significant temporal trend, combined with the similarity in values between pre-restoration sites and remnant prairies, suggests that these mineral soil pools were not substantially depleted prior to restoration, or that they possess high inherent resilience. Rather than observing a recovery trajectory, our data indicate that mineral soil C stocks are maintained across this restoration gradient. This stability may point out that legacy effects from prior land use, while potentially influencing soil structure, did not result in a consistent loss of total soil carbon that requires a recovery period (Larson et al., 2020). Consequently, the decoupling observed between aboveground vegetation increases and soil C stability suggests that even where restoration successfully increases plant-derived inputs, those inputs might not currently be driving net increases in mineral soil C storage within this timeframe (Ambaw et al., 2026; Li et al., 2025), but may be mediated by microbial processing, mineral interactions, and nutrient availability(Bai & Cotrufo, 2022; Cotrufo et al., 2013; She et al., 2024; Wardle, 1992; Wiesmeier et al., 2019b).

Changes in soil properties, specifically the depth-specific shifts in surface %C and BD, could provide evidence of gradual soil development during restoration. While significant variability in these near-surface properties was observed at later restoration stages, these changes did not translate into significant increases in total MS carbon storage. This decoupling suggests that early restoration may be characterized primarily by surface-level physical and chemical changes rather than net accumulation of soil C at depth (Arije et al., 2024; Hernández et al., 2013). Improvements in porosity and surface organic matter concentrations may indicate a shift toward

remnant-like soil conditions, but the stability of the total soil C pool suggests that carbon accumulation lags these structural changes. This pattern is consistent with the view that soil C recovery is a gradual process that reflects the long-term integration of soil development, organic matter inputs, and carbon stabilization mechanisms (Baer et al., 2002; Fornara & Higgins, 2022).

Fine roots play an important role in linking above- and belowground C dynamics. The significant but non-linear response of FR carbon across the restoration gradient, together with relatively high values in remnant prairies, suggests an increase in belowground allocation as plant communities develop. This pattern is consistent with global grassland studies, where fine roots represent a dominant pathway for carbon inputs to soils and can equal or exceed aboveground contributions (Jackson et al., 1997, 2017; McCormack et al., 2015). Yet, the absence of a corresponding increase in mineral soil C, despite greater FR inputs, indicates that enhanced belowground allocation does not immediately translate into stabilized soil C (Malhotra et al., 2025; McCormack et al., 2015). Recent global analyses further show that the relationship between fine-root inputs and mineral-soil C storage is highly variable and ecosystem-dependent, even in grasslands, where coupling is generally stronger than in forested systems (Malhotra et al., 2025). The FR carbon stored in remnant prairies suggests a more developed belowground system with sustained inputs, whereas restored prairies may be progressing toward similar trajectories despite delayed soil C responses.

Conversely, coarse root C remained consistently low across all restoration stages, reflecting the herbaceous-dominated structure of prairie ecosystems. While coarse roots can represent a substantial C pool in woody systems due to their slow turnover and long residence times (Mokany et al., 2006), their minimal presence here indicates a limited contribution to overall carbon storage. Slightly higher values in remnant prairies likely reflect minor woody components or legacy root biomass (An et al., 2026b), although these contributions remain negligible relative

to other pools. This reinforces that C storage in prairie systems may be primarily driven by herbaceous vegetation and fine root dynamics rather than persistent woody biomass.

Surface litter and organic layers (LL + SOL) further illustrate the role of disturbance in regulating C dynamics. In fire-maintained systems such as prairies, these pools reflect cycles of production and removal rather than continuous accumulation (Clarke et al., 2026; Hou et al., 2021; Neary & Leonard, 2020; Xu et al., 2022). The strong, non-linear increases observed in LL + SOL carbon likely capture periods of accumulation between disturbance events. Prescribed fire, a common management practice in these systems, consumes litter and surface organic material (Gates et al., 2017), limiting long-term build-up despite increasing productivity (Neary & Leonard, 2020). These dynamics highlight the importance of disturbance regimes in regulating carbon storage and emphasize that productivity increases do not necessarily correspond to sustained accumulation in surface pools.

Changes in plant functional composition strongly regulate the distribution of C across ecosystem pools. Graminoid dominance across restoration stages and in remnant prairies is consistent with common trends in prairie ecosystems, where perennial C_4 grasses drive C inputs and maintain system structure (Fornara & Tilman, 2008; Huang et al., 2024; Ott & Hartnett, 2015). The persistence of graminoid dominance across all time points, including pre-restoration conditions, underscores the strong role of environmental filtering and disturbance regimes in shaping community structure. In southeastern prairie systems, frequent prescribed fire likely reinforces this pattern by promoting fire-adapted grasses and suppressing woody encroachment (Larson et al., 2020). Similar dominance patterns have been observed across grassland systems globally, where productivity and community composition are heavily influenced by dominant functional groups rather than species richness alone (Adler et al., 2011; Baer et al., 2002).

Despite consistent graminoid dominance, the relative contributions of subordinate functional groups varied across the restoration gradient. Forbs showed a marked increase during

intermediate restoration stages, reaching peak proportional contributions at 4–5 years. This transient increase is consistent with patterns of successional turnover observed in restored grasslands, where multiple functional groups temporarily co-occur before being filtered by competition and disturbance (Grman et al., 2021; Hernández et al., 2022). Although such shifts in functional composition are often associated with increased complementarity and potential enhancement of ecosystem processes (Chen et al., 2018; Tilman et al., 2001; Y. Yang et al., 2019), the present study did not directly quantify ecosystem functioning. Instead, the observed increase in forb contribution likely reflects shifts in biomass allocation and resource use rather than a measurable increase in ecosystem productivity.

Importantly, these temporal shifts in relative C allocation among functional groups did not translate into big differences in overall multivariate community composition. The NMDS ordination revealed considerable overlap among plots across restoration stages, and PERMANOVA results indicated no significant effect of time since restoration on functional group composition. This suggests that, although the proportional contributions of graminoids, forbs, and other groups change through time, overall community composition remains highly variable and does not follow a single, directional trajectory. These patterns are consistent with the thought that grassland communities can exhibit multiple assembly pathways under similar environmental conditions, resulting in compositional variability even when dominant functional structure is maintained (Adler et al., 2018). Rather than converging toward a single compositional endpoint, prairies in restoration appear to maintain a consistent functional framework (graminoid dominance) while allowing flexibility in the contributions of subordinate groups (Baer et al., 2016; McCain et al., 2010; Pfeifer-Meister et al., 2012). This interpretation is supported by the broad overlap observed in ordination space and the absence of clear clustering by restoration time, indicating that plots with similar functional composition occur across multiple stages. Similar outcomes have been reported in restoration studies where

communities achieve comparable functional characteristics without converging taxonomically or compositionally (Grman et al., 2021; Newbold et al., 2020).

Woody vegetation, vines, and mosses contributed minimally to total groundlayer C across the time since restoration gradient, although woody vegetation showed relatively higher contributions in remnant prairies. This difference suggests that remnant systems may retain greater plant structural complexity (Polley et al., 2005) despite similar levels of overall C storage. The generally low contribution of woody vegetation in ongoing restoration plots likely reflects the influence of prescribed fire, which limits the accumulation of long-lived woody biomass and maintains herbaceous dominance. While these minor functional groups contribute little to total C, they may still play roles in structuring habitat heterogeneity and influencing localized processes.

Carbon storage in ongoing restoration prairie systems reflects interacting aboveground and belowground processes rather than a single, consistent pathway of recovery. Although groundlayer carbon increased during intermediate and late restoration stages, these changes primarily reflect shifts in biomass rather than broader ecosystem functioning. While restored systems approached remnant prairies in terms of groundlayer C magnitude, this apparent convergence likely reflects the moderate quality of remnant sites included in this study. In less degraded, fire-maintained prairie systems, carbon storage may be substantially higher, and recovery trajectories may diverge more strongly over similar timescales. Differences in functional composition, such as the greater relative contribution of woody vegetation in remnants, further indicate that restored prairies may approximate, but not fully replicate, reference conditions. As one of the first assessments of carbon dynamics in southeastern U.S. prairies, this study provides an important baseline for understanding restoration trajectories in the region. Future work should incorporate additional metrics such as functional diversity indices, other nutrient processes, and microbial dynamics, to better understand the mechanisms

driving carbon stabilization and long-term ecosystem development in restored prairie systems. Overall, these findings indicate that restoration alters the relative contributions of plant functional groups to carbon storage while maintaining compositional variability, suggesting that prairie restoration can support carbon sequestration and ecosystem resilience even as systems follow diverse trajectories toward remnant conditions.

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Appendix B. Supplementary material

Table S6. **Statistical output of models evaluating the effects of restoration time (continuous), ecosystem moisture (dry vs mesic), and their interaction on carbon pools.** F-values and p-values are derived from Type II ANOVA of linear models. For leaf litter and soil organic layer (LL + SOL), linear model results are presented for consistency with other pools; however, generalized additive models (GAMs) were additionally used to evaluate the effects of time, moisture, and their interaction structure (bottom), confirming the significance of temporal trends and the absence of moisture and interaction effects. Reported values correspond to the main effects of time and moisture. Bolded p-values indicate $p \leq 0.05$.

	Overall model			**Time since restoration**		**Moisture level**		**Time × Moisture**	
	F-statistic	p-value	Adj. R^2	F-value	p-value	F-value	p-value	F-value	p-value
GLV	5.87	**0.001**	0.25	7.78	**0.007**	5.80	**0.02**	0.02	0.88
LL+SOL	6.89	**0.0007**	0.28	15.85	**0.0003**	0.19	0.66	2.89	0.09
Mineral soil	1.68	0.19	0.04	0.48	0.49	3.06	0.09	0.85	0.36
Fine root	3.03	**0.02**	0.18	5.54	**0.007**	0.64	0.43	1.97	0.15
Coarse root	0.83	0.49	-0.01	0.68	0.415	0.54	0.47	0.91	0.35
Total	3.00	**0.04**	0.12	7.44	**0.009**	1.89	0.17	0.95	0.34

	Time since restoration		**Moisture level**	
	F-value	p-value	F-value	p-value
LL+SOL	11.31	**0.002**	0.00	0.81

Table S7. **Mean (± SD) carbon storage (Mg C ha^{-1}) across ecosystem pools at each observed time point along the restoration chronosequence.** Values represent descriptive summaries of observed data and are provided to illustrate variability along the continuous time gradient. Statistical tests evaluating the effects of time, ecosystem moisture, and their interaction are presented separately in Table S1.

Mean (Mg C ha^{-1}) (%)	0	< 1	1	4	5	9	Remnant
GLV	2.31 (2.70)	1.83 (0.95)	0.82 (0.14)	3.04 (1.56)	2.94 (0.85)	3.26 (1.09)	2.14 (1.00)
LL+SOL	0.55 (1.10)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.99 (1.80)	0.39 (0.69)
Mineral soil	52.68 (7.45)	48.31 (4.56)	53.80 (8.34)	52.30 (8.19)	67.80 (3.75)	52.68 (5.22)	57.47 (9.64)
Fine root	1.10 (0.81)	0.66 (0.42)	2.42 (1.46)	2.30 (1.17)	1.00 (0.53)	1.72 (0.90)	2.80 (1.58)
Coarse root	0.00001 (0.00002)	0.00012 (0.00001)	0.0080 (0.003)	0.00045 (0.0004)	0.000 (0.001)	0.00026 (0.0002)	0.0010 (0.0007)
Total	56.64 (5.23)	50.80 (4.48)	57.05 (9.72)	57.65 (7.14)	71.74 (4.49)	59.66 (4.32)	62.81 (9.96)

Table S8. **Mean (± SD) mineral soil carbon concentration (%C) and bulk density (g cm^{-3}) at each observed time point along the restoration chronosequence.** Values represent descriptive summaries of observed data and are provided to characterize variability across the continuous restoration gradient. Inferential statistics from models evaluating the effects of time and moisture are presented separately in Table S1.

Mean (Mg C ha^{-1}) (SD)	0	< 1	1	4	5	9	Remnant
% Carbon	1.41 (0.31)	1.04 (0.15)	1.27 (0.23)	1.18 (0.22)	1.58 (0.07)	1.38 (0.14)	1.58 (0.28)
Bulk Density	1.37 (0.13)	1.65 (0.06)	1.51 (0.06)	1.55 (0.11)	1.47 (0.02)	1.36 (0.16)	1.29 (0.14)

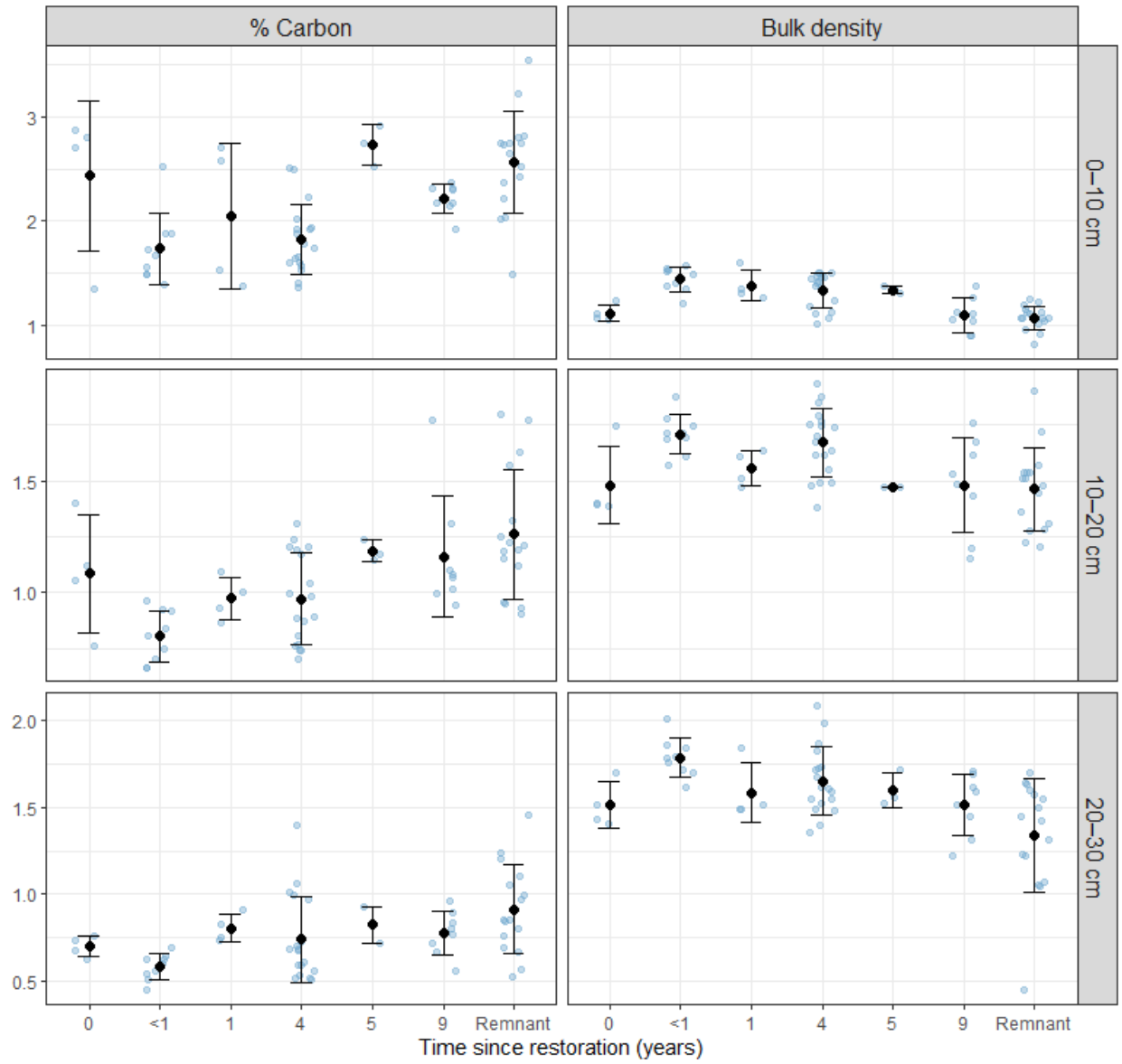

Figure S2. **Mineral soil carbon concentration (%C) and bulk density (g cm^{-3}) across a restoration chronosequence, shown by soil depth (0–10, 10–20, 20–30 cm).** Points represent individual plot-level observations, with overlaid means (± SD). Percent carbon values are expressed as percentages. Time since restoration is treated as a continuous variable, with axis labels corresponding to observed sampling times, including remnant prairies.

Table S9. **Mean carbon stored (Mg C ha^{-1}) and proportional contribution (%) from observed data of ground-layer functional groups across restoration stages (continuous), including pre-restoration (t = 0), early (<1 and 1 year), intermediate (4 and 5 years), late (9 years), and remnant prairies.** Functional groups include graminoids, forbs, woody plants, vines, and mosses. Percent values represent the relative contribution of each functional group to total groundlayer vegetation carbon within each restoration stage. Total C values represent the sum of all functional groups (100%).

Mean (Mg C ha^{-1}) (%)	0	< 1	1	4	5	9	Remnant
Graminoid	2.22 (96.22)	1.29 (70.25)	0.60 (73.11)	1.91 (62.79)	1.85 (62.95)	2.41 (73.94)	1.38 (64.78)
Forb	0.004 (0.18)	0.30 (16.35)	0.036 (4.41)	1.10 (36.11)	1.08 (36.87)	0.81 (24.74)	0.31 (14.41)
Woody	0.002 (0.10)	0.18 (9.67)	0.07 (8.38)	0.02 (0.69)	0.00 (0.00)	0.03 (0.88)	0.31 (14.68)
Vine	0.08 (3.48)	0.04 (2.19)	0.12 (14.09)	0.01 (0.37)	0.01 (0.18)	0.01 (0.18)	0.12 (5.68)
Moss	0.00 (0.00)	0.03 (1.54)	0.00 (0.00)	0.001 (0.04)	0.0001 (0.00)	0.008 (0.26)	0.01 (0.45)
Total C	2.31 (100)	1.83 (100)	0.82 (100)	3.04 (100)	2.94 (100)	3.26 (100)	2.14 (100)

Table S10. **Summary of non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA)** evaluating variation in groundlayer vegetation composition based on carbon contributions of plant functional groups across the restoration gradient. PERMANOVA tested the effect of time since restoration on functional group composition. Functional group scores (NMDS1 and NMDS2) represent the relative position of each group in ordination space, indicating their contribution to compositional variation.

	Distance	Dimensions (k)	Stress	R^2	F-value	p-value
NMDS + PERMANOVA	Euclidean	2	0.036	0.006	0.36	0.716

	NMDS1	NMDS2
Graminoid	1.34	-0.30
Forb	-0.53	1.83
Woody	-2.28	-1.29
Vines	-3.74	-0.70
Moss	-2.77	-0.49

Table S11. **Site locations in the Interior Plateau, southeastern U.S.**, along with ecosystem type sampled, soil moisture level, and time since restoration of each site when the sample collection occurred.

Site name	Ecosystem type	Soil moisture level	Location	Latitude/Longitude	Time since restoration	Land Management
Barnet Woods State Natural Area	Prairie	Dry	Clarksville, Montgomery Co., TN	36°31'33.74'' N 87°33'41.87'' W	Remnant	Annually herbicide treatment Biannual bush hogging No prescribed fire
	Crop field	Dry			Pre-restoration	No restoration management
Best Hope Farm	Prairie	Dry	White Bluff, Dickson Co., TN	36° 4'50.58" N 87°15'27.25" W	Remnant	Annually herbicide treatment (2022-2024) Prescribed fire (2-year interval; starting 2023)
					4 years	Hardwoods clearcut and herbicide treatment (2020) Seeding mix native graminoids and forbs (2021) Prescribed fire (2024)
					5 years	Herbicide treatment (2019) Seeding mix native graminoids and forbs (2020) Bush hogging (2022) Prescribed fire (2-year interval; starting 2023)
Baker Natural Area	Prairie	Dry	Russellville, Logan Co., KY	36°51'17.9" N 86°55'00.4" W	Remnant	Seeding mix native graminoids and forbs as maintenance (2023)

Dunbar Cave State Park	Prairie	Dry	Clarksville, Montgomery Co., TN	36°33’36.43” N 87°18’13.52’’ W	9 years	Annually herbicide treatment (2015-2016) Seeding native graminoids (2017) Prescribed fire (2020) Prescribed fire (dormant season 2024-2025)
		Mesic			9 years	Annually herbicide treatment (2015-2016) Seeding native graminoids (2017) Prescribed fire (2020) Prescribed fire (dormant season 2024-2025)
Fort Campbell Army Base	Prairie	Dry	Hopkinsville, Christian Co., KY + Clarksville, Montgomery Co., TN	36°39’13” N 87°27’35” W	Remnant	Annually prescribed burn (since 2021)
					4 years	Annually prescribed burn (since 2021) Mowing (3-year interval)
Google Prairie	Prairie	Dry	Clarksville, Montgomery Co., TN	36°37’01.26’’ N 87°16’09.00’’ W	<1 year	Annually herbicide treatment (2024-2025) Seeding native graminoids (2025)
					4 years	Herbicide treatment (2020; spotted 2025) Seeding native graminoids (2021) Prescribed fire (dormant season 2023-2024) Endangered species plugs planting (2025)
		Mesic			<1 year	Annually herbicide treatment (2024-2025) Seeding native graminoids (2025)
					4 years	Annually herbicide treatment (2024-2025) Seeding native graminoids (2021) Prescribed fire (dormant season 2023-2024

Guthrie Prairie	Prairie	Mesic	South Guthrie, Montgomery Co., TN	36°38’29.67’’ N 87°09’26.26’’ W	Remnant	Mowing (2021, 2023) Herbicide treatment (2024-2025) Prescribed fire (2024)
Montgomery Bell State Park	Prairie	Dry	Burns, Dickson Co., TN	36°03’45.0’’ N 87°17’45.0’’ W	1 year	Hardwoods clearcut (2023) Seeding mix graminoids and wildflowers (2023) Herbicide treatment (2024-2025)
Wessyngton Plantation	Grazing fields	Dry	Cedar Hill, Robertson Co., TN	36°31’8.48’’ N 87° 0’32.81’’ W	Pre-restoration	No restoration management