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RESEARCH ARTICLE

Savanna soil carbon accrual occurs through particulate organic matter from grass rather than tree biomass, regardless of atmospheric CO_2 levels

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Abstract

1. Afforestation schemes in savannas are increasingly promoted as a carbon storage strategy despite threats to biodiversity. We also lack a clear understanding of how trees and grasses differentially contribute to the major carbon store in savannas, that is, soil organic carbon (SOC) and its fractions. Because SOC fractions vary in their persistence, saturation potential, and vulnerability to loss, it is crucial to understand how shifts in tree cover and biomass will influence both the amount and stability of SOC, particularly under disturbance regimes and future climate scenarios such as rising atmospheric CO_2.
2. Using an Open-Top Chamber system, we examined the responses of five C_3 leguminous savanna tree species and the C_4 grass species *Themeda triandra* to grass-tree competition under ambient (400 ppm) or elevated (550 ppm) atmospheric CO_2. We hypothesised that SOC increases will mostly depend on plant biomass rather than plant life form; and that CO_2 fertilisation will benefit C_3 savanna trees more than C_4 grasses, leading to a loss in labile grass root exudates and thus the mineral-associated organic carbon (MAOC) that depends on these exudates.
3. We found that grasses, not trees, were associated with increased SOC in savanna soil to a depth of 0.3 m due to their relatively large biomass, regardless of CO_2 concentration. Soil planted with both grass and trees had 10% more carbon and 8% more nitrogen compared to trees only. End-member mixing models indicated that 50% to more than 90% of this carbon was grass-derived. Savanna SOC accumulation occurred primarily through below-ground and indirectly through above-ground biomass, which drove the formation of the occluded particulate organic

carbon fraction (oPOC) according to a structural equation model. Similar results were observed for soil nitrogen. The responsiveness of oPOC (and not MAOC) in this savanna soil is different to temperate grassland soils, emphasising the need to understand savanna SOC dynamics.

4. While recognising the limitations of pot culture, our results provide a clear message for policymakers and land managers: Conservation of grassy biomes such as savannas, and not afforestation, aligns with both climate and biodiversity goals.

KEYWORDS
afforestation, climate change, elevated carbon dioxide, nitrogen, woody thickening

1 | INTRODUCTION

Soil is the largest terrestrial store of carbon (C) on Earth, yet we know little about its storage mechanisms in savannas, which cover ca. 20% of the land area (Sankaran et al., 2005). Savannas are a type of open, grassy ecosystem characterised by a continuous layer of mostly C_4 grasses with a low density of trees (Lehmann et al., 2011) and 70% of its ecosystem C resides belowground (Scharlemann et al., 2014). Developing new knowledge about soil C storage pools is critical to understanding the real-world efficacy of ongoing efforts to sequester C in savannas via increasing tree biomass and under climate change, for example via AFR100 afforestation schemes. These schemes have come under heavy criticism for misclassifying ancient grassy biomes such as savannas and thus conflating afforestation with forest restoration (Bond et al., 2019; Parr et al., 2024). Not only does afforestation threaten savanna biodiversity (White et al., 2024; Wieczorkowski et al., 2024) but it remains unclear whether afforestation will increase net ecosystem C. While increased tree density indisputably increases aboveground C in tree trunks/branches/foliage (Abreu et al., 2017), the impact on belowground C stores and their long-term persistence is much less certain.

Besides tree planting, woody plants have increased by 8% in Africa over the last three decades (Venter et al., 2018) and expanded globally (Deng et al., 2021) causing increases in tree cover in historically open ecosystems. Multiple factors are likely responsible for this woody plant encroachment (WPE) including altered disturbance regimes and climate change, specifically wetter and warmer conditions and rising atmospheric CO_2 (Stevens et al., 2017; Venter et al., 2018). Theoretically, elevated CO_2 concentrations can have a 'CO_2-fertilisation' effect on C_3 trees because their photosynthetic pathway is relatively less efficient in CO_2 fixation than that of C_4 grasses, and is not saturated under ambient CO_2 conditions (Ehleringer et al., 1997; Ehleringer & Björkman, 1977). Growth of C_4 grasses can respond to elevated CO_2 particularly under water limitation (Ward et al., 2001). Grasses can also benefit indirectly under elevated CO_2 if reduced stomatal conductance suppresses water loss (and uptake) by adjacent trees resulting in increased soil water availability (Lecain et al., 2003). The responses of grasses to elevated CO_2 depends on C_3/C_4 type or even C_4 biochemical subtype (Wand et al., 2001) and may reverse in the long-term due to down-regulation (Reich et al., 2018). Generally, while a positive growth response of C_3 plants under elevated CO_2 is expected, it is highly variable across geographies and time, possibly occurring only under ideal conditions of for example limited competition or herbivory (Raubenheimer & Ripley, 2022).

Alteration of the relative dominance of grasses and trees in savanna ecosystems is expected to have knock-on effects for the relative quantity and quality of biological inputs to the soil and thus soil C sequestration. Both the quantity and quality (C:N ratio and other plant chemistry) of these inputs are known to drive soil organic carbon (SOC) accrual (Wiesmeier et al., 2019). For instance, the substantial fine root systems of savanna grasses contribute large amounts of C inputs to the soil via biomass carbon, root litter and exudates (Pellegrini et al., 2021). Indeed, the role of grasses in SOC storage was recently illustrated by a Europe-wide study that found higher stocks in grasslands compared to forests (Cotrufo et al., 2019). In a semi-arid African savanna, grasses contributed the bulk of SOC in soil with or without herbivory (Wigley et al., 2020). Compared to plant biomass, the intrinsic properties of leaf litter are thought to exert less influence on SOC over timescales of decades to millennia (von Lützow et al., 2006; Wiesmeier et al., 2019). However, other inputs to the soil may affect the amount and permanency of SOC. For example, increasing density of leguminous trees commonly found in savannas has been linked to increased SOC (Sitters et al., 2012). The mechanism for this likely includes the addition of soil N via the N_2-fixing bacteria associated with these trees (Ludwig et al., 2004), which in turn promotes microbial decomposition and soil organic matter (SOM) formation. However, increasing tree density has had varying effects on SOC (Zhou et al., 2017) with little to no information on how SOC amounts and permanency will change under increased atmospheric CO_2 concentrations. Thus, studies that control tree and grass presence under varying CO_2 would be valuable.

Another area of uncertainty is SOC dynamics in savannas. SOC inventories have relied historically on bulk soil in many ecosystems (Cotrufo et al., 2019). Within the different soil pools or fractions, mineral-associated organic matter (MAOM) is important for C sequestration because of its persistence and slow turnover rate (Poeplau et al., 2018). This fraction commonly results from the utilisation of dissolved organic matter (DOM) such as root exudates by soil microorganisms with subsequent binding to clay minerals and physical

protection in small aggregates (Six et al., 2002). While this fraction is stable and persistent, it has a high N demand (due to its origins from soil microbes) and readily saturates due to a finite number of mineral surfaces onto which it can stabilise (Cotrufo et al., 2013, 2019; Six et al., 2002). Particulate organic matter (POM) comprises mostly structural C compounds of plant origin, is low in N, and is biochemically recalcitrant. While POM has a higher turnover rate than MAOM, it appears not to saturate because it is independent of binding sites (Cotrufo et al., 2019). The individual stabilities and turnover rates of these fractions determine the SOC sequestration potential of a soil, providing more information than bulk SOC alone (Cotrufo et al., 2019). A lot of our knowledge about these fractions comes from European grasslands where grasses are thought to facilitate high C concentrations in the MAOM fraction via root exudates (DOM). However, Cambardella and Elliot (1992) suggested that POM is more important in sandy savanna soils where low clay percentages limit MOAM formation. Thus, it is possible that the capacity and mechanisms of C storage in savanna soils are very different to those in temperate grasslands upon which our knowledge is based.

To address the existing knowledge gaps, we formulated two hypotheses regarding the impacts of tree-grass interactions on SOM and SOC. First, we expect that the total plant biomass will affect SOC concentration more than the plant life form (grasses vs. trees) or species of tree. An alternative is that the combination of grasses and leguminous N_2-fixing trees will result in the highest SOM and SOC sequestration potential than either alone due to the synergistic effects of C-rich exudates from grasses and available N from trees. Second, we expect that CO_2 fertilisation will enhance C_3 tree competitiveness, leading to reduced grass fine roots, exudates and DOM, and less MAOM formation. Following from this, we expect the soil $\delta^{13}C$ signature to reflect the greater contribution of C_3 trees to C sequestration under CO_2 fertilisation.

2 | MATERIALS AND METHODS

We tested our hypotheses using a pot experiment at the Rhodes University Elevated CO_2 Facility (RUECF; Makhanda, Eastern Cape, South Africa; Figure 1a). This facility houses sixteen 3-m-diameter Open-Top Chambers (OTCs) as described by Ripley et al. (2022) and Singini et al. (2025). For detailed information on methods, see the Supporting Information.

FIGURE 1 The grass-tree interaction experiment at the Rhodes University elevated CO_2 facility showing (a) Open-Top Chambers (OTCs); (b) pot culture of trees and grass in one of the OTCs; (c) collection of soil into 10-cm sections during harvest; and (d) roots and nodules of the leguminous tree *Vachellia robusta*.

2.1 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Soil	Pot (0–0.3 m depth)	$n = 6$ soil samples per CO_2/tree/grass combination = 136 samples; $n = 6$ unplanted pots; total samples = 142
Plant life form	Plant life form (tree seedling or grass)	$n = 6$ biomass samples per treatment as above

2.2 | Plant species

Five N_2-fixing C_3 tree species from the Fabaceae family were selected because they are widespread across southern African savannas: *Vachellia karroo* Hayne, *Vachellia sieberiana* (DC.) Kyal. & Boatwr. var. *woodii* (Burtt Davy) Kyal. & Boatwr., *Vachellia grandicornuta* (Gerstner) Seigler & Ebinger *Vachellia robusta* ssp. *clavigera* (E.Mey.) Kyal. & Boatwr., and *Senegalia caffra* (Thunb.) P.J.H. Hurter & Mabb. The selected grass (*Themeda triandra*) is a common C_4 grass in savannas (Figure 1b).

2.3 | Experimental conditions

Each pot contained 17.5 L of local savanna soil, a Eutric cambisol (Table S1). Soil was left unsterilised to supply the natural assemblages of mycorrhizal fungi and rhizobial bacteria. Planted pots contained one of the five savanna tree species either with or without grasses, or with grasses alone. Grasses and trees were grown from seed in seed trays and grasses transplanted into pots ca. 18 months before trees to impose competitive pressure on the latter as would be seen in the field with tree seedlings recruiting in an established grass layer. Potted plants were transferred into OTCs at either an ambient atmospheric CO_2 (aCO_2) treatment of 400 ppm (median daytime CO_2 concentration was 398 ppm with 90% of values falling between 383 and 411 ppm), or elevated atmospheric CO_2 (eCO_2) treatment of 550 ppm (median daytime CO_2 concentration was 545 ppm with 90% of values falling between 496 and 588 ppm). The CO_2 source was derived from fossil (coal) fuel ($\delta^{13}C$ of ca. −24‰) with the supply being adjusted depending on plant drawdown, but we expected the 550 ppm plants to be relatively more influenced by the $\delta^{13}C$-CO_2 of the source. The 400 ppm level approximates the 'green road' in the Shared Socioeconomic Pathway predictions, that is SSP-1.9, while 550 ppm approximates the 'middle of the road' between SSP-2.6 and SSP-4.5 (Meinshausen et al., 2020). Plants received ambient rainfall through the roof of the OTCs, except for two manual waterings during an extended dry period to prevent mortality. On average, plants received ca. 0.30 mm day^{-1}. Six unplanted pots with soil at aCO_2 were used to account for changes to soil C and N in the absence of plants. Plants were harvested ca. 30 months after the start of the experiment.

2.4 | Plant and soil sampling

Aboveground plant biomass was harvested by clipping at soil level followed by oven-drying at 60°C for 72 h, weighing and grinding to a fine powder. The soil column was sectioned into 10-cm layers from the surface downwards (Figure 1c) and immediately placed into bags made from shade netting (40%) to minimise material loss. For each section, the roots and soil were loosened by dropping the section ca. 10 cm onto a metal pan. The root ball and other large pieces of root were gently shaken free of soil and set aside before emptying the soil into a metal pan, gently homogenising and subsampling (ca. 150 g). Soil from the top 30 cm was combined (ca. 450 g), homogenised, air-dried, sieved to 2-mm before storing at room temperature until further processing. Remaining soil was returned to the mesh bags with roots to extract both fine and coarse roots as well as root nodules by washing over stacked 1- and 2-mm sieves. Roots were separated into fine (<2 mm) and coarse (>2 mm) fractions, oven-dried at 40°C for 48 h, followed by 70°C for an additional 24 h, weighed and ground to a fine powder.

2.5 | Soil microbial biomass

Soil microbial biomass was measured based on its proportional relationship with microbial C using the routine chloroform fumigation-extraction method based on Vance et al. (1987), followed by combustion of the extracts and infrared detection of the resulting C (see Supporting Information for more detail).

2.6 | SOM fractions

Air-dried soil was fractionated by particle size as described by Leuthold et al. (2023), excluding the optional density fractionation step because this did not alter the yields of fractions. Briefly, soils were dispersed and agitated in water, after which the floating free particulate organic matter (fPOM) was collected under vacuum filtration and the supernatant kept as DOM. The pellet was further extracted followed by wet-sieving where the <53 µm fraction is designated as MAOM and the 53–2000 µm fraction as occluded particulate organic matter (oPOM). The C in SOM fractions was expressed as free particulate organic carbon (fPOC), occluded particulate organic carbon (oPOC), mineral-associated organic carbon (MAOC), and dissolved organic carbon (DOC). Total SOC was calculated from the sum of all fractions. To ensure accuracy in this approach, the recovery rates of all solid fractions were assessed, and sample processing was repeated if recoveries fell outside the range <95%–>105%. As for C, the fractions of soil organic nitrogen (SON)

were expressed as fPON, oPON, and MAON (DON was not measured). The results were expressed as g C or N kg^{-1} soil by multiplying the C or N% values by 10 and subsequently by the fraction (0–1) that fPOC/N, oPOC/N, or MAOC/N occupied in the bulk soil sample. The SOC sequestration potential (C/N_{SOM}) was calculated according to Cotrufo et al. (2019) as:

$$C/N_{SOM} = \frac{C}{N_{MAOM}} \times f_{MAOM} + \frac{C}{N_{oPOM}} \times (1 - f_{MAOM})$$

where C/N_{SOM}, C/N_{MAOM}, and C/N_{oPOM} are the C/N ratios of the total SOM, MAOM, and oPOM respectively, and f_{MAOM} is the fraction (0–1) of MAOM in the bulk soil.

2.7 | Elemental analysis of soil fractions

Carbon in the DOM fraction was determined using non-dispersive infrared detection of CO_2 after combustion and removal of carbonates. Carbon and N in solid fractions (fPOM, oPOM, and MAOM) were determined using isotope ratio mass spectrometry after removal of carbonates and combustion (see Supporting Information for more detail). Carbonates were removed because they can increase the $\delta^{13}C$ signal (Ramnarine et al., 2011) thus confounding any interpretation regarding the C_3- or C_4 plant origin of the carbon.

2.8 | Isotopic mixing model

The relative contribution of trees or grasses to SOC and its fractions was estimated using a simple end-member mixing model (Post, 2002), commonly used in tracking vegetation influence on soil, e.g. Poeplau et al. (2018). To establish the correct end-members for the model, we measured the C_4 (grass) and C_3 (tree) leaf signatures of the plants grown under both aCO_2 and eCO_2. Separate models at each CO_2 concentrations used these measured values of tree-, grass- and soil-$\delta^{13}C$ values as below.

$$\text{Grass derived SOC}\,(\%) = 1 - \left(\frac{\partial^{13}C[\text{Soil}] - \partial^{13}C[C_4\ \text{Grass}]}{\partial^{13}C[C_3\ \text{Tree}] - \partial^{13}C[C_4\ \text{Grass}]} \right)$$

2.9 | Data analysis

All statistics and plots were performed in R v.4.5.0 (RCoreTeam, 2023). Data was log transformed when necessary to achieve a normal distribution. We used *t*-tests for general comparisons between SOM in planted and unplanted pots. We tested hypotheses via linear mixed effects models using the *lmer* function in the 'lme4' package (Bates et al., 2015) for each response variable, with grass-tree treatment and CO_2 concentration as fixed effects, and plant species and OTC identity as random effects. The most parsimonious model was selected by backward elimination of fixed- and random effect terms using the *step* function, but since grass-tree treatment was of interest it was always maintained in the model. Linear models were used if all random effects were eliminated during backwards elimination. Final models are presented in Table S2 (Supporting Information). For plots, the adjusted means from these models were computed using *lsmeans* from the 'emmeans' package (Lenth, 2024) with differences displayed as different letters using the *cld* function from the 'multcompView' package (Hothorn et al., 2008) after a post hoc Sidak test.

We then plotted Pearson correlations between both plant and soil data using the *pairs.panels* function in the 'psych' package (Revelle, 2024). This guided the development of a structural equation model (SEM) using the 'lavaan' package (Rosseel, 2012) to explain which plant and soil factors contribute directly and/or indirectly to SOC formation. The model was constructed by postulating how plant biomass fractions (total aboveground plant biomass; fine and coarse roots) and microbial biomass would directly or indirectly affect soil C fractions. The most parsimonious, best fit model was a simple mediation model using soil fractions and plant biomass only. Specifically, the direct effects on SOC were belowground biomass, oPOC and MAOC, and the indirect effects were belowground biomass through oPOC or MAOC, aboveground biomass through belowground biomass, and aboveground biomass through oPOC.

3 | RESULTS

3.1 | Soil characteristics

The MAOM fraction had the highest C and N concentrations (Table 1). About 5% of the SOC was in DOC, 7% in fPOC, 27% in oPOC and 66% in MAOC (planted soil), with a similar distribution of percentages in SON fractions. Planting increased the C and N in all fractions (g kg^{-1} soil; Table 1) and increased the fPOC, MAOC, and fPON percentages.

3.2 | Plant biomass effect on SOM

Generally, aboveground biomass of grasses (Figure 2A) was higher than that of trees (Figure 2B) and less affected by interspecific competition (two-fold compared to a three-fold reduction). Grasses grown alone had more aboveground biomass than the combined output of grasses and trees grown together (data in Figure 2). Grass, total tree, and tree leaf biomass were unaffected by eCO_2 ($p = 0.522$, 0.220, and 0.721, respectively). While the total biomass of plants was positively associated with SOC ($r = 0.45$; Figure S1), this relationship was not related to the above-ground biomass of either lifeform (Figure 2C). In fact, SOC decreased with increasing tree biomass. Below-ground biomass was, however, positively correlated with SOC ($r = 0.45$, $p < 0.0001$; Figure 2D), and this was largely due to fine- ($r = 0.44$; Figure S1) rather than coarse roots. While we could not separate tree and grass roots,

TABLE 1 Concentration of C and N in soil organic matter and its fractions of a savanna soil at aCO_2 (400 ppm).

	Unplanted	Planted	Unplanted	Planted
Soil organic matter	**Carbon (g C kg^{-1} soil)**		**Percent of total (%)**	
Total SOC	$7.2^a \pm 0.19$	$8.1^b \pm 0.06$		
DOC	$0.43^a \pm 0.027$	$0.37^b \pm 0.005$	$6^a \pm 0.3$	$5^b \pm 0.1$
fPOC	$0.27^a \pm 0.030$	$0.57^b \pm 0.046$	$4^a \pm 0.4$	$7^b \pm 0.3$
oPOC	$1.7^a \pm 0.09$	$2.2^b \pm 0.06$	$26^a \pm 1.0$	$27^a \pm 0.6$
MAOC	$4.3^a \pm 0.11$	$5.3^b \pm 0.05$	$63^a \pm 0.8$	$66^b \pm 0.7$
	Nitrogen (g N kg^{-1} soil)		**Percent of total (%)**	
Total SON	$0.69^a \pm 0.015$	$0.73^b \pm 0.005$		
fPON	$0.014^a \pm 0.0015$	$0.029^b \pm 0.0023$	$2^a \pm 0.2$	$4^b \pm 0.2$
oPON	$0.17^a \pm 0.011$	$0.21^b \pm 0.006$	$27^a \pm 1.7$	$28^a \pm 0.8$
MAON	$0.46^a \pm 0.008$	$0.52^b \pm 0.005$	$71^a \pm 0.8$	$72^a \pm 0.9$

Note: Different letters indicated a significant difference between unplanted and planted soil per fraction at the $p < 0.05$ level after *t*-tests.
Abbreviations: DOC, dissolved organic C; fPOC/fPON, free particulate organic C/N; MAOC/MAON, mineral-associated organic C/N; oPOC/oPON, occluded POC/N; SOC/SON, soil organic C/N.

visual inspection suggested that much of the fine root mass was from grasses. The SON was also well correlated with plant biomass and fine roots (Figure S1). Grass-tree treatment was a significant term in all plant models, with plant species also being significant for trees (Table S2).

3.3 | Plant life form effect on SOM

We presented the main effects of grass-tree treatment and CO_2 concentration on SOM (SOC and SON) separately because there were no interacting effects. If significant, CO_2 concentration effects were plotted as insets within the main plots. The SOC concentration was higher with grasses than without (Figure 3A), and the lowest SOC and SON concentrations occurred when there were trees only (Figure 3A,B). Despite the positive effect of grasses on SOC and SON, there were no changes in the SOC sequestration potential (C/N_{SOM}; Figure 3C). According to a simple regression analysis, SOC was highly related to SON ($r = 0.87$; $p < 0.001$; Figure S1). The most parsimonious models for SOC and C/N_{SOM} included grass-tree treatment and OTC identity, while the SON model included treatment only (Table S2).

3.4 | The oPOM fraction responded to grass presence

Changes in SOC in these sandy savanna soils were more associated with increases in oPOC (Figure 4B; $r = 0.50$; $p < 0.001$; Figure S1) than in MAOC (Figure 4C; $r = 0.24$; $p < 0.001$; Figure S1) or fPOC (Figure 4A; $r = -0.30$; $p > 0.05$; Figure S1). Similarly, oPON (but not fPON or MAON) increased with grass addition (Figure S4). The expected increases in DOC with grass presence did not occur (Figure 4D), and microbial biomass also did not respond to grass presence or eCO_2 ($p = 0.963$ and 0.351 respectively) ranging widely between 0.3 and 115.3 g C kg^{-1} soil with an average of 33.9 ± 2.15 g C kg^{-1} soil. The most parsimonious models for SOM fractions included grass-tree treatment and the OTC identity, except for fPOM where treatment and plant species were significant terms in models (Table S2). Plotted on a per tree basis, grass addition did not however affect fPOC or fPON (Figure S2).

3.5 | Stable isotope changes

The δ^{13}C-SOC, δ^{13}C-fPOC and δ^{13}C-MAOC values were best described by models that included not only grass-tree treatment but also CO_2 concentration (Table S2). While the presence of grass in tree pots increased δ^{13}C in the direction of a grass signal for fPOC (Figure 5B; Figure S2C), the soil δ^{13}C was generally decreased by eCO_2 (Figure 5A,B,D insets). This was likely because eCO_2 (550 ppm) plants acquired relatively more of the fossil fuel-derived CO_2 source mixed into the air, which would also alter the isotopic signature of plant C inputs to the soil. Grass addition to tree pots increased the δ^{15}N of both fPON and oPON (Figure S4) compared to when either life form was grown separately. Overall δ^{15}N was positively correlated with nodule biomass ($r = 0.26$, $p < 0.01$; Figure S1).

The simple end-member mixing model suggested that >90% of the C in SOC was from C_4 grasses (Figure 6A) for both CO_2 concentrations. The calculated contribution of C_4 grasses to oPOC and MAOC was 90% or more, and between ca. 50% and 63% for fPOC (Figure 6B). Plots for fPOC are presented separately due to rank deficiency where this fraction was very small and sometimes absent from samples. The eCO_2 increased the proportion of C from C_4 grasses in SOC (Figure 6) and significantly increased oPOC relative to that at 400 ppm CO_2. These end-member mixing models were

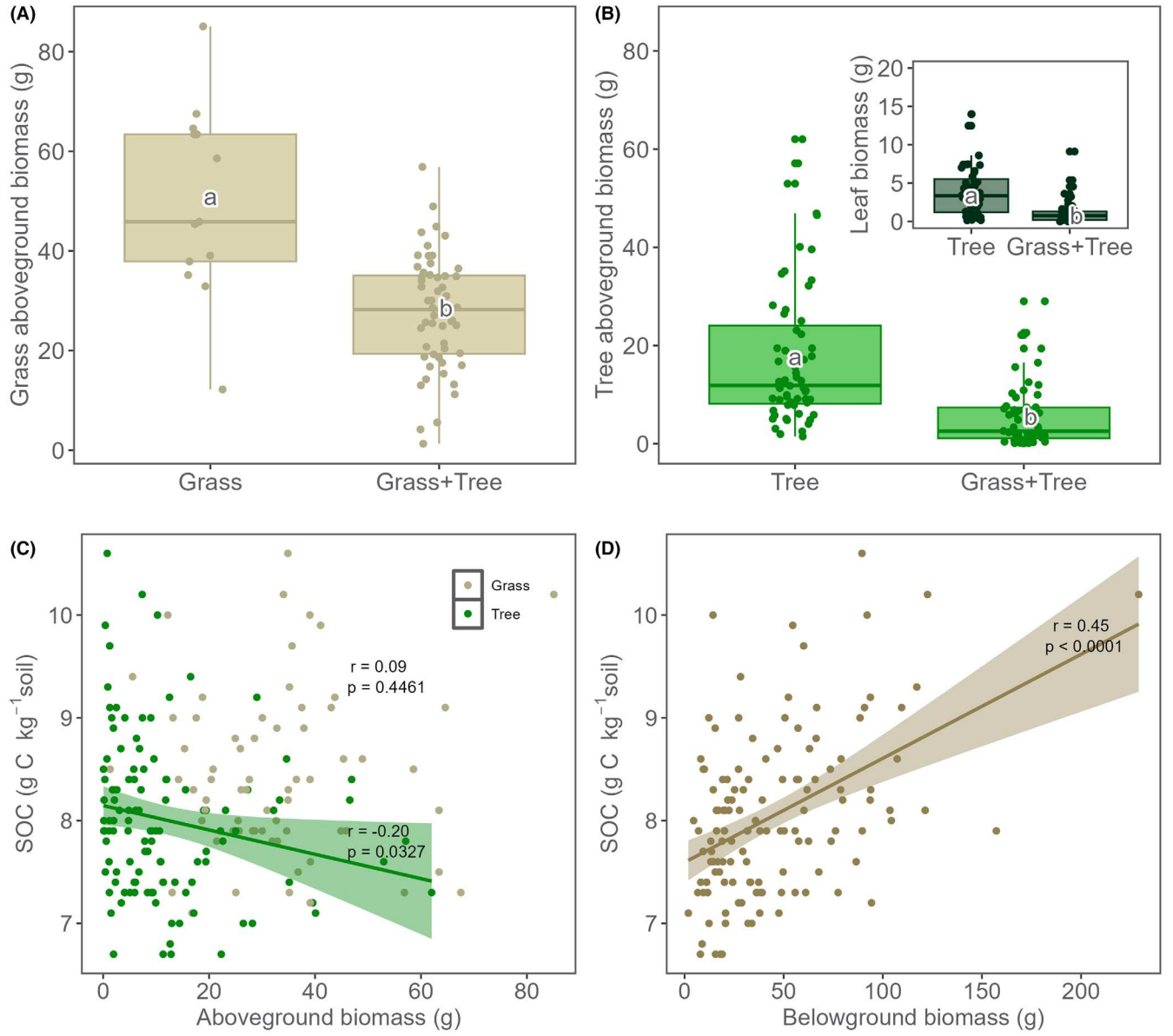

FIGURE 2 The effect of tree competition on grass (*Themeda triandra*) biomass (A) or grass competition on tree (*Senegalia caffra* and *Vachellia* spp.) biomass (B) and of plant biomass on soil organic carbon (SOC; C, D). Letters in (A) and (B) indicate significant difference at the $p < 0.05$ level after a post hoc Sidak test and linear mixed effects models. Since there was no interaction between grass-tree treatment and CO_2 concentration, and the latter was not significant, only the treatment effect is shown. Total aboveground biomass of grasses and trees together in the Grass+Tree treatment was 32 ± 1.5 g.

calculated based on the C_3 and C_4 $\delta^{13}C$ values for the respective CO_2 treatments, which in turn were influenced by the inclusion of fossil fuel-derived CO_2 and are also influenced by the legacy SOC $\delta^{13}C$ of the soil. These issues and the potential contributions of decomposition to uncertainty in this model are later discussed.

3.6 | Pathways to SOC formation

We used significant correlations between SOC, plant and soil attributes (Figure S1) to inform a SEM model of SOC formation in the savanna soil (Figure 7). Preliminary models that included more predictors (e.g. microbial biomass as a mediator of MAOC or oPOC, fine roots, or tree leaf C:N ratio) resulted in a relatively poorer fit compared to the final, most parsimonious model. The correlation coefficients (R^2) indicate the proportion of variance explained by the model. The SOC concentration was directly associated with the amount of belowground biomass (pathway d; Figure 7), which in turn was strongly correlated with aboveground biomass (pathway a). As expected, SOC concentration was also linked to oPOC and MAOC concentrations. The indirect pathway from belowground biomass to MAOC to SOC (b2×c2) was significant but this was not the case for oPOC (indirect pathway b1×c1). Instead, oPOC was predicted by aboveground biomass (pathway e).

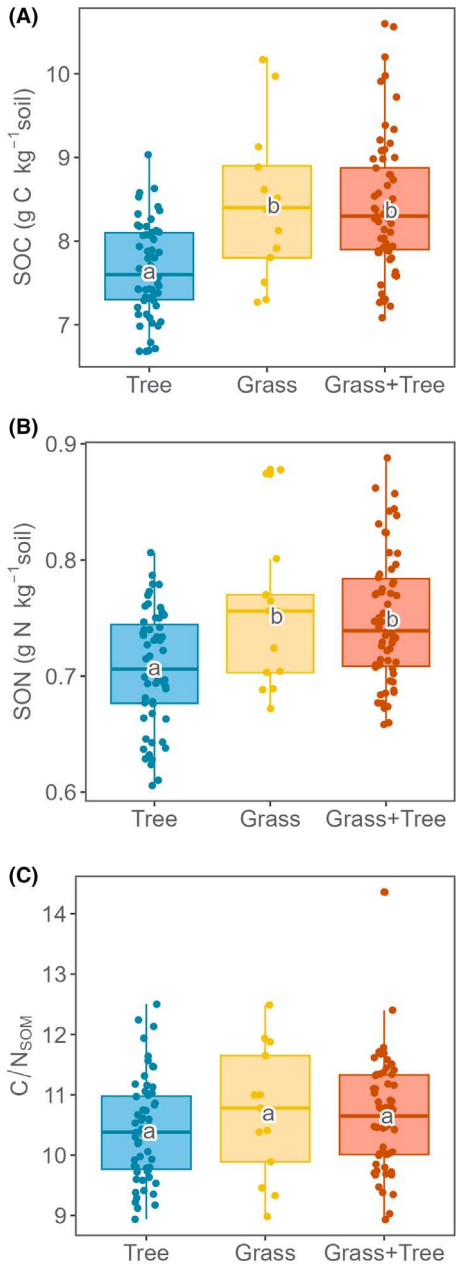

FIGURE 3 Soil organic matter and carbon sequestration potential in a savanna soil with trees only (various *Vachellia* spp., and *Senegalia caffra*), grass only (*Themeda triandra*) or both at 400 or 550 ppm atmospheric CO_2 concentrations, shown as (A) soil organic carbon (SOC); (B) soil organic nitrogen (SON); and (C) SOC sequestration potential (C/N_{SOM}). Only the treatment effect is shown for reasons given in Figure 2. Letters indicate significant difference at the $p < 0.05$ level after a post hoc Sidak test and linear mixed effects model.

4 | DISCUSSION

4.1 | SOC and SON increases linked to plant biomass

As hypothesised, SOC increases were related to plant biomass rather than plant life form, although much of this biomass was grass roots. The SOC and SON increased by ca. 10% and 8% respectively in our savanna soil (0–0.3 m) when planted with grasses (or grasses plus trees) relative to trees alone, regardless of atmospheric CO_2 concentration. Nitrogen-fixation by savanna trees is promoted by grass competition (Cramer et al., 2007). This was shown to be the case in a related study, especially at eCO_2 (Telford et al., 2025), providing an explanation for the increased SON when grasses and trees coexisted. The relatively lower SON when trees were grown alone could have been due to relatively less N_2 fixed (Telford et al., 2025), a higher N uptake compared to soil N inputs, and/or more N allocated to aboveground tree biomass. The C inputs into the soil were similar between trees species and CO_2 concentrations. This despite evidence that trees such as *V. karroo*, and *V. sieberiana* have been shown to upregulate their photosynthetic capacity and increase growth rates under eCO_2 compared to for example *V. robusta* (Singini et al., 2025).

It is notable that the competitive coexistence of grasses with trees had less effect on grass compared to tree productivity in our study. This dominance of grasses is understandable given that each pot has a limited amount of water and N, and the higher water- and N-use efficiency of C_4 grasses (Taylor et al., 2010) would allow them to convert more resources to biomass than the C_3 trees would. The key role of biomass in our results suggests that if trees were larger than in our experiment, they would contribute relatively more to SOC than the 0%–17% that we found. But we speculate that the amount of photosynthetically active grass biomass in savannas is often higher or comparable to trees. For instance, the active biomass of grasses was 1530 kg ha^{-1} (Grunow et al., 1980) compared to 1100 kg ha^{-1} in tree leaves (Rutherford, 1979) in a South African savanna (Nylsvley Nature Reserve), while the total non-living woody biomass was 16,273 kg ha^{-1}.

Increases in tree biomass via WPE have been reported to result in increases (Abreu et al., 2017; Zhou et al., 2017), decreases (Jackson et al., 2002), and to have no effect (Hughes et al., 2006) on SOC. Trees contributed 24% of the total SOC in Kruger National Park (Zhou et al., 2023). Across tropical savannas globally, trees contribute an average of 42% of the total SOC within the surface 1-m of soil (Zhou et al., 2023). The variable effects of plant biomass on SOC partially depend on soil texture, precipitation, and fire (Jackson et al., 2002; Pellegrini et al., 2020; Zhou et al., 2023). For example, more sandy arid areas have relatively higher contributions to SOC from grasses (Zhou et al., 2023). Our study supports this, as SOC in a sandy soil increased with increasing belowground biomass associated with fine grass roots.

4.2 | Much of SOC was grass-derived

Our end-member model suggests that much of the SOC was grass-derived. However, the actual proportions from C_4 grasses (ca. 50%–>90%) should be interpreted with caution. Zhou et al. (2023) previously showed that grass-derived C accounted for over half of the SOC to a soil depth of 1 m at a site in Kruger National Park,

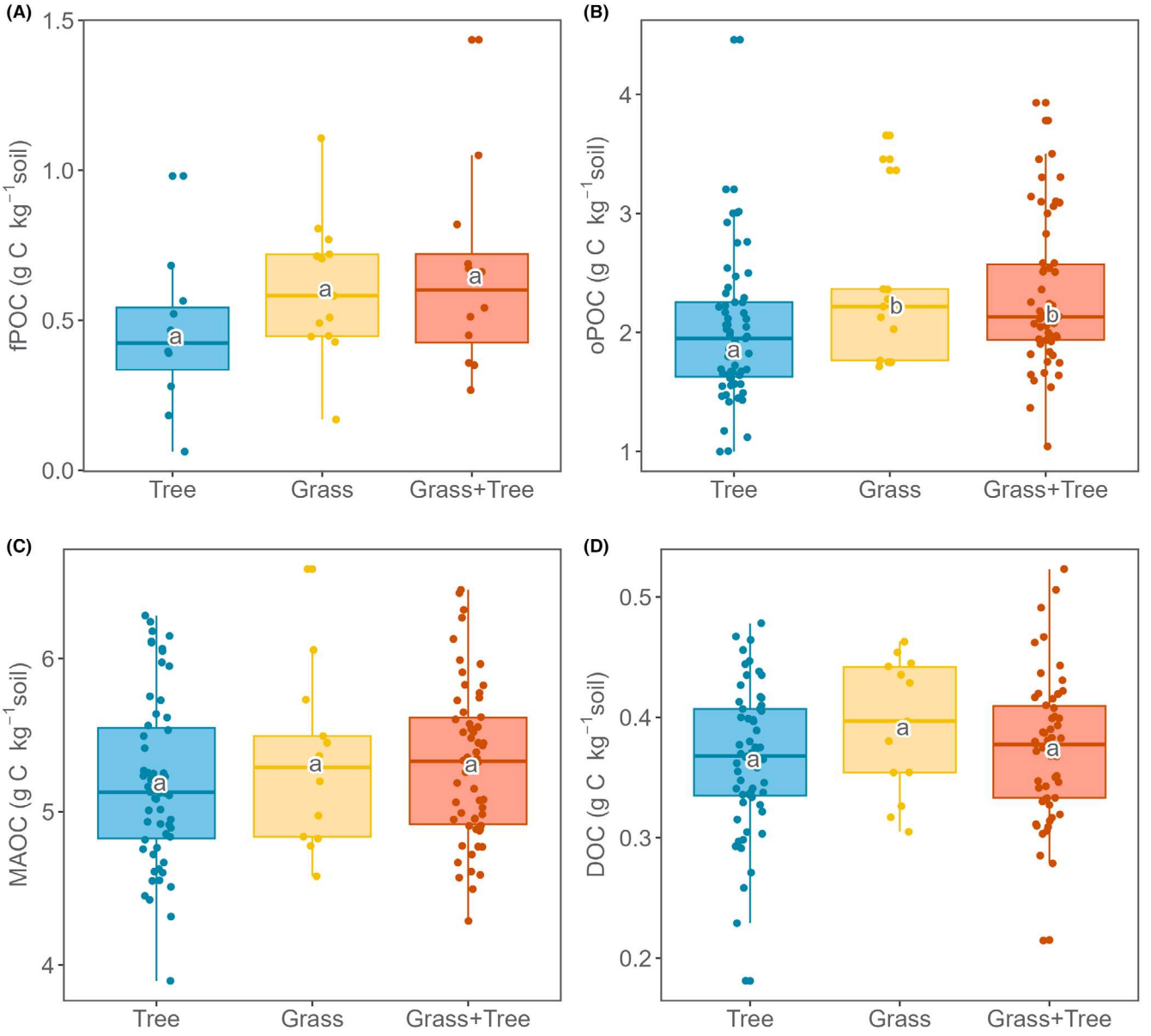

FIGURE 4 Carbon concentration of different soil fractions under the same conditions described in Figure 3, where the fractions shown are (A) free particulate organic carbon (fPOC); (B) occluded POC (oPOC); (C) mineral-associated organic carbon (MAOC); and (D) dissolved organic carbon (DOC). Only the treatment effect is shown for reasons given in Figure 2. Letters indicate significant difference at the $p < 0.05$ level using tests described in Figure 3.

South Africa. The higher estimate from our study may be due to the shallower soil depths where SOC is expected to be relatively high. However, both the Zhou et al. (2023) and our study may overestimate the proportion of SOC from grasses. Commonly used end-member mixing models consider plant-based drivers of $\delta^{13}C$, but not changes due to soil decomposition (Wynn et al., 2006) and microbial CO_2-fixation (Nel & Cramer, 2019), which could drive soil $\delta^{13}C$ values closer to atmospheric values and thus towards those of C_4 grasses. Furthermore, the apparent increase in the proportion of SOC derived from grasses at eCO_2 is at least partly because the fossil fuel-derived CO_2 increased the disparity in $\delta^{13}C$ between the reference C_3 and C_4 plants at 550 ppm (resulting in an estimate of >100% contribution from grasses).

The hypothesis that grasses with trees would synergistically increase SOC because of N_2-fixation by trees contributing to grass growth and microbial activity was not supported as SOC was similar when grass was grown alone or with trees. The isotopic enrichment of soil ^{15}N was consistent with previous evidence showing that grass-tree competition stimulates N_2-fixing savanna trees to increase nodulation and thus N acquisition so as to better survive grass competition (Cramer et al., 2007; Telford et al., 2025). The positive correlation between nodule biomass and soil $\delta^{15}N$ in our study is likely the consequence of nodules becoming enriched with ^{15}N (Shearer et al., 1983) and subsequent nodule turnover. The relatively positive ^{15}N values from the senesced nodules (Telford et al., 2025) appear to have increased the $\delta^{15}N$ in both the fPON and oPON fractions.

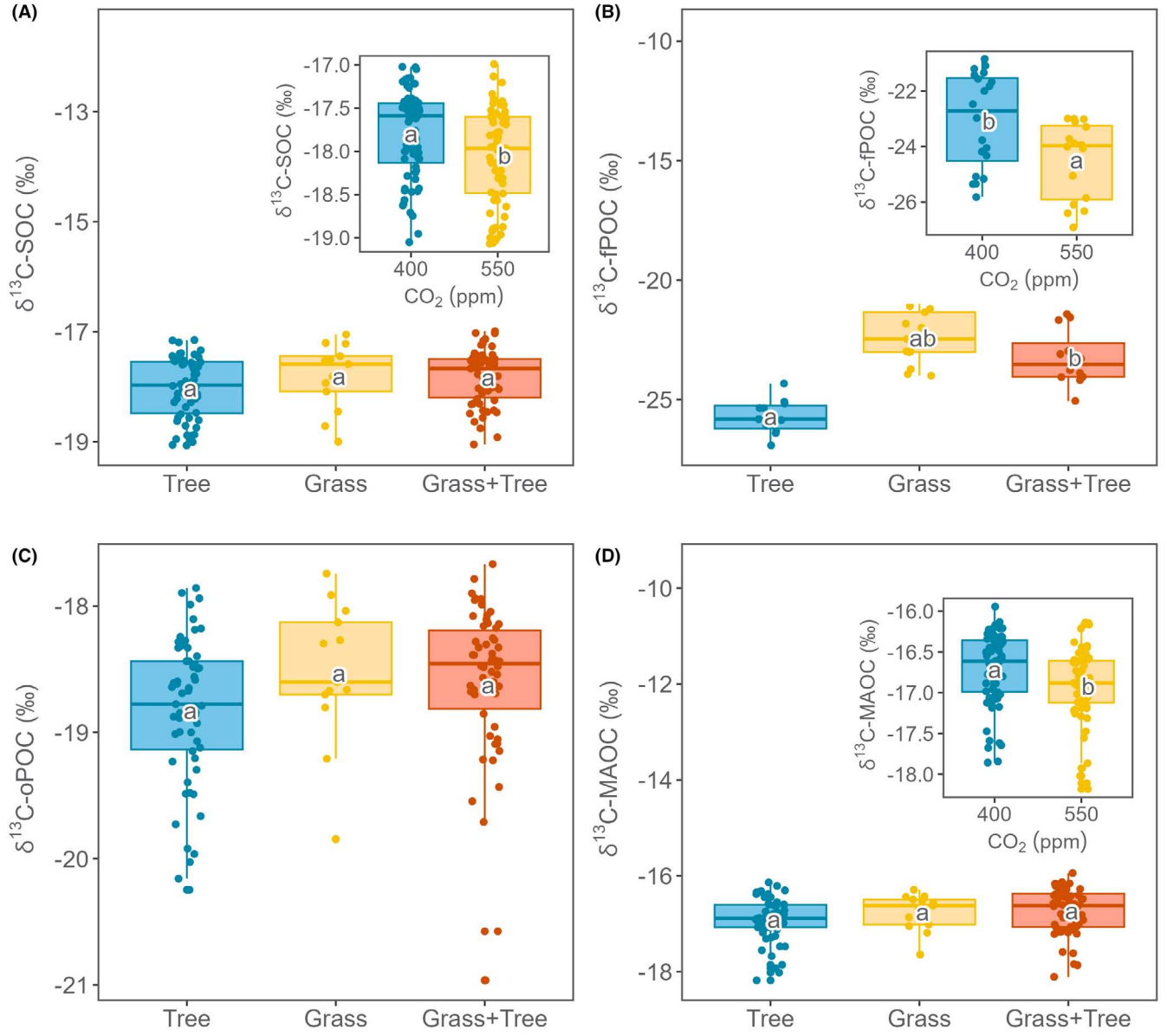

FIGURE 5 Stable carbon isotope ratios ($\delta^{13}C$) of different soil fractions under the same conditions described in Figure 3, where the fractions shown are (A) soil organic carbon (SOC); (B) free particulate organic carbon (fPOC); (C) occluded particulate organic carbon (oPOC); and (D) mineral-associated organic carbon (MAOC). Since there was no interaction between grass-tree treatment and CO_2 concentration, only significant main effects are shown (with CO_2 concentration as insets if significant). Letters indicate significant difference at the $p < 0.05$ level using tests described in Figure 3.

4.3 | Carbon dynamics and constraints in savannas

A novel aspect of our study is that we link grass-associated increases in SOC in a savanna soil with increases in the oPOC fraction. The role of oPOC in SOC formation is strongly supported by our SEM. The responsiveness of oPOC within the ca. 2.5 years of our study aligns well with the theory that oPOC turns over relatively quickly, that is <10 years to decades (Lavallee et al., 2020; von Lützow et al., 2007). POM can be partly broken down by in vivo transformation or ex vivo modification into smaller molecular weight compounds (Cotrufo et al., 2019) and incorporated into MAOM if mineral surfaces are available for binding this OM (Guo et al., 2022). The MAOC fraction can also be expected to change via the MEMS pathway, that is rapid utilisation of DOC via microbes and subsequent binding to mineral surfaces (Cotrufo et al., 2013). However, MAOC was unresponsive to treatments in our savanna soil.

The lack of change in MAOC in our study may relate to its long turnover times (decades to centuries; Lavallee et al. (2020)) but other short-term studies have shown changes in MAOC. For example, restoration of cropland to grassland in temperate areas showed a ca. three times greater change in MAOC compared to POC after only 1–3 years (subset of a meta-analysis; Zhang et al. (2024)). Besides turnover times, the very low clay percentage (0.3%) in our savanna soil likely meant that binding sites for organic molecules were limited or even

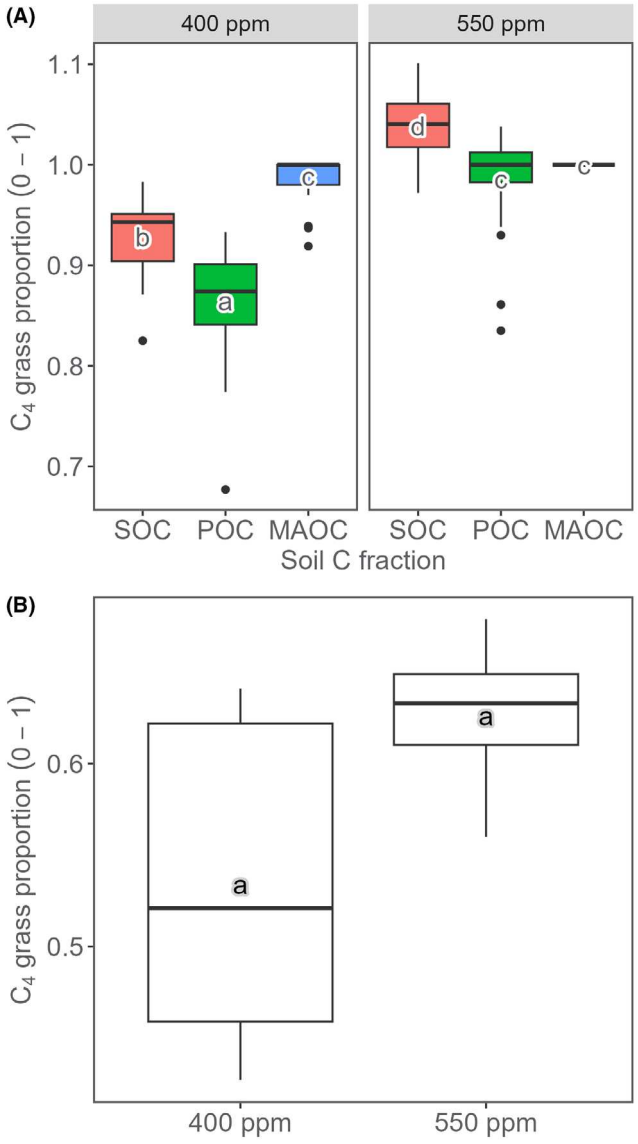

FIGURE 6 Relative proportion of carbon contributed by the C_4 grass *Themeda triandra* to different soil fractions at 400 or 550 ppm atmospheric CO_2 concentrations according to an end-member mixing model, showing (A) soil organic carbon (SOC), occluded particulate organic carbon (oPOC), and mineral-associated organic carbon (MAOC); and (B) free particulate organic carbon (fPOC). Letters indicate significant difference at the $p < 0.05$ level using tests described in Figure 3.

saturated (Robertson et al., 2019). Possibly, new MAOC was formed in response to plant C inputs but substituted existing C rather than accumulating. A study that argues against the phenomenon of SOC saturation (Heinemann et al., 2024) only considered soils with relatively high clay percentages (10%–21%). Thus, while the proportion of MAOC in our study was comparable to that found across the temperate grasslands of Europe (66% in our soil compared to 50%–75% in Lugato et al. (2021)), the absolute amounts of SOC and MAOC were lower (24 and 10 mg C ha^{-1} in our soil compared to ca. 80 and 50 mg C ha^{-1}). Clay-rich savanna soils such as the 'cotton-soils' in Kenya (Veblen, 2012) and the lower cumulative ends of catena sequences (Theron et al., 2020) may have relatively high capacities to bind and store organic C. It is also possible that the relatively arid savanna climates limit the amount and duration of inputs from root exudates and thus the pathway from DOM to MAOM. The SOC sequestration potential index (C/N_{SOM}) proposed by Cotrufo et al. (2019) depends on the C:N ratio of POM and MAOM (and their relative proportions). In our study, the lack of response in this index is likely because only oPOC and oPON responded to treatments, and not MAOC and MAON. Overall, long-term data on SOC fractions across a gradient of soil types and rainfall in savannas would help to better understand its SOC dynamics.

4.4 | Little CO_2-fertilisation effect

Elevated atmospheric CO_2 did not alter the competitive balance between C_4 grasses and C_3 savanna trees and did not alter SOC or its fractions, that is our second hypothesis was not supported. It follows that the soil $\delta^{13}C$ values did not reflect a relatively greater contribution of C_3 trees to C sequestration under CO_2 fertilisation as also hypothesised. Although the fossil fuel CO_2 used in the eCO_2 treatment negatively influenced $\delta^{13}C$ values overall, it also increased the proportion of SOC derived from grasses. The fact that $\delta^{13}C$ decreased at eCO_2 in fPOC, MAOC and total SOC, however, shows that these pools were somewhat dynamic and affected by eCO_2. Possibly, a stronger effect of CO_2 would be observed in the field where environmental variability, species interactions, and longer-term acclimation processes may influence plant and soil responses.

4.5 | Implications for soil conservation and C offsetting

The responsiveness of savanna oPOC to treatments such as biomass inputs can be seen as a double-edged sword. On the one hand, oPOC represents a significant and non-saturating pool of C, but on the other, it is particularly vulnerable to C loss through climate and land use change (Lugato et al., 2021). A modelling study by Lugato et al. (2021) suggests that oPOC is especially prone to degradation as environmental conditions shift. While POM is recalcitrant due to its chemical composition, its degradation can suddenly increase when microbial activity is reactivated, such as when frozen soils warm or waterlogged soils dry (Kleber, 2010), because it is not well protected within stable aggregates. In contrast, MAOM is protected by various mineral associations such as occlusions (in organo-mineral clusters and fine aggregates), micropores, or sorption to mineral surfaces (von Lützow et al., 2007) with a relatively slower turnover rate. The vulnerability of oPOC makes it particularly important to avoid any soil disturbance, for example erosion, tillage and tree planting, as well as to carefully manage natural disturbances such as fire and herbivory within ecological bounds. On the other hand, the capacity of oPOC to accumulate indefinitely means that the potential of savanna soils to increase SOC via this fraction may

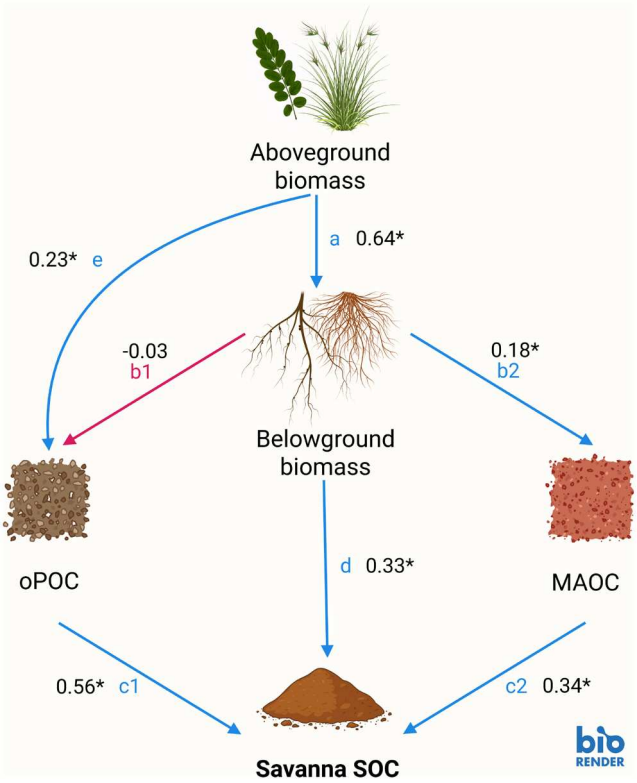

FIGURE 7 Structural equation model (SEM) predicting the formation of soil organic carbon (SOC) in a savanna soil via plant biomass, occluded particulate organic carbon (oPOC) and mineral-associated organic carbon (MAOC). Each arrow and associated letter plus number indicates a pathway in a process, either directly (a, d, e,) or indirectly via mediators (b1×c1 and b2×c2). Blue and pink pathways indicate positive and negative relationships, respectively. The regressions coefficient (R^2) or variance explained for each pathway are given above the arrows where * indicated *p*-values <0.01. The overall model fits the data well where χ^2/df = 0.27 (i.e. is non-significant), the Comparative Fit Index = 1.00 and Tucker–Lewis Index = 1.02 (i.e. are >0.95), root mean square of approximation = 0.00 (i.e. is <0.06) and standardised root mean square residual = 0.02 (i.e. is <0.08).

have been underestimated. Certainly, these fractions are not built into models commonly used in C offset projects. In addition, oPOC has a relatively high C:N ratio and thus a lower N cost per unit of C sequestered compared to MAOC (Cotrufo et al., 2019). Given that N availability is one of the main limiting factors for soil C sequestration and that soil N may decrease under climate change (Mason et al., 2022), C storage that depends on oPOC could be distinctly advantageous.

4.6 | Knowledge gaps

The responsiveness of oPOC in this savanna soil differs from temperate grassland soils, emphasising the need to understand SOC dynamics in tropical savannas and not extrapolate from temperate environments. Currently, the mechanisms, saturation limits, and stoichiometric constraints that govern SOC accumulation in savanna soils are largely unknown. Experiments that trace the accumulation, turnover and losses of C across a gradient of soil types, climates and fire regimes by using for example isotopically labelled organic matter, would help resolve the mechanism to SOC accumulation in savanna soils. Related to this is that savannas remain significantly underrepresented in the global SOC maps. Recently a global map of MAOC and oPOC as well as their turnover was generated by Zhou et al. (2024) using a dataset of 8341 observations. These maps improve on a previous global map of MAOC (Georgiou et al., 2022) by harmonising methods, elucidating drivers, and providing a benchmark for next generation Earth system models. For savannas, most of these observations were in South America (*n* = 308), Africa (*n* = 137) and North America (*n* = 100), with very few in Australia (*n* = 11) and none in Asia (using Olson and Dinerstein ecoregions; Dinerstein et al., 2017). Crucially, the savanna dataset (*n* = 556) was much smaller than those for example forests (*n* = 5423) and the total dataset (*n* = 8343). Expanding SOC fraction data in savannas is part of the overall need for more soil data to improve representation in Earth system models and their predictive capacity.

5 | CONCLUSION

Our study addresses a critical knowledge gap about the effect of tree-grass interactions and elevated atmospheric CO_2 on C accumulation and storage mechanisms in savannas, thus directly responding to ongoing afforestation efforts and encroachment threats. While recognising that pot culture conditions differ to those in the field, our results support the growing body of evidence showing the value of the grass component alongside trees for SOC storage. Tree planting and encroachment have quantified negative impacts on biodiversity. However, our data also calls into question assumed links between tree cover and SOC in savanna ecosystems, and afforestation remains an uncertain strategy for increasing SOC pools. The responsiveness of oPOC but not MAOC to plant-derived C inputs in our study suggests that MAOC is saturated in many sandy savanna soils and that detailed tracer studies across diverse soil textures and climate conditions are needed to elucidate SOC dynamics in savannas. Clearly there is much to learn about the grass-centric SOC and its fractions in savannas that are not yet accurately represented by global C models or considered in C offsetting. But it is clear that the assumed links between tree biomass and SOC in savannas do not fit a universal model and discounts the significant role of grasses in forming resilience SOC stores. A clear message for policymakers and land managers is that the conservation of grassy biomes such as savannas, and not afforestation, aligns with both biodiversity and climate goals.

AUTHOR CONTRIBUTIONS

Brad S. Ripley, Colin P. Osborne and Caroline E. R. Lehmann conceived the ideas for the overall savanna study, with assistance from Sarah L. Raubenheimer, Kim J. Simpson, Edith J. Singini, and Elizabeth

M. Telford. Then, Heidi-Jayne Hawkins and Michael D. Cramer conceived the ideas for this soil study in discussion with aforementioned project leads. Heidi-Jayne Hawkins and Michael D. Cramer collected the data, Heidi-Jayne Hawkins processed the samples and analysed the data. Heidi-Jayne Hawkins and Michael D. Cramer led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

Elizabeth M. Telford is an Associate Editor of *Functional Ecology* but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are in the main article, the Supporting Information, and the Zenodo Data Repository https://doi.org/10.5281/zenodo.17437473 (Hawkins 2026). Further information and requests for resources should be directed to and will be fulfilled by the lead contact, H-JH (heidi.hawkins@uct.ac.za).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Correlation matrix using the Pearson method between measured plant and soil variables of interest in a savanna soil with trees only (various *Vachellia* spp., and *Senegalia caffra*), grass only (*Themeda triandra*) or both at 400 or 550 ppm atmospheric CO_2 concentrations.

Figure S2. Free particulate organic matter fractions under the same conditions described in Figure S1, presented on a per plant species basis where the latter was a significant term in linear mixed effects models, shown as (A), fPOC (free particulate organic C); (B) fPON (free particulate organic N), and (C) δ^{13}C-fPOC (stable carbon isotope ratios in fPOC).

Figure S3. Nitrogen concentration of different soil fractions under the same conditions described in Figure S1, where the fractions shown are (A) free particulate organic nitrogen (fPON); (B) occluded PON (oPON); and (C) mineral-associated organic nitrogen (MAON).

Figure S4. Stable nitrogen isotope ratios ($\delta^{15}N$) of different soil fractions under the same conditions described in Figure S1, where the fractions shown are (A) soil organic nitrogen (SON); (B) free particulate organic nitrogen (fPON); (C) occluded PON (oPON); and (D) mineral-associated organic nitrogen (MAON).

Appendix S1. Details of methodology.

Appendix S2. Supplementary references.

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