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Root decomposition in silvopastures is influenced by grazing, fertility, and grass species

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Abstract

Grass root production and decomposition is a major source of C entering soils, although rates are largely unknown based on edaphic and management factors. Therefore, study objectives were to evaluate four explanatory variables including forage species (native and nonnative), fertility (poultry litter and a control), soil moisture (udic and aquic), and pasture management (grazed and an ungrazed control) in order to evaluate driving factors for root turnover and subsequent soil organic matter formation in silvopastoral systems using the root litter bag technique. Native grass root decomposition was accelerated relative to the nonnative forage based on root mass balance, as well as the exponential decay function, likely owing to greater five- and six-C sugars and more digestible root tissues of native grasses. These physiochemical results suggest more favorable microbial food sources, which culminate in faster decomposition and greater microbially derived organic matter. Overall, there was greater root sloughing and subsequent soil organic matter formation potential with native grass species and poultry litter applications, with soil moisture affecting decomposition to a lesser extent. This study contributes to the understanding of complex interactions of grass species, soil moisture, nutrients, and grazing, which controls primary productivity, as well as nutrient cycling and C sequestration in silvopastures.

1 | INTRODUCTION

Decomposition cycles and processes are a major source of fixed C in terrestrial ecosystems and are important aspects of ecosystem function, although root decomposition rates are largely unknown, especially in silvopastoral systems. Silvopasture is the intentional combination of agroforestry and pastures (either through grazing or hay management), and such systems can be a mechanism to mitigate risk by diversifying markets. It is also recognized by its ecosystem services, including increased soil organic C (SOC) storage (Lorenz & Lal, 2014; Nair, 2011) and reduced losses of nutrients by leaching and runoff (Nair & Graetz, 2004).

Belowground biomass (root litter) turnover is a key component of C sequestration in grasslands (Siqueira da Silva et al., 2015). As belowground plant tissues slough off and decompose, a significant pool of nutrients and C is released to soils (Adams et al., 2021; Garcia-Pausas et al., 2012). The litterbag technique is the most commonly applied method for measuring fine-root decay and turnover (Silver & Miya, 2001) and thus has provided us with our current understanding of

Abbreviations: ADF, acid digestible fiber; AU, animal units; DNS, data not shown; NDF, neutral detergent fiber; OM, organic matter; PLS, pure live seed; RTD, root tissue density; SOC, soil organic carbon; SRL, specific root length.

elemental and C fluxes through decaying fine roots. This method generally entails the digging of live roots, weighing and drying, then root placement in litterbags, and in situ rhizosphere replacement, followed by timed retrieval of bags. Thereafter, root decay is temporally quantified (Dornbush et al., 2002). Some research has shown that fine-root turnover rate via the litter bag method is underestimated, although this is still the most widely used and acceptable method.

Residue and root composition affects nutrient mineralization and immobilization processes, which can in turn affect nutrient cycling, plant production, and soil health (Dubeux et al., 2006). Biotic and abiotic factors are key drivers in affecting root decomposition. For example, soil fertility has proven to increase fine root turnover (Puttaso et al., 2011), as has stocking rate (Dubeux et al., 2006; Thomas, 1992), and soil conditions such as moisture and temperature, although their combined effects and interactions are largely unknown.

Nutrient depositions from animal excreta or fertilizers may also directly or indirectly affect residue and root decomposition through sustaining soil biota and increasing above- and belowground plant growth (Garcia-Pausas et al., 2012). Poultry litter (combination of bedding and manure) is a common nutrient source for pastures, given the proximity of cow-calf operations to broiler houses (Yang et al., 2019), although its impact on root chemical composition and decomposition has not been reported to date.

Paul (1970) outlined the role of plant compounds in soil humus formation and identified the importance of C/N ratios, polyphenols, and nutrient contents when quantifying organic matter (OM) turnover from roots. Minderman (1968) estimated that 10% of phenolic, 50% of lignin, 75% of the cellulose, and 99% of simple sugars are lost during the first year of litter fall in forest environments. Interactions between phenolic compounds (lignin), cell wall components, carbohydrates, and C/N ratios are thought to control the relative rates of decomposition of plant tissue in soils. To test these hypotheses, the present study evaluates four explanatory variables including forage species (native and nonnative), fertility (poultry litter and a control), soil moisture (udic and aquic), and pasture management (grazed and an ungrazed control) in order to assess the driving mechanisms of root turnover in silvopastoral systems. Authors hypothesize that native grass roots will have greater decomposition rates owing to less recalcitrance and that increased fertility (poultry litter) will accelerate fine root turnover.

Core Ideas

- Root decomposition is a major source of C entering soils in grasslands.
- Native grass roots decomposed quicker than roots from the nonnative forage.
- Poultry litter increased root decay, with soil moisture affecting rates to a lesser extent.
- Root chemical composition and management were driving factors for decomposition.
- This work contributes to understanding factors affecting root decomposition.

2 | MATERIALS AND METHODS

2.1 | Site description

This study was conducted on a 4.25-ha paddock located at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, AR (36.09° N, 94.19° W). The site is located in the Ozark Highlands, Major Land Resource Area 116A (Soil Survey Staff, 2019a). Information on previous site history is described by Thomas et al. (2008), DeFauw et al. (2014), and Sauer et al. (2015). Briefly, soil in most of the experimental area is mapped as Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) with some Pickwick silt loam (fine-silty, mixed, semiactive, thermic Typic Paleudults) and small areas of Johnsburg silt loam (fine-silty, mixed, active, mesic Aquic Fragiudults), and Nixa cherty silt loam (loamy-skeletal, siliceous, active, mesic Glossic Fragiudults; Soil Survey Staff, 2019b). The field also contains a dissimilar inclusion at this site that is lower in elevation and was not captured in the mapping unit. The wetter location within the study site is classified as fine, mixed, active, thermic Typic Endoaqualfs, an "aquic" soil moisture regime.

During tree establishment in 2000, 15 rows of three species including northern red oak (*Quercus rubra* L.), eastern black walnut (*Juglans nigra* L.), and pecan (*Carya illinoinensis* Wangenh. K. Koch) were oriented east–west at 15-m spacing. In 2014, the eastern black walnut trees were replaced with rows containing three species, including American sycamore (*Plantanus occidentalis* L.), cottonwood (*Populus deltoides* W. Bartram ex Marshall), and pitch/loblolly pine (*Pinus rigida* Mill. × *Pinus taeda* L.). Two forage species treatments were established in the alleys between tree rows, including a cool-season species (orchardgrass [*Dactylis glomerata* L., var. Tekapo]), which was seeded fall 2015 at 17 kg pure live seed (PLS) ha^{-1}, and a native warm-season mix (8:1:1 big bluestem [*Andropogon gerardii* Vitman], little bluestem [*Schizachyrium scoparium* L.], and indiangrass [*Sorghastrum nutans* L.]), which was seeded spring 2016 at 10 kg PLS ha^{-1}. Alleys were planted with a Haybuster 107C no-till drill (DuraTech). Prior to establishment, Cornerstone Plus [*N*-(phosphonomethyl) glycine] was used to kill existing vegetation at a rate of 2.2 kg ha^{-1} (41% a.i.). After establishment, alleys were treated with Plateau (ammonium salt of imazapic)

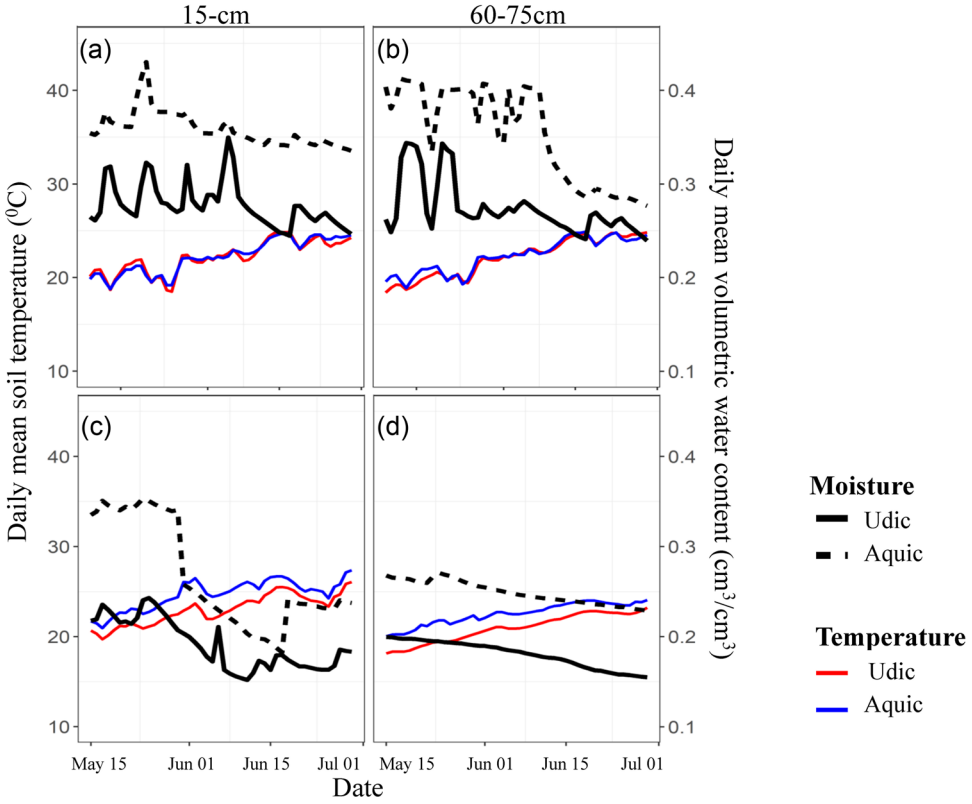

FIGURE 1 Daily mean volumetric soil water content and daily mean soil temperature for the wet (aquic) and dry (udic) treatments from May to July in (a) 2017 at 15 cm, (b) 2017 at 60–75 cm, (c) 2018 at 15 cm, and (d) 2018 at 60–75 cm

at a rate of 0.28 kg ha^{-1} (23.6% a.i.; Niyigena et al., 2021). The site receives an average (30-yr mean) annual precipitation of 1,232 mm and has an average ambient temperature of 14.5 ˚C (NCDC, 2019a, 2019b).

2.2 | Treatment implementation and field management

Independent or explanatory variables were tested and included forage species (native grass mixture and nonnative orchardgrass), fertility (poultry litter and an unfertilized control [0 kg N ha^{-1}]), soil moisture regime (udic and aquic), and pasture management (grazed and ungrazed) in order to assess mechanisms for root decomposition. Each main effect is described below.

The primary treatment, or forage species, was implemented as described above with three replications. The second main effect, or soil moisture regime, was implemented by random placement of litter bags in known wet and dry areas within the field (Figure 1). This treatment was confirmed by placement of volumetric water content sensors at two depths (15 and 60–75 cm) from the soil surface (Figure 1). Water content measurements were recorded every 4 h and logged on a Decagon EM50 data logger (METER Group) throughout the experimental period from May 2017 to July 2018. Soil moisture data were averaged each day and expressed as daily mean volumetric water content for further analysis (Figure 1). The third main effect, or fertility, was implemented by orchardgrass and native grass receiving locally sourced poultry litter applied at a rate of 84 kg N ha^{-1} on 21 Mar. 2017 and on 21 Mar. 2018 (4.94 Mg ha^{-1}, fresh weight basis). Chemical composition of poultry litter was 2.69%, 0.70%, 1.12%, and 6.1 for N, P, K, and pH, respectively, during 2017 and 1.98%, 0.58%, 1.02%, and 6.2 for N, P, K, and pH, respectively, during 2018 (Arkansas Diagnostic Laboratory). To implement the fourth main effect or grazing treatment, heifers (*Bos taurus* L.) grazed the site at a rate of 1.92 animal units (AU) ha^{-1} from 11 May to 23 June in 2017, and in 2018, 2.20 AU ha^{-1} grazed the site from 24 May to 6 July. To exclude cattle from grazing, 0.25-m^2 enclosures were placed and secured over litter bag installation areas (per main effect). Weather variables were measured by a micro-meteorological weather station approximately 500 m from the experimental site.

Soil samples (per species, fertility, and soil moisture regime) were collected in triplicate 20 March 20. 2017 and 16 Mar. 2018 at the 0-to-15-cm depth using a 2-cm push probe and composited. Plant material was manually removed, and samples were dried in a forced-air oven at 70 ˚C for 48 h. Samples were subsequently ground and sieved to pass a 2-mm

mesh. A modified hydrometer method was used to determine soil texture (Gee & Or, 2002). Additionally, total C and N were determined via combustion using a VarioMax CN analyzer (Elementar Americas). Mehlich-3 extractable soil elemental concentrations (i.e., Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Ti, and Zn) were determined using a 1:10 soil mass/extractant solution volume ratio (Tucker, 1992) and analyzed by inductively coupled argon-plasma spectrometry (ICP, Agilent Technologies). Weight-loss-on-ignition was used to determine soil OM concentration after 2 h at 360 ˚C (Schulte & Hopkins, 1996).

2.3 | Root/litter bag technique for determining root decomposition

Root decomposition was measured using the litter bag technique. Root samples (<2-mm diameter) of both grass species in fertilized and nonfertilized alleys were collected at a depth of 15 m between 19 April and 4 May 4 in 2017 and between 1 and 8 May in 2018. To process, roots were clipped from green vegetation at the stolon, rinsed with tap water to remove sediment, dried in a forced-air oven at 55 ˚C, and 5–6 g of material was packed into nylon monofilament mesh liquid filter bags (75 μm, 105 × 203 mm; The Cary Company; Dubeux et al., 2006). Packed bags were reinstalled into respective treatments (species, fertility, soil moisture regime, and grazing regimes) at a 15-cm soil depth on 9 May 2017 and on 15 May 2018. Root bags were collected 20, 34, and 48 d after installation. Each incubation time (per fixed effect) was replicated three times, resulting in 48 bags (2 forage species × 2 fertility levels × 2 soil moisture regimes × 2 grazing levels × 3 replications) per collection date per year.

2.4 | Sample processing and compositional and chemical analyses

At the end of each incubation time, foreign material was removed from root bags and samples were weighed upon removal from the field. Subsequently, original root tissue and retrieved samples were dried at 70 ˚C for 48 h and reweighed to determine moisture content. After drying, samples were ground using a Wiley mini-mill (Thomas Scientific) to pass through a 1-mm screen. Total C and N were determined via high-temperature combustion using a VarioMax C/N analyzer (Elementar Americas). Lignin, OM, acid digestible fiber (ADF), and neutral detergent fiber (NDF) were determined using an ANKOM 2000 Fiber Analyzer (ANKOM Technologies; Van Soest & Robertson, 1980). Hemicellulose was calculated by ADF minus NDF (Ashworth et al., 2016). Root nutrients and metals were extracted using Mehlich-3 and measured by inductively coupled plasma using a 7300 ICP-OES DV (Perkin-Elmer). Root elements (percentage) of each remaining nutrient was calculated based on each nutrient content before and after the incubation period. Total ash was determined based on ASTM standard E1755-01 (Sluiter et al., 2005). One gram of ground, prepared root tissue (sieved to 1 mm) was placed in an oven-dried, porcelain crucible overnight at 105 ˚C. Crucibles were placed in a muffle furnace at 575 ˚C for 4 h; then, crucibles were removed and cooled to room temperature in a glass desiccator. The material retained in the crucible was weighed and ash concentration was expressed as grams per kilogram. Ash measurements were assayed in triplicate for each plot.

2.5 | Root morphology quantification of grass species

A subset of root samples from the four treatments (two grass species and two fertilized and nonfertilized) were set aside for later processing on a root-scanning system. Root samples of both grass species at each fertility level were collected per plot (replicated thrice) and weighed to constant mass (1.0 ± 0.001 g; $n = 12$ total). Weighed samples were scanned (Epson perfection V800, SEIKO Epson Corporation) at 400 dpi and analyzed using WinRhizo (Regent Instruments) software. Root surface area and volume were analyzed per root diameter class (0–0.5, 0.5–1.0, 1.0–1.5, 1.5–2.0, and >2.0 mm).

2.6 | Analysis of data and model development

2.6.1 | Soil data analysis

Soil physical (sand, silt, clay, C, and OM) and chemical (N, P, K, Ca, Mg, and S) properties were analyzed with a mixed model (version 9.4, SAS Institute) consisting of the random effects of replication and year, with fixed effects being forage species, poultry litter fertility, and soil moisture regime. When main effect differences were found, pairwise post-hoc comparisons were performed by the SAS macro 'pdmix800' (Saxton, 1998), with Fisher's LSD at a Type I error rate of 5% (SAS Institute, 2009).

2.6.2 | Mass and composition changes based on main effects

Remaining root mass percentage, composition (NDF, ADF, lignin, hemicellulose, and ash), and nutrient or metal data (N, Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Ti, and Zn) were analyzed with a mixed model

(version 9.4, SAS Institute) consisting of the random effects of replication and year. Under this model, forage species (native and nonnative) were the whole plot, with soil moisture regime (udic and aquic) being the split plot, fertility (poultry litter and a control) the split-split plot, pasture management (grazed and an ungrazed control) the split-split-split plot, and timing (0, 20, 34, and 48 d after installation) the split-split-split-split plot. When main effect differences were found, pairwise post hoc comparisons were performed by the SAS macro 'pdmix800' (Saxton, 1998), with Fisher's LSD at a Type I error rate of 5% (SAS Institute, 2009).

Analysis of variance was conducted on root morphology and characterization data (e.g., root tissue density [RTD], root length to dry mass ratio, etc.) as a split-plot design with grass species as a whole plot and fertility as the split-plot factor. Data were analyzed using R programming language (R Core Team, 2019).

2.6.3 | Decay function model development

Recovered root mass at each incubation time period was expressed as percentage relative to the initial root mass (beginning of litterbag placement time [time = 0]). Nutrient contents of recovered root (N, C, P, C/N, hemicellulose, lignin, and lignin/N) were compared and decay functions were developed based on initial mass, nutritive, and compositional constituents. A single exponential decay model (Wagner & Wolf, 1999) was used to estimate the percent of original biomass that remained after incubation, remaining root N, C, P, C/N, hemicellulose, lignin, and lignin/N ratio. The equation used for the exponential model was $X = B_0 \exp^{(-kt)}$, where X is the proportion of remaining root mass (or nutrient content and composition) at day t, B_0 is the initial value of root mass (nutrient or composition), and k is the relative decomposition/decay rate over incubation time period (Dubeux et al., 2006). Decay rate was explored at four factor levels: forage species (native and nonnative), soil moisture regime (udic and aquic), fertility (poultry litter and a control), and pasture management (grazed, and ungrazed control). Root incubation time (t) ranged from 0 to 48 d after root bag installation per year. A nonlinear least square function available in R software (R Core Team, 2019) was used to estimate the best parameter value (B_0 and k) for each study variable (root mass, nutrient, and composition). Decay rate was statistically tested against the null hypothesis of $k = 0$.

3 | RESULTS AND DISCUSSION

In 2017, total daily rainfall for the study period was greater than 2018 and the 30-yr average (272, 118, and 235 mm, respectively; Figure 2; NCDC, 2019a, 2019b). Daily air temperatures also varied more in 2017 relative to 2018 (Figure 2). However, mean daily air temperature in 2017 (20.9 ˚C; NCDC, 2019a) was similar to the 30-yr average (21.0 ˚C; NCDC, 2019b). In 2018, daily air temperature for the study site was 2.9 ˚C greater than average (23.9 ˚C; Figure 2). Daily surface volumetric soil water content ranged from 0.18 to 0.43 $cm^3\ cm^{-3}$, with the average being 0.32 $cm^3\ cm^{-3}$ for the wet (aquic) treatment, whereas it ranged from 0.15 to 0.35 $cm^3\ cm^{-3}$ with the average being 0.24 $cm^3\ cm^{-3}$ for the dry (udic) treatment (Figure 1). Similarly, daily soil surface temperature ranged from 19 to 27 ˚C for the wet treatment, whereas it ranged from 18 to 26 ˚C for dry treatments (Figure 1). The overall greater soil temperature in the wet treatment (2018) might be due to potential lateral flow from the surrounding landscape, as well as due to the higher specific heat capacity of water present in wetter soils.

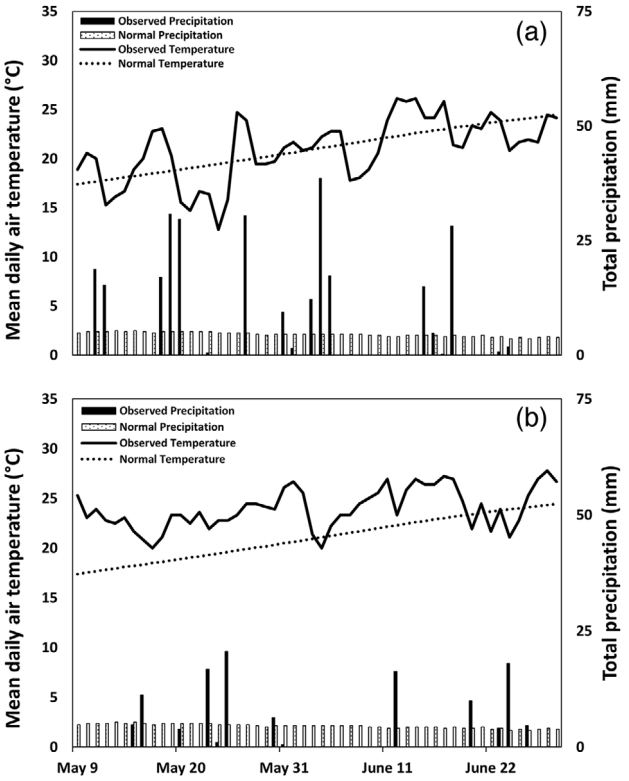

FIGURE 2 Daily mean and normal temperatures, precipitation (vertical bars), at the Agricultural Research and Extension Center, University of Arkansas, Fayetteville, in (a) 2017 and (b) 2018. Normal temperature data were obtained from the U.S. NOAA from 1971 to 2000 (U.S. Department of Commerce)

3.1 | Soil variation based on treatments

There were no three-way (forage species × poultry litter fertility treatment × soil moisture regime) interactions for sand, silt, clay, C, N, P, K, Ca, Mg, and S ($P \geq .05$; Table 1).

TABLE 1 Soil properties measured at a silvopasture site in Fayetteville, AR, from 2017 and 2018 (analyzed across years as there were no year effects; $P \geq .05$)

Spp	Fert	Moisture	Sand	Silt	Clay	OM	C	N	P	K	Ca	Mg	S
			%						mg kg^{-1}				
NG	F	W	19a[a]	61a	20a	3.18a	1.71a	0.15a	43a	88a	1,538a	54a	13a
NG	F	D	21a	61a	18a	2.97abc	2.02a	0.20a	56a	193a	1,558a	75a	11a
NG	NF	W	18a	62a	20a	2.48c	1.46a	0.13a	17a	82a	1,160a	48a	8a
NG	NF	D	18a	63a	19a	3.15ab	1.46a	0.14a	52a	77a	1,174a	63a	9a
OG	F	W	18a	62a	20a	2.75bc	1.61a	0.15a	58a	85a	1,521a	60a	10a
OG	F	D	19a	60a	21a	2.85abc	1.69a	0.16a	57a	109a	1,377a	65a	9a
OG	NF	W	18a	63a	19a	3.00ab	1.74a	0.16a	64a	83a	1,457a	48a	11a
OG	NF	D	19a	61a	20a	2.85abc	1.63a	0.15a	55a	90a	1,378a	66a	10a

Note. Samples were collected at 0–15 cm at four factor levels: Forage species (NG = native grass mix, and OG = nonnative orchardgrass), moisture (W = wet/mesic, and D = dry/xeric), and fertility (F = fertilized with poultry litter, and NF = un-fertilized control). Spp = forage species, Fert = fertility, OM = soil organic matter.
[a]Different letters indicate a significant difference at $P \leq .05$ within a given analyte.

However, there was a three-way interaction for soil OM. Specifically, OM was greatest ($P \leq .05$) under the native warm-season grass mix under fertilized and the aquic soil moisture regime, with lowest soil OM contents occurring in the native grass roots aquic and not fertilized with poultry litter and orchardgrass aquic treatment combinations (Table 1). For percent C, differences occurred for species × poultry litter fertility ($P \leq .05$; data not show [DNS]), with the greatest C occurring under the native grass fertilized roots. Therefore, in general, there was the greatest soil OM and C storage under the fertilized native grass mix management strategy.

3.2 | Treatment effects on root composition and mass

Overall, across years (as year had minimal effect on all main effects; $P \geq .05$), remaining root mass percentage (delta values were derived by subtracting Day 0 by each retrieval day × 100) varied ($P \leq .05$) based on forage species, fertility, grazing management, and retrieval day, but not by soil moisture regime ($P \geq .05$; Table 2). Remaining root mass percentage varied by forage species × fertility and by forage species × fertility × grazing management × soil moisture regime ($P \leq .05$; Table 2). For the former, the control (0 kg N ha^{-1}) and orchardgrass had the greatest remaining mass, followed by poultry litter-amended orchardgrass, indicating less C mineralization and greater recalcitrance for this nonnative species. Remaining mass was 11 and 3.3% lower for native warm-season grass roots under poultry litter and unfertilized conditions (relative to unfertilized orchardgrass roots), respectively (DNS). For the latter interaction, the least amount of root decomposition occurred for poultry litter-amended orchardgrass under wet soils and grazed conditions, which did not differ from unfertilized and ungrazed soils (Table 3). Conversely, poultry litter-amended native grass roots under nongrazing and dry (udic) soil conditions had the greatest decomposition, which did not differ from wet and grazed soil conditions (Table 3). Therefore, there was greater root decomposition and subsequent soil C formation with native grass species under poultry litter applications and grazing over time, with soil moisture regimes affecting remaining root mass to a lesser extent (Tables 1 and 2).

Root compositional (ADF, NDF, lignin, hemicellulose, and ash) changes were evaluated, with main effects having minimal impacts. Overall, root NDF, ADF, hemicellulose, and ash differed only by forage species, with ash varying by root bag retrieval day × forage species × fertility ($P \leq .05$). Specifically, native grass roots had greater hemicellulose and lower ash (inorganic, nondigestible fraction) content (Table 4), indicating greater degradability owing to greater five- and six-C sugar levels and less nondigestible inorganic fractions (Ashworth et al., 2016), compared with cool-season forage roots. These results suggest less favorable microbial food sources in the rhizosphere of orchardgrass (Ashworth et al., 2017; Gurmessa et al., 2021). However, greater metal content (i.e., Al, As, Cd, Co, Cr, Cu, Fe, and Zn) and root P and K were observed in the remaining root mass for the cool-season forage, indicating greater nutrient retention in root tissue throughout the summer grazing season for orchardgrass (Table 4). In addition, root P differed based on forage species × fertility applications, with fertilized orchardgrass roots having the greatest P retention, followed by nonfertilized orchardgrass, which was not different from the native grass mix with poultry litter applications (DNS).

Root tissue of C_4 grasses have previously been shown to have a high immobilization potential (i.e., high C/N ratio; Silver & Miya, 2001). Native grass roots in this experiment had 26% greater root C compared with orchardgrass ($P \leq .05$; Table 4). Root C/N ratio was affected by the interaction of grazing × soil moisture regime and forage species × grazing. Over all species and fertility treatments, grazing under

TABLE 2 Analysis of variance of remaining percentage root biomass (delta [change] was derived by subtracting Day 0 by each retrieval day [i.e., 20, 34, and 48 days after installation])

Effect[a]	Num. df	Den. df	*F* value	*Pr* > *F*
Spp	1	288	32.31	<.0001
Moisture	1	288	0.58	.4466
Spp × Moisture	1	288	1.12	.2918
Fert	1	288	15.94	<.0001
Spp × Fert	1	288	7.85	.0055
Fert × Moisture	1	288	0.36	.5487
Spp × Fert × Moisture	1	288	0.17	.683
Grazing	1	288	5.71	.0176
Spp × Grazing	1	288	1.8	.1809
Grazing × Moisture	1	288	1.41	.2358
Spp × Grazing × Moisture	1	288	2.24	.1361
Fert × Grazing	1	288	0.13	.7202
Spp × Fert × Grazing	1	288	0.06	.8056
Fert × Grazing × Moisture	1	288	0.83	.3643
Spp × Fert × Grazing × Moisture	1	288	6.64	.0106
Time	2	288	41.71	<.0001
Spp × Time	2	288	0.3	.744
Moisture × Time	2	288	0.74	.4783
Spp × Moisture × Time	2	288	1.11	.3321
Fert × Time	2	288	0.6	.5502
Spp × Fert × Time	2	288	1.23	.2955
Fert × Moisture × Time	2	288	0.08	.9249
Spp × Fert × Moisture × Time	2	288	0.41	.6671
Grazing × Time	2	288	0.83	.4387
Spp × Grazing × Time	2	288	0.41	.6668
Grazing × Moisture × Time	2	288	0.67	.5119
Spp × Grazing × Moisture × Time	2	288	0.64	.5301
Fert × Grazing × Time	2	288	0.82	.4397
Spp × Fert × Grazing × Time	2	288	0.38	.6841
Fert × Grazing × Moisture × Time	2	288	0.5	.6059
Spp × Fert × Grazing × Moisture × Time	2	288	0.26	.7692

Note. Num. df , numerator degree of freedom, Den. df, denominator degree of freedom.

[a]Spp, forage species (native and nonnative); moisture. soil moisture (wet/aquic and dry/udic); fert, fertility (fertilized with poultry litter and a control); grazing (grazed and an ungrazed control); time, root bag time point retrieval (Days 20, 34, and 48).

wet soil conditions resulted in the greatest C/N ratio, suggesting less C mineralization potential compared with grazed and ungrazed conditions and under wetter soils (DNS). Silver and Miya (2001) found that the C/N ratio is negatively correlated with root N concentration, which was also found in this study (0.93% N for the native grass mix and 1.09% for orchardgrass). In the C_4 grass green panicum (*Panicum maximum* Jacq. var. *trichoglume*), net root N mineralization did not occur until 50–100 d after incubation (Robbins et al., 1989). Additionally, during the first week of soil incubation of another C_4 grass, creeping signalgrass [*Brachiaria humidicola* (Rendle) Schweick.], 600–800 g kg^{-1} of all soil N was immobilized in the microbial biomass fraction, with 300–500 g kg^{-1} remaining immobilized after 150 d (Cantarutti, 1996). Therefore, despite the C_4 species in this study having less recalcitrance, most N may be immobilized and nonmicrobially available. Root N for both species also varied based on fertilizer applications × grazing management in this study, with sloughed roots from fertilized plants having higher overall root tissue N (under both grazed and ungrazed conditions, DNS). However, limitations with the litter bag technique exist and should be considered, such as macroinvertebrates are excluded from litter bags due to mesh size, thus lowering actual in situ decomposition (Wieder & Lang, 1982).

TABLE 3 Average remaining percentage root biomass (delta [change] was derived by subtracting Day 0 by each retrieval day [i.e., 20, 34, and 48 days after installation]) based on forage species (big bluestem [native grass mix] and orchardgrass [nonnative]) × soil moisture (wet/aquic and dry/udic) × fertility (fertilized with poultry litter and a control [0 kg N ha^{-1}]) × grazing (grazed and an ungrazed control) interactions ($P \leq .05$)

Forage species	Fertility	Grazing	Moisture	Mean
Native grass mix	Poultry litter	Grazed	Udic	75.61bcde[a]
Native grass mix	Poultry litter	Grazed	Aquic	69.41ef
Native grass mix	Poultry litter	Ungrazed	Udic	63.66f
Native grass mix	Poultry litter	Ungrazed	Aquic	71.49de
Native grass mix	Control	Grazed	Udic	79.83abc
Native grass mix	Control	Grazed	Aquic	79.26abc
Native grass mix	Control	Ungrazed	Udic	77.19abcd
Native grass mix	Control	Ungrazed	Aquic	74.79cde
Orchardgrass	Poultry litter	Grazed	Udic	78.09abc
Orchardgrass	Poultry litter	Grazed	Aquic	82.62a
Orchardgrass	Poultry litter	Ungrazed	Udic	79.01abc
Orchardgrass	Poultry litter	Ungrazed	Aquic	79.06abc
Orchardgrass	Control	Grazed	Udic	81.43ab
Orchardgrass	Control	Grazed	Aquic	81.73ab
Orchardgrass	Control	Ungrazed	Udic	78.82abc
Orchardgrass	Control	Ungrazed	Aquic	82.21a

[a]Different letters indicate a significant difference at $P \leq .05$.

In addition, drying of roots prior to incubation periods may affect initial chemical and physical composition and subsequent decomposition; however, despite these limitations, this approach represents a classic approach to estimating decomposition rates.

3.3 | Root decay as affected by management, soil conditions, and forage species

Remaining root mass decreased ($P \leq .05$) over the incubation time period regardless of species, fertility, soil moisture regime, and grazing management (Table 5, root mass decomposition rate, k). Semmartin et al. (2004) also reported substantial mass loss (75–45% remaining mass) after 1 yr of incubation in the soil. Overall, in this study, the C_4 native grasses had a higher decomposition rate (average $k = 0.009$; Table 5) compared with the nonnative C_3 species (orchardgrass, average $k = 0.005$). This result is contrasting to those reported by Fornara et al. (2009), who reported greater root mass loss in C_3 plants. Poultry litter nutrient applications increased ($P \leq .05$) root decomposition rates (higher k value for root mass) in the native grass treatment, except under wet and ungrazed conditions (Figure 3, Table 5). For orchardgrass, poultry litter applications increased ($P \leq .05$) decomposition rates (higher k value for root mass) only in drier and grazed condition (Figure 4, Table 5). Averaged over fertility and moisture factor levels (Table 5), grazing resulted in slower root decomposition in both C_3 (root mass k value for ungrazed = 0.006 vs. grazed = 0.005) and C_4 (ungrazed = 0.009 vs. grazed = 0.008) grass species. This was counter to expectations in that grazing is thought to promote root decay and decomposition (Sun et al., 2018). When combined over fertility and grazing factors, aquic soil conditions increased root decomposition of native grass roots, whereas it decreased orchardgrass root decomposition. Semmartin et al. (2004) also reported higher decomposition rates under wet and grazed conditions owing to higher N and P contents.

Root N disappearance (N mineralization) rate was higher in the native grass mix ($k = 0.008$ when averaged for native grass), whereas it was slower for the nonnative orchardgrass species ($k = -0.001$, Table 5). This result is again contrary to the findings from Fornara et al. (2009), who reported negative effects of C_4 species on N mineralization. Generally, higher C/N ratios in plant tissues increase immobilization and lower C/N ratios increase N mineralization (Aber & Mellilo, 1991). Our experiment reported a higher C/N ratio in the native grass mix (47.2 vs 30.5 in orchardgrass; Table 4), yet the root N mineralization was higher in the native grass mix. Overall, poultry litter applications increased the N-disappearance rate in native grass species (Figure 5, Table 5). The greatest N disappearance rate was observed under wet, grazed, and poultry litter-amended treatment combinations for the native grasses (Table 4). Fahnestock and Delting (2002) reported four times higher N mineralization on grazed sites compared with ungrazed sites. Combined over fertility and moisture levels, grazing did not affect N disappearance rates for the native grass mix ($k = 0.008\%$ d^{-1} for both grazed and ungrazed

conditions; Table 5). When combined over fertility and grazing levels, soil moisture increased the N disappearance rate (0.007% d^{-1} in udic vs. 0.009% d^{-1} in aquic conditions) in the native grass mix (Table 5).

Decomposition of forage root C affects potential soil C pools as the root system of grasslands accounts for up to 60% of the net primary productivity (Jackson et al., 2017). Over the incubation period, root C decreased or remained unaffected (Table 5). The only treatment where root C content decreased was orchardgrass with poultry litter applications, aquic, and ungrazed conditions. The C/N ratios increased (negative *k* value) over the incubation period for native grasses and poultry litter applications. Nonnative orchardgrass had a decreasing C/N ratio under poultry litter, udic, and grazed treatments over the summer grazing period. Root P decay rate was affected in the native grass mix only, suggesting greater P mineralization in native grasses compared with introduced forage species (orchardgrass; Table 5).

Disappearance rate of lignin and lignin/N ratio were not affected ($P \geq .05$, Table 5) by any treatment level. Although lignin has been previously considered a reliable predictor of root decay rate (Prescott, 2010; Sumiyoshi et al., 2017), we found no such result in our study. Jo et al. (2016) also reported similar results of no relationship between root lignin and decomposition rates. Schmidt et al. (2011) reported that the molecular structure controlling decomposition rate needs reevaluation to consider several other microenvironmental factors where belowground plant tissue is decomposing. Overall, study results reveal that root decomposition varies widely based on management and edaphic factors.

3.4 | Root morphology differences in C_3 and C_4 grasses

Fine and small roots are classified as <5 mm, with coarse roots being >5 mm, both of which are major components of belowground biomass, and their vertical distributions define subsequent soil physical and biological properties (Tufekcioglu et al., 1998). For example, fine roots represent the dynamic portion of belowground biomass and greater nutrient sinks, both of which are important for net primary production in grasslands. In this study, root surface area and root volume (0–15 cm) were affected by grass species and fertility (Table 6), especially in smaller root diameter classes (0–0.5, 0.5–1.0, and 1.0–1.5 mm). Overall, the native grass mix had lower surface areas (129 vs. 146 cm^2) and lower volumes (1.70 vs. 1.84 cm^3) compared with the nonnative orchardgrass, respectively. Root diameter class had varying effects from poultry litter applications (Table 6). Root diameter finer than 0.5 mm had decreased surface area and volume in native grass roots × fertility, whereas opposite effects were observed for the nonnative orchardgrass, as surface area and volume increased

TABLE 4 Initial root elements cell wall composition (neutral detergent fiber [NDF], acid detergent fiber [ADF], lignin, ash, and hemicellulose), and metal content (Al, As, Cd, Co, Cr, Cu, Fe, and Zn) per forage species ($P \leq .05$) averaged across years (2017–2018) in a silvopastoral study in Fayetteville AK

Species	C/N	P	C	NDF	ADF	Lignin	Ash	Hemi.	Al	As	Cd	Co	Cr	Cu	Fe	Zn
		mg kg^{-1}	%						mg kg^{-1}							
Native grass mix	47.24b[a]	872.87b	43.12	70.53a	55.39a	36.61a	3.93b	15.44a	2,714.81b	1.37b	0.25b	2.15b	7.89b	10.26b	3.354.63b	37.3b
Orchardgrass	30.5a	1,283.36a	32.46b	63.37b	50.42b	34.70a	9.11a	13.94b	6,132.96a	3.10a	0.41a	5.39a	16.56a	15.767a	7.603.27a	53.58a

[a]Different letters indicate a significant difference at $P \leq .05$ within a given analyte.

TABLE 5 Decay rates (k) for root mass and root nutrients derived from exponential decay function: $X = B_0 e^{(-kt)}$, where X is proportion of remaining biomass (or nutrient) at day t, B_0 is the disappearance coefficient, and k is the relative decomposition rate

Forage	Fertility	Moisture	Grazing	Root mass	N	C	C/N	P	Hemi	Lignin	Lignin/N
				% d^{-1}			ratio d^{-1}	mg kg^{-1} d^{-1}	% d^{-1}		ratio d^{-1}
NG	F	Aquic	G	0.011[a]	0.011[a]	−0.001	−0.009[a]	0.013[a]	−0.005	0.004	−0.008
NG	F	Aquic	U	0.009[a]	0.009[a]	0	−0.007[a]	0.013[a]	−0.004	0.003	−0.006
NG	F	Udic	G	0.008[a]	0.008[a]	−0.001	−0.007[a]	0.004	0	0.008	0.002
NG	F	Udic	U	0.011[a]	0.009[a]	0	−0.008[a]	0.012[a]	−0.002	0.002	−0.008
NG	NF	Aquic	G	0.007[a]	0.008[a]	0	−0.005[a]	0.008	0.007	−0.002	−0.009
NG	NF	Aquic	U	0.009[a]	0.006[a]	0.001	−0.003	0.009[a]	0.01	−0.002	−0.006
NG	NF	Udic	G	0.006[a]	0.004	0	−0.003	0.006	0.007	−0.002	−0.003
NG	NF	Udic	U	0.007[a]	0.006[a]	0.001	−0.004	0.011[a]	−0.004	−0.002	−0.007
OG	F	Aquic	G	0.005[a]	0.001	0.002	0.002	0.008	0.005	−0.011	−0.013
OG	F	Aquic	U	0.005[a]	0.001	0.005[a]	0.004	0.006	0.013	−0.006	−0.008
OG	F	Udic	G	0.006[a]	−0.004[b]	0.002	0.006[a]	0.002	0.013	−0.006	−0.002
OG	F	Udic	U	0.006[a]	−0.002	0.002	0.003	0.007	0.013	−0.005	−0.003
OG	NF	Aquic	G	0.005[a]	−0.001	−0.002	−0.001	0.006	−0.005	−0.006	−0.005
OG	NF	Aquic	U	0.005[a]	0	−0.001	−0.001	0.006	0.006	−0.009	−0.008
OG	NF	Udic	G	0.004[a]	−0.001	0.002	0.002	0.004	0.001	−0.002	−0.001
OG	NF	Udic	U	0.006[a]	−0.003	0.001	0.003	0.003	0.004	−0.004	−0.001

Note. Decay rate was explored at four factor levels: forage species (NG = native grass mix, and OG = nonnative orchardgrass), fertility (F = fertilized with poultry litter, and NF = unfertilized control [0 kg N ha^{-1}]), moisture (wet [aquic] and dry [udic]), and grazing (G = grazed, and U = ungrazed). Time (t) ranged from 0 to 48 d. Hemi = Hemicellulose.

[a]Significant at $P \leq .05$ against the null hypothesis $k = 0$.

[b]Negative decay rate indicates increasing root nutrient content over time.

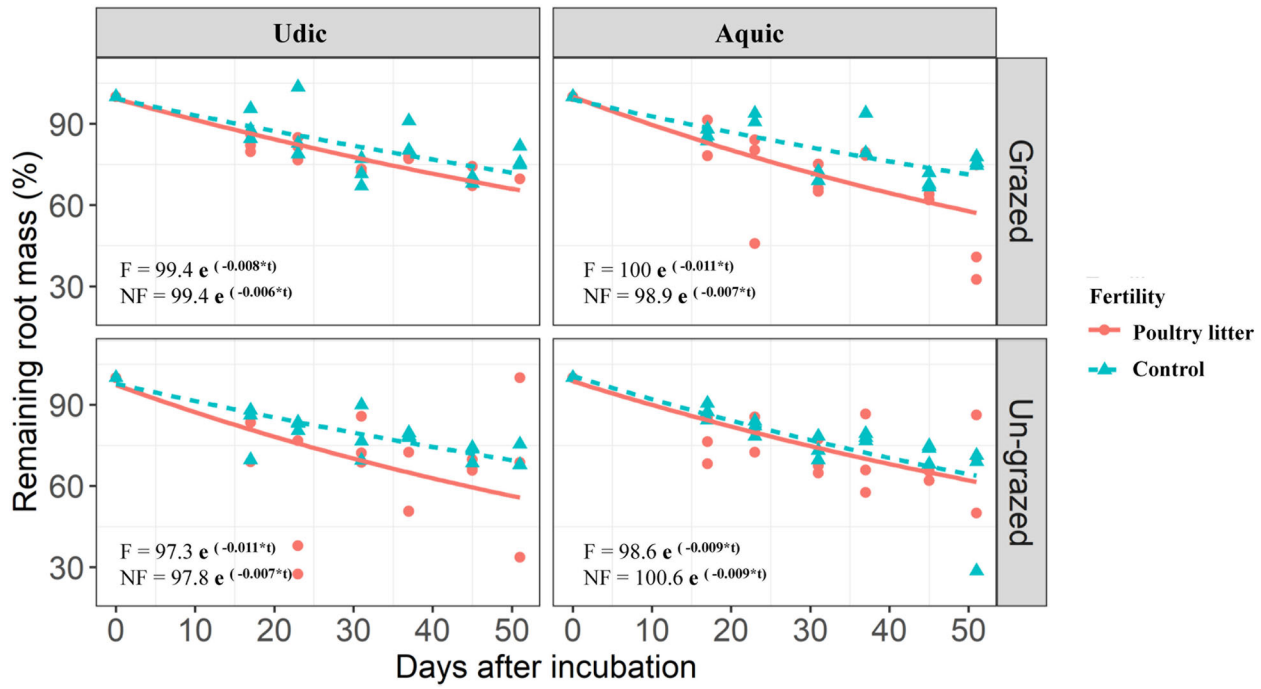

FIGURE 3 Native grass root biomass decay over the incubation time period (Days 0–48) for each treatment level: fertility (poultry litter [F] and an unfertilized control [NF, 0 kg N ha^{-1}]), soil moisture (wet [aquic] and dry [udic]), and grazing (grazed and ungrazed) from 2017 to 2018. Exponential decay function: $X = B_0 e^{(-kt)}$, where X is the proportion of remaining biomass at day t, B_0 is the initial biomass, and k is the relative decomposition rate

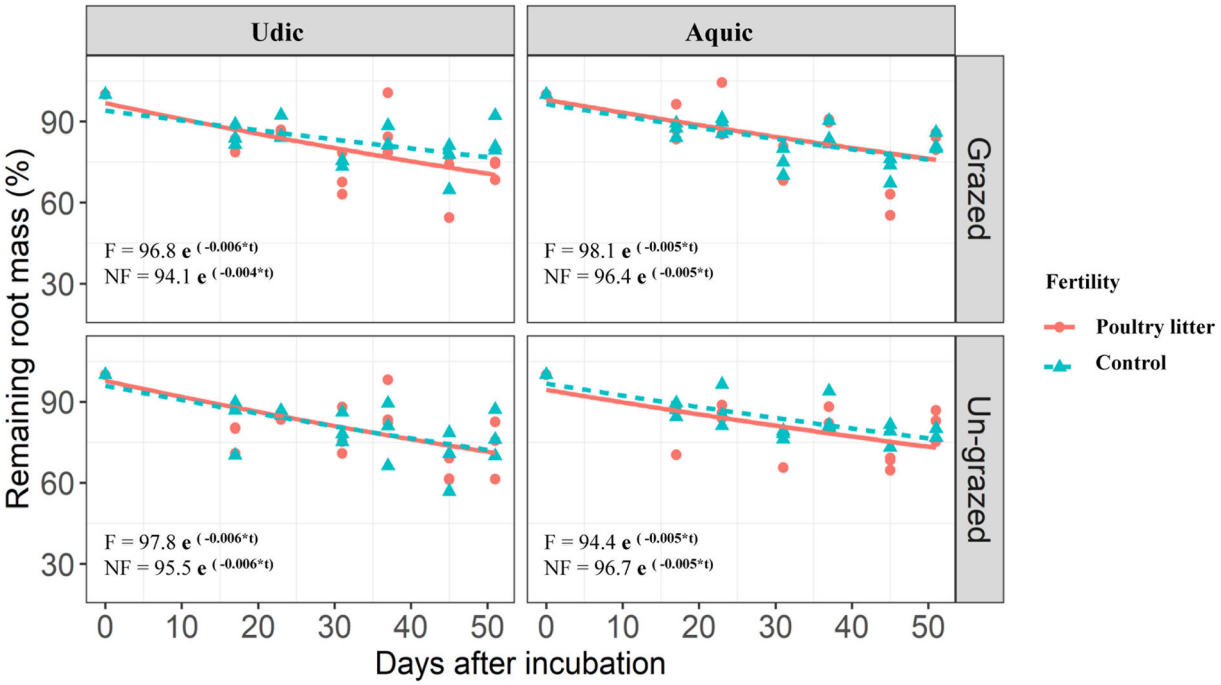

FIGURE 4 Orchardgrass root biomass decay over the incubation time period (Days 0–48) for each treatment level: fertility (poultry litter [F] and an unfertilized control [NF, 0 kg N ha^{-1}]), soil moisture (wet [aquic] and dry [udic]), and grazing (grazed and ungrazed) from 2017 to 2018. Exponential decay function: $X = B_0 e^{(-kt)}$, where X is the proportion of remaining biomass at day t, B_0 is the initial biomass, and k is the relative decomposition rate

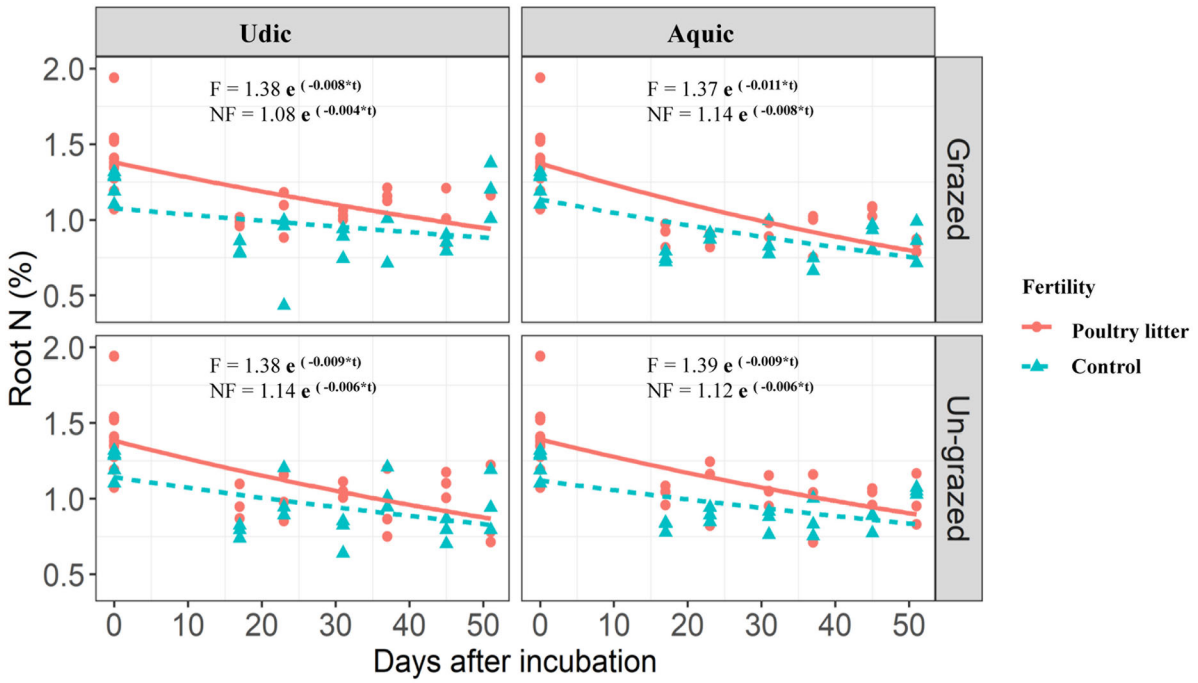

FIGURE 5 Native grass root N (%) decay over the incubation time period (Days 0–48) for each treatment level: fertility (poultry litter [F] and an unfertilized control [NF, 0 kg N ha^{-1}]), soil moisture (wet [aquic] and dry [udic]), and grazing (grazed and ungrazed) from 2017 to 2018. Exponential decay function: $X = B_0 e^{(-kt)}$, where X is the proportion of remaining biomass at day t, B_0 is the initial biomass, and k is the relative decomposition rate

TABLE 6 Analysis of variance of root surface area and volume for each root diameter class from 2017 to 2018 in a silvopastoral system in Fayetteville, AR

Effect[a]	Num. df[b]	Den. df	Root diameter class			
			0–0.5 mm	0.5–1.0 mm	1.0–1.5 mm	1.5–2.0 mm
			Surface area			
			cm^2			
Spp	1	1	0.21[c]	0.34	0.25	0.13
Fert	1	6	0.58	0.06	0.01	0.46
Spp × Fert	1	6	0.04	0.01	0.01	0.17
			Root volume			
			cm^3			
Spp	1	1	0.21	0.86	0.26	0.12
Fert	1	6	0.44	0.03	0.01	0.47
Spp × Fert	1	6	0.03	0.01	0.02	0.16

[a]Spp, forage species (native and nonnative); Fert, fertility (fertilized with poultry litter and a control).
[b]Num. df, numerator degree of freedom; Den. Df, denominator degree of freedom.
[c]ANOVA probability levels significant at $P \leq .05$.

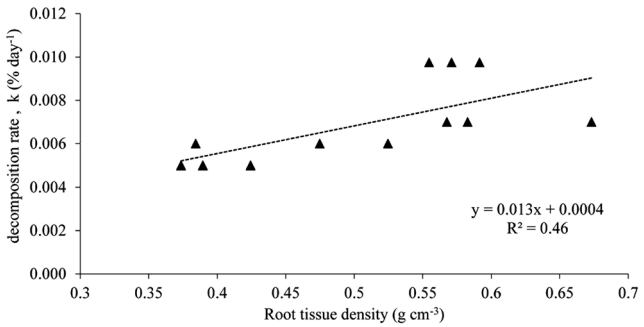

FIGURE 6 Relationship between root-mass decomposition rate (k) and the root tissue density derived from two grass species (native and nonnative) at two fertility levels (poultry litter and an unfertilized control [0 kg N ha^{-1}]) from 2017 to 2018. Decomposition rate were averaged over moisture (aquic and udic) and grazing (grazed and ungrazed) treatments

(DNS). Fertility showed a more pronounced effect on finer root class (0–0.5 mm) compared with coarser root diameter (>0.5 mm; DNS), suggesting that the addition of micro- and macronutrients from poultry litter applications provided important food sources for microbial metabolism in the rhizosphere of these forage crops (Ashworth et al., 2017; Yang et al., 2019). Root tissue density (RTD, ratio of dry mass to volume) was higher in the native grass mix (0.59 vs. 0.42 g cm^{-3} in orchardgrass, $P \leq .05$). There was an interaction ($P \leq .05$) for poultry litter application × grass species for RTD. Poultry litter applications increased RTD in native grass mix (0.57 to 0.61 g cm^{-3}), whereas it decreased for the nonnative orchardgrass (0.45 to 0.40 g cm^{-3}). Although Ryser (1996) showed RTD decreases with fertilizer application, we saw this phenomenon only in nonnative orchardgrass. Additionally, root decomposition rate (k) was positively correlated with RTD (Figure 6), as greater root decomposition occurred in the native grass mix with higher RTD. Usually roots with lower sugar content and larger diameters are expected to have slower decomposition rates (Baddeley & Watson, 2005; Bai et al., 2017), whereas roots with higher specific root length (SRL, root length to dry mass ratio) have faster decomposition rates (Weemstra et al., 2016). However, in this study, orchardgrass had a greater SRL (920 vs. 779 cm g^{-1} in the native grass mix), as well as a lower C/N ratio (Table 4). These results might be attributed to greater five- and six-C sugars and more digestible belowground tissues of the native grass mix (10% greater hemicellulose and 11% greater NDF, Table 4). In addition, Birouste et al. (2012) reported that root chemical composition was the driving factor for decomposition rates between species rather than root morphology. Our result is similar to Jo et al. (2016), who reported a negative correlation between SRL and decomposition rates. Jo et al. (2016) also reported higher RTD in native tree species compared with nonnative species similar to what we observed in our study for grass species. This work is important for understanding nutrient, C mineralization, and root compositional effects on SOC sequestration and root turnover under differential animal inputs, grazing, soil moisture regimes, and grass species in grassland systems.

4 | CONCLUSIONS

Belowground biomass (root litter) turnover is an important component of the C cycle and C sequestration in terrestrial ecosystems. Results from the litter bag retrieval method showed that native grass species have greater decomposition rates than non-native (orchardgrass) forages based on delta root mass and the decay rate derived from an exponential function. Specifically, unfertilized orchardgrass had the

greatest remaining mass, followed by fertilized orchardgrass; indicating less C mineralization and greater recalcitrance for this non-native species. In tandem, greatest soil OM and C storage occurred under the fertilized native grass mix management strategy. In addition, fertilized native grass roots under non-grazing and udic (dry) soil conditions had the greatest decomposition, which did not differ from native grass aquic (wet) and grazed soils. Therefore, there was greater root decomposition (less recalcitrance) with native grass species and poultry litter applications, with soil moisture regimes affecting remaining root mass to a lesser extent. Native grass roots also had greater hemicellulose and lower ash content, indicating greater degradability owing to greater five- and six-carbon sugar levels and less nondigestible inorganic fractions, compared with cool-season forage roots. These results indicate the existence of less favorable microbial food sources in the rhizosphere of orchardgrass. In addition, the RTD (ratio of dry mass roots to volume) was greater in the native grass mix (C_4) relative to the cool-season forage (C_3), with poultry litter applications increasing the RTD in C_4 species, while applications decreased RTD with C_3 species. This study contributes to the current understanding of complex edaphic interactions of grass species, soil moisture, soil temperature, fertility, and grazing and their impacts on root decomposition, which helps control grassland primary and net productivity, as well as nutrient cycling and C sequestration.

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AUTHOR CONTRIBUTIONS

Amanda J. Ashworth: Conceptualization; Data curation; Formal analysis; Project administration; Supervision; Visualization; Writing-original draft. Taylor Adams: Data curation. Tulsi Kharel: Software; Visualization; Writing-review & editing. Dirk Philipp: Project administration. Phillip Ray Owens: Investigation; Project administration. Thomas Sauer: Investigation; Project administration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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