



Winter cover crops impact on soybean yield and quality in Mississippi Delta

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Abstract Cover crops (CCs) improve soil quality, nutrient cycling and main crop yield. However, the benefits vary by CCs, main crops, soils, and climates. For the adoption of CC in cropping systems, it is crucial to identify the CC mix that enhances crop yield, quality and soil sustainability. Therefore, we evaluated the effects of a winter grass mix (GM), a legume mix (LM), and their combination (GM+LM) CCs on soybean (*Glycine max* L.) yield and seed quality. The study was a randomized complete block design with four replications. CCs were planted in winter, terminated in spring, followed by soybean planting in 2022 and 2023. Seed yields were measured at harvest, and seed samples were analyzed for quality: protein, oil, fatty acids, and sugars and nutrient compositions. Overall, the incorporation of winter-mix CCs into soybean production resulted in a 3–14% yield reduction compared to winter-fallow. However, LM CCs produced 7–10% higher yields than GM CCs and had superior nutrient uptake capabilities. Soybean seed fiber, oil, and protein content remained unaffected by CC treatments while variations in fatty and amino acid contents were noted among CC treatments and

years. The GM treatments resulted in higher phosphorus, magnesium, iron, and manganese content in soybean seeds compared to fallow. In the two-year study, soybean seed yield declined under CC treatments, but LM provided higher yield and better seed quality than GM. Further research across soybean varieties, soils, and climates is needed to quantify and recommend suitable CC mixes for seed yield and quality in the region.

Keywords Multispecies winter cover crops · Cover crop biomass · Nitrogen · Soybean yield · Soybean seed quality

Introduction

Soybean [*Glycine max* (L.) Merr.], one of the major row crops grown in Mississippi, increased its production area from 861,000 hectares in 2023 to 930,000 hectares in 2024, generating an estimated economic value of \$1.3 billion (USDA-NASS, 2024a, 2024b). Row crop production in the Mississippi Delta region (MDR) predominantly relies on conventional tillage practices for weed management and planting with fields often left fallow during the winter and early spring. However, this practice leaves the fields vulnerable to soil and nutrient loss from high precipitation during the fallow period, which spans from winter to early spring (November to April), before the main crop planting season. For instance, Tang et al.

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(2018) estimated an average monthly precipitation of ~127 mm during this fallow period, compared to an average of around 82 mm during the crop-growing season in the MDR.

Planting cover crops (CCs) provides multiple agronomic and environmental benefits, including improvements in soil quality (Blanco-Canqui 2018; Bhandari et al. 2023; Lal 2015), enhanced soil carbon sequestration and water retention (Jian et al. 2020; Qin et al. 2021), stimulation of soil biota (Getahun and Keefer 2023), and reductions in soil erosion and nutrient losses (Dabney et al. 2001; USDA-NRCS 2019), which in some cases contribute to improved crop yields (Blanco-Canqui 2018; Lal 2015). A wide range of CC species, including grasses and legumes, have been evaluated either as monocultures or mixtures, with positive outcomes reported across diverse regions of the United States (Acharya et al. 2019; Appelgate et al. 2017; Brakie and Shadow 2020; Debiasi et al. 2025; Steele et al. 2012). However, several studies caution that CCs may reduce the yield or quality of the subsequent cash crop due to competition for soil nutrients and water (Abdalla et al. 2019; Deines et al. 2023; Euteneuer et al. 2022; Koehler-Cole et al. 2020; Mubvumba et al. 2023; Noland et al. 2018; Reddy et al. 2003; Reese et al. 2014), whereas other research has reported no significant yield response during the initial years of CC adoption (Chu et al. 2017; Finney and Kaye 2016). More recently, multispecies CCs have been associated with significantly higher yields compared with single-species CCs (Acharya et al. 2019; Chalise et al. 2019; Chu et al. 2017), highlighting the context-dependent nature of CC impacts on yield and soil health.

These inconsistent findings emphasize the need for a deeper understanding of CC effectiveness, including biomass production, soil nitrate retention, moisture dynamics, nitrogen availability, and nutrient uptake (Blanco-Canqui 2018; Duiker 2014). Winter grass mix CCs (GM) were reported to scavenge nutrients from the soil, improve water infiltration, and minimize soil loss (Blanco-Canqui et al. 2015; Haruna et al. 2020). In contrast, legume mix CCs (LM) were reported to add N to the soil by fixing atmospheric N and from the rapid decomposition of their biomass due to their lower carbon-to-nitrogen (C:N) (Mubvumba and Tyler 2024). A survey found that the farmers who grew CCs mostly used mixtures of legumes and small grains (SARE 2017). However,

producers in the MDR remain reluctant to incorporate CCs especially for soybeans due to uncertainties and a lack of information on CC impacts in location-specific crop production systems (Mubvumba and Tyler 2024; Wallander et al. 2021).

Beyond yield, seed quality is equally important, as higher protein content and increased oleic acid levels are linked to greater profit potential for soybean producers (Merriitt 2017; Held and Dinterman 2025). Notably, limited literature exists on the effects of mixed CCs on subsequent soybean yield, seed quality, and nutrient composition in the Mid-South region. In this context, we hypothesize that integrating CC mixes, such as grass, legume, or combinations, during the winter fallow period in MDR soybean production systems enhances soil-environmental qualities and returns seed yield and quality to producers. As such, this study evaluates the effects of winter cover crop treatments, a grass mix (GM), a legume mix (LM), and a combination of both (GM+LM) on soil variables at termination, soybean yield, seed quality, and nutrient compositions.

Materials and methods

Site description, and experimental design

The study was initiated in 2021 at the USDA-ARS Crop Production Systems Research Unit farm, Stoneville, MS. Cover crops (CCs) were planted annually between the last week of October and the first week of November. The main crop was continuous soybeans (*Glycine max* L.) planted in 2022 and 2023. The soybean seed were purchased from Farmers Feed & Supply Company, 608 N Main St, Leland, MS, 38756. Cover crop cultivar treatments included winter grass CC mix (GM) [wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and rye (*Secale cereal* L.)], and winter legumes cover crops mix (LM) [Austrian winter pea (*Pisum sativum* L.), crimson clover (*Trifolium incarnatum* L.), hairy vetch (*Vicia villosa* Roth)], LM+GM, [wheat+oats+rye+crimson clover+hairy vetch+berseem clover (*Trifolium alexandrinum* L.) and turnip (*Brassica rapa* L.)], and fallow (no cover crops). For GM treatment, wheat, oats, and rye were seeded at 16.8 kg ha⁻¹. Similarly, for LM, Austrian winter pea, crimson clover, and hairy vetch were planted at 16.8 kg ha⁻¹. For LM+GM

treatment, rye, wheat, oats and crimson clover were planted at 16.8 kg ha⁻¹, purple turnip, berseem clover was planted at 11.2 kg ha⁻¹ and hairy vetch and Austrian winter pea were planted at 22.4 kg ha⁻¹ seeding rates.

The soil was Commerce very fine sandy loam (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts). The experimental design was a randomized complete block with four replications, and cover crops were randomly assigned within blocks. Cover crops (blooming and milk stages) were terminated one week before soybean planting using paraquat [dichloride 0.77 kg ha⁻¹ paraquat dichloride (Gramoxone SL2.0, Syngenta)]. Standard agronomic practices for soybean production including herbicide applications were followed. Crop management, detailed site descriptions, and cover crops seeding rates were discussed in Kharel et al. (2023, 2025). Briefly, the production system is no till, CCs were planted on November 3, 2021, and 2022, and terminated on May 4, 2022, and May 12, 2023, respectively. Soybean was planted following cover crop termination on May 11, 2022, and May 18, 2023, and harvested on October 6, 2022, and October 10, 2023. The plots were surface-irrigated using polyethylene tubes positioned at the head of the field, with water delivered down the furrows on land with a slope of less than 2%. Irrigation was initiated when visual symptoms of crop water stress appeared and was terminated at the R6 growth stage. In 2022, potassium (K) fertilizer was aerially applied using agricultural aircraft at 55.6 kg K ha⁻¹ (67 kg K₂O ha⁻¹) as part of the farm's biennial K application practice, while no other fertilizers were applied because soil tests indicated sufficient nutrient availability. The middle two rows were harvested for yield using a Massey 8XP 2-row combine, and soybean seed subsamples were ground and analyzed for quality and nutrient composition.

Cover crop biomass and nutrient analysis

Cover crops (CCs) biomass was collected using two randomly placed 1 m² sampling frames from each plot in 2022 and 2023 prior to termination. However, the biomass in fallow (mostly weeds) was collected only in 2023. The biomass was dried in a greenhouse and then in an oven until constant weight was achieved, to determine the average dry biomass.

Sub-samples were collected, and nutrient analysis of CC biomass was conducted only in 2023. The total nitrogen (TN) content of the cover crops was determined using an Elementar Vario Max combustion analyzer method described by McGeehan and Naylor (1988), while phosphorus (P), K, and other nutrients were analyzed after acid digestion (Lancaster extraction, Cox, 2001) using inductively coupled plasma-optical emission spectrometry (ICP-OES) by acid digest according to Miller (1998). Weather data were collected from the Delta Agricultural Weather Center located in Stoneville, MS, ~2.0 km from the study site. (<http://deltaweather.extension.msstate.edu/stoneville-aws>, accessed in May 2024).

Soil nutrient analysis

Soil samples were collected following harvesting soybeans to assess the impact of cover crops on yields and quality. A tractor-mounted hydraulic Giddings machine (Giddings Machine Company, Inc.) equipped with a 5-cm diameter soil probe, was used for collecting soil cores to a depth of 30 cm in increments of 0–5, 5–15, and 15–30 cm. Three cores were collected per plot and pooled to form one composite sample per depth for each plot. Soil P, K, calcium (Ca), magnesium (Mg), and zinc (Zn) were extracted using the Lancaster method (Cox 2001) and determined by ICP-OES following Miller (1998), using the same procedure applied to cover-crop biomass. Total soil nitrogen (TN) and organic carbon (OC) were measured using an Elementar Vario Max combustion analyzer (McGeehan and Naylor 1988). Soil pH was determined using a pH meter in a soil/water ratio of 1:2 after shaking for 15 min, following the method described by Schofield and Taylor (1955). Soil cation exchange capacity (CEC) was determined by displacing magnesium (Mg²⁺), calcium (Ca²⁺), potassium (K⁺), and sodium (Na⁺) cations with 1N ammonium acetate (NH₄OAc) at pH 7. The amount of adsorbed ammonium acetate on the exchange sites was determined to calculate the CEC (US EPA 1986).

Seed protein, oil, fatty acids, and sugars

Mature seeds were collected at harvesting (moisture content of ~13%) and were ground using a Laboratory Mill 3600 (Perten, Springfield, IL). Seed protein, oil, fatty acids, amino acids, and sugars were determined

in mature seed by a near-infrared reflectance spectrometer (NIRS) DS3-F Analyzer using ISIScan Nova operating software (FOSS North America, Inc., Eden Prairie, MN, USA). The instrument utilized silicon detectors for 850–1100 nm and lead sulfide detectors for 1100–2500 nm, covering a wavelength range of 850–2500 nm.

Statistical analysis

Response variables (soil nutrients, soybean seed yields, seed protein, oil, fatty acids, sugars, and nutrient concentrations) were analyzed using the GLIMMIX procedure in SAS Version 9.4 (SAS Institute Inc., Cary, NC), which integrates characteristics of generalized linear and mixed models (SAS Institute 2017). Treatments were designated as fixed effects, whereas blocks were treated as random effects for the measured parameters. For year-specific analyses, blocks were nested within the year to control unit-level or spatial variation every year, which means that each year has its own set of blocks, and the blocks do not exist across multiple years, meaning interaction between year and treatment was not evaluated. Mean separations were determined using Fisher's protected least significant difference (LSD) at $p \leq 0.10$ when the analysis of variance (ANOVA) was significant at $p \leq 0.10$, respectively.

Results

Environmental conditions during each experiment year

Annual precipitation in 2022 and 2023 was 1140 mm and 873 mm, respectively (Fig. 1a). Peak values occurred in March 2022 (156 mm) and April 2023 (149 mm), while September recorded the lowest (1 mm in 2022; 20 mm in 2023) (Delta AWS, 2024). Both years were drier than the 30-year mean (115 mm monthly; 616 mm during May–October), with mean monthly precipitation of 95 mm (2022) and 73 mm (2023). Despite historically adequate moisture during the crop-growing season, cumulative precipitation during soybean growth was markedly lower in 2022 (416 mm) and 2023 (303 mm) compared to the long-term average (616 mm), indicating potential water limitations. Although precipitation during the

soybean growing season was lower than the typical water requirement of 508–660 mm (Kranz and Specht 2012), supplemental irrigation compensated for the deficit. The average monthly maximum temperature in May (30.3 °C) and June (32.7 °C) of 2022 slightly exceeded the 30-year average but was lower in July and August (Fig. 1b). Conversely, the monthly average maximum temperature from June to October 2023 exceeded the 30-year average. Throughout the growing season (June–October) in 2023, the average monthly maximum temperature trended higher than the 2022 growing season and 30-year average values.

Cover crops biomass and nutrient compositions

There was no significant difference in CCs biomass yield at termination among treatments for either 2022 or 2023. However, the CC biomass was significantly greater in 2023 (3.88 MT ha⁻¹) compared to 2022 (2.20 MT ha⁻¹) across all CC treatments. Although not significant, the LM had 24% and 33% greater CC biomass than LM+GM and GM treatments in 2022. In 2023, all the CCs (LM, GM and LM+GM) had 20%, 26% and 29% greater biomass compared to the fallow (no cover crop treatments) (Fig. 2).

The nutrient content analysis of CCs biomass indicated that LM+GM CCs contained 21% and 19% more total nitrogen (TN) compared to fallow and grass-mix CC (GM), though these differences were not statistically significant (Fig. 3). Similarly, the LM exhibited 19% and 17% more TN content than GM and fallow treatments. The fallow had greater P ($p=0.01$) and S ($p=0.07$) content compared to GM ($p=0.01$). Notably, in 2023, LM+GM demonstrated 33% greater and significantly higher ($p=0.05$) K content than fallow (Fig. 3). Additionally, although not significant, LM biomass showed 2% and 20% more K content than GM and fallow, respectively. These findings suggest that legume CCs can potentially contribute more N and K to the soil after decomposition compared to GM and fallow treatments.

The analysis also revealed that, compared to fallow (18 mg kg⁻¹), the LM+GM and LM biomass exhibited a notable increase in Zn content of 23% (24 mg kg⁻¹) and 19% (22 mg kg⁻¹), respectively. Compared with GM, the biomass of LM+GM contained 31% more Zn (24 vs. 16 ppm), 47% more B (21 vs. 11 mg kg⁻¹), and 26% more Fe (56 vs. 41 mg kg⁻¹). Similarly, LM biomass showed 27%

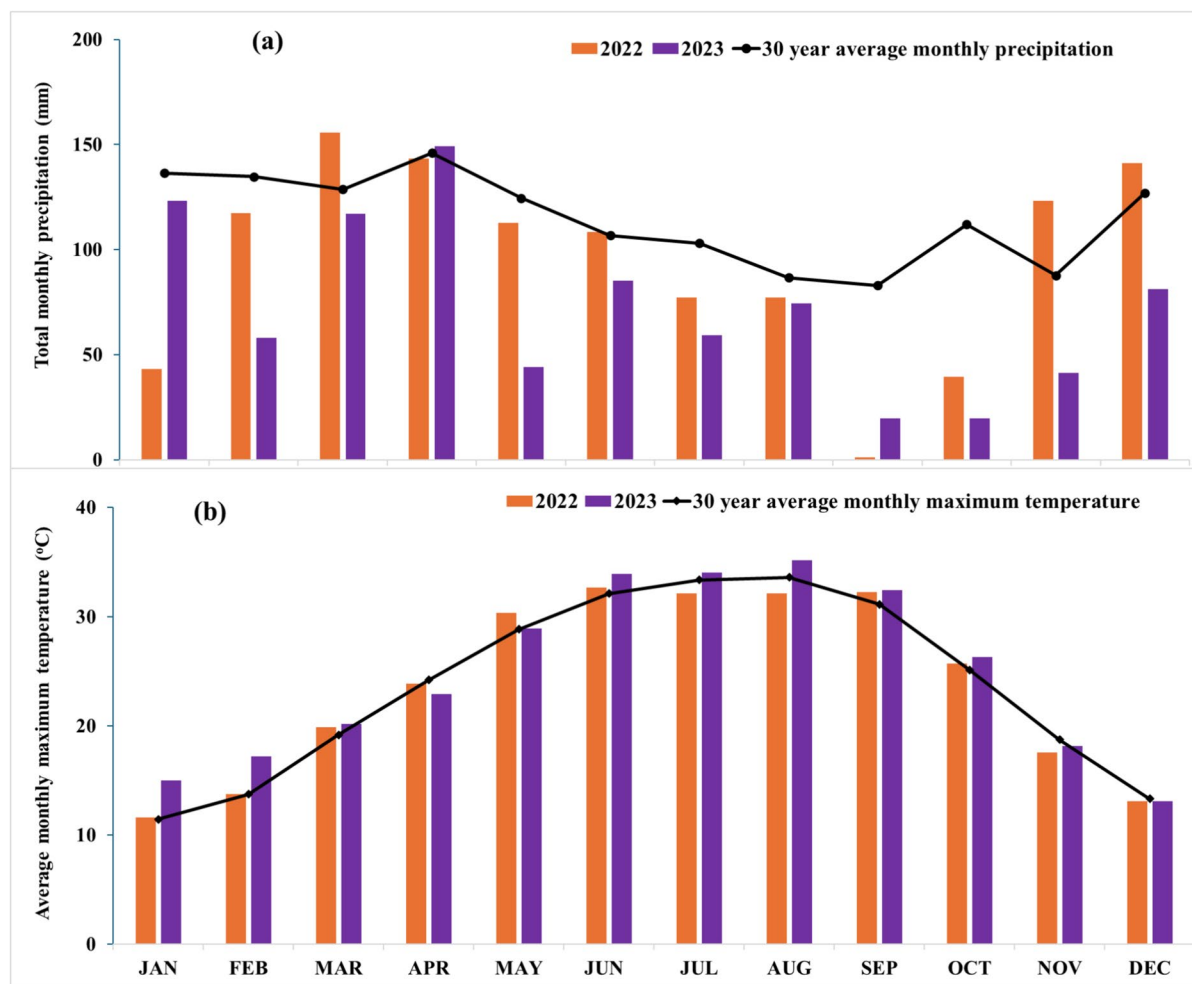


Fig. 1 Monthly weather data during the study period **a** total monthly precipitation from January 2022 to December 2023 and 30-year average monthly total precipitation (1993–2023);

and **b** average monthly maximum temperature data from January 2022 to December 2023 and 30-year average monthly maximum temperature (1993–2023) (Delta AWS, 2024)

more Zn (22 vs. 16 mg kg⁻¹), 49% more B (22 vs. 11 mg kg⁻¹), and 57% more Fe (95 vs. 41 mg kg⁻¹). This may be attributed to the rapid growth and high nutrient uptake efficiency of sparsely spontaneously growing weed species. Likewise S content was 36% and 23% higher in LM (0.096 mg kg⁻¹) and LM+GM (0.070 mg kg⁻¹) compared to GM (0.074 mg kg⁻¹), respectively.

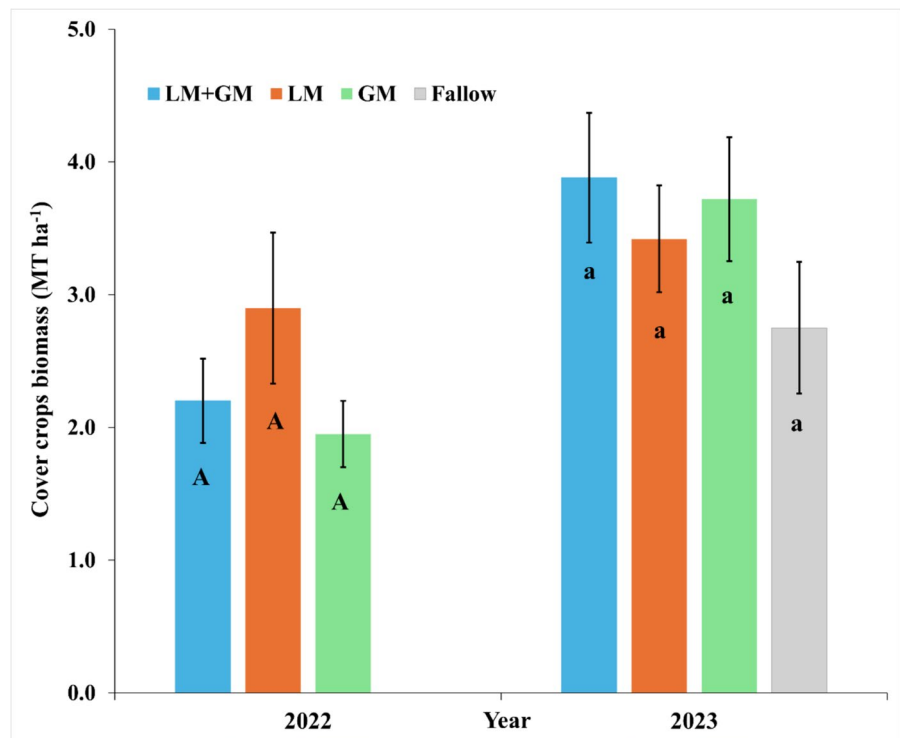
Soil variables at termination as affected by winter cover crops mix

In 2022, the GM treatment exhibited significantly higher soil pH at 0–5 cm depth compared to the LM

and fallow (no cover crops) treatments (Table 1). Conversely, the LM treatment demonstrated significantly higher soil pH at the 5–15 cm ($p \leq 0.01$) and 15–30 cm ($p \leq 0.02$) depths compared to the GM and fallow treatments. This trend continued in 2023, with the LM treatment showing a significantly higher soil pH at 15–30 cm depth compared to the GM treatment.

The total nitrogen (TN) content in 2022 was significantly higher ($p=0.03$) at the 15–30 cm depth in fallow soils compared to the LM treatment, while TN content was comparable between the GM and LM+GM treatments. In contrast, during 2023, the TN content in the LM was significantly greater at the 0–5 cm depth ($p=0.05$) compared to the LM+GM

Fig. 2 Cover crops (CC) biomass by CC treatments (LM+GM=Legume-mix + grass-mix cover crops; LM=Legume-mix cover crops, GM=grass-mix cover crops, fallow=no cover crops) in 2022 and 2023 growing season. The fallow biomass was not collected in 2022. Uppercase and lowercase letters indicate treatment differences within a single year, and different letters indicate statistical differences ($p < 0.10$) of means. Error bars represent standard error



treatment and exhibited a trend of greater TN content than the fallow and GM treatments. Although the difference was not statistically significant, the greater total nitrogen (TN) content at the 0–5 cm depth in the LM treatment may be attributed to the 17% higher N concentration in cover crop biomass compared to fallow. This difference could potentially contribute approximately 20 kg N ha⁻¹ more to the soil under LM cover crops than under fallow following biomass decomposition. Notably, although there was no significant difference in biomass among CC treatments, the LM produced 24–33% more biomass in 2022 than the GM and LM+GM treatments (Fig. 2), potentially contributing to higher TN concentration in the topsoil (0–5 cm depth) after decomposition. Based on 2023 N concentration data, estimated N contribution was greatest for LM (54 kg N ha⁻¹), followed by LM+GM (41 kg N ha⁻¹) and GM (29 kg N ha⁻¹), with LM and LM+GM contributing 25 and 12 kg N ha⁻¹ more than GM, respectively.

The extractable P concentration in soil at the 5–15 cm and 15–30 cm depths was significantly higher with LM+GM compared to the fallow and LM treatments. The CCs biomass at termination was 14% and 29% greater in the LM+GM compared to

the LM and fallow treatments, respectively, which may have contributed to the increased P content in the soils after decomposition. In contrast, the extractable K concentration at 15–30 cm depth was significantly higher in the fallow treatment compared to the LM treatment; however, fallow had the lowest K concentration in biomass.

In 2022, the soil organic matter (SOM) was significantly greater in the LM and LM+GM compared to the fallow treatment at the 5–15 cm depth. However, SOM was higher in the fallow treatment than in the LM treatment at 15–30 cm depth. In 2023, the SOM was greater at 0–5 cm with the LM and GM than the LM+GM treatment, but these treatments were statistically similar across all other depths and CC treatments.

Soybean seed yield, fiber, oil and protein content

The analysis of variance revealed a significant effect ($p=0.008$) of CCs on soybean yield (Table 2). In 2022, the differences were not statistically significant; however, the fallow treatment showed numerical yield increases of 5% (4223 kg ha⁻¹), 12% (3949 kg ha⁻¹), and 8% (4099 kg ha⁻¹) over

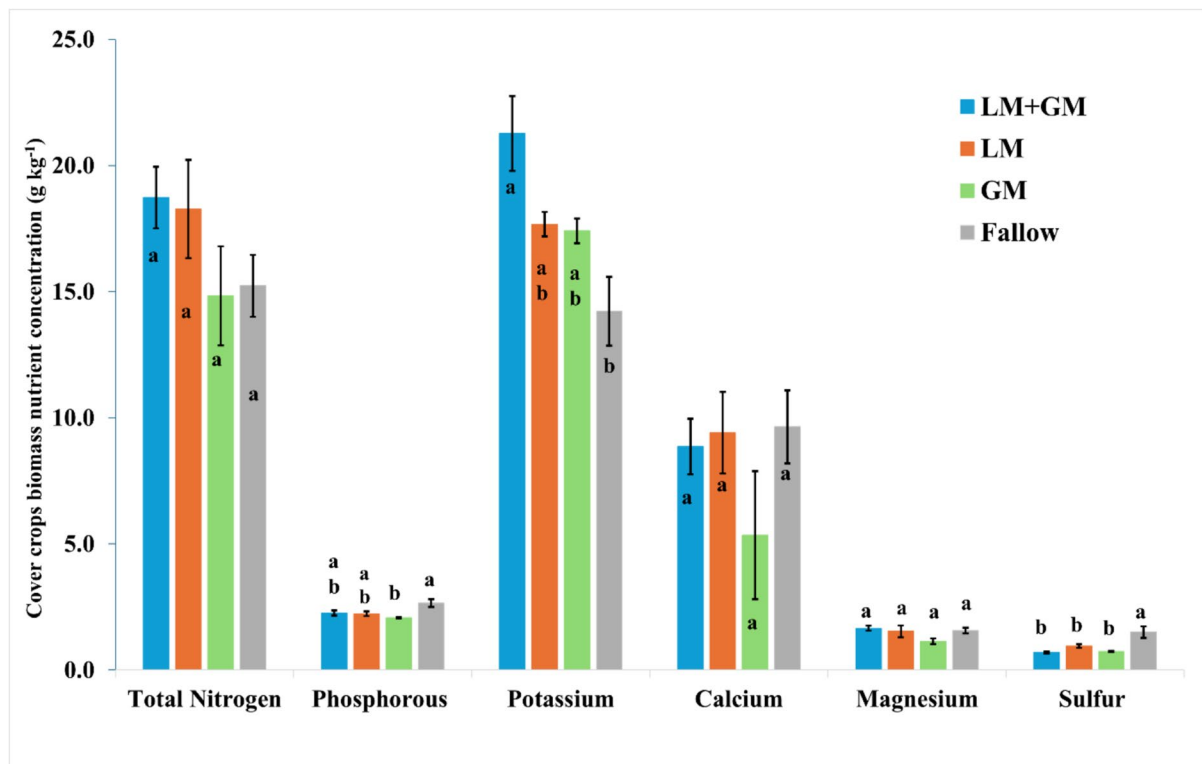


Fig. 3 Cover crop biomass nutrient concentrations at termination in 2023 (LM+GM=Legume-mix + grass-mix cover crops; LM=Legume-mix cover crops, GM=Grass-mix cover crops, fallow=no cover crops). Error bars represent standard error

the LM, GM, and the combined LM+GM treatments, respectively (Fig. 4). In 2023, the fallow treatment produced a significantly higher yield (4598 kg ha^{-1}) than GM but was not significantly different from LM or LM+GM. However, the fallow treatment resulted in 3% (4478 kg ha^{-1}) and 9% (4231 kg ha^{-1}) greater yield compared to LM and LM+GM, respectively (Fig. 4).

The average yield across the CC treatments compared to fallow showed a decrease of 331 kg ha^{-1} in 2022 and 351 kg ha^{-1} in 2023, resulting in an overall average yield reduction of approximately 7.5%. Based on the average soybean market price in November 2023 (USDA-NASS 2024a, b), the estimated economic losses were approximately \$158 per hectare in 2022 and \$168 per hectare in 2023 with CCs. Averaged across years, income reductions relative to the fallow treatment were \$76 for LM, \$165 for LM+GM, and \$248 for GM per hectare, corresponding to yield decreases of 159 kg ha^{-1} , 345 kg ha^{-1} , and 519 kg ha^{-1} , respectively, representing a

substantial financial loss for producers, despite the yield loss not being statistically significant.

Although statistically insignificant, the results showed that, when comparing CC treatments, the LM achieved 7% (2022) and 10% (2023) greater yield compared to the GM. Additionally, the combined LM+GG-CC exhibited 3% and 6% higher yield compared to GM in 2022 and 2023, respectively. Furthermore, the 2023 nutrient content analysis of CC biomass substantiated these findings: compared to GM, both LM and LM+GG-CC exhibited increases of 19–21% in total N, 7–8% in P, 2–18% in K, 26–31% in Mg, 27–31% in Zn, and 26–58% in Fe.

The fiber, oil, and protein content in soybean seed were significantly influenced by year but were unaffected by CC treatments (Table 3). In 2022, oil content was notably higher compared to 2023 across all CC treatments, whereas protein content was significantly greater in 2023 than in 2022, regardless of the CC treatments, including fallow. Across years and treatments, soybean yield and seed protein content

Table 1 Soil chemical properties and nutrient concentrations as affected by the cover crops and soybean production

Treatments	2022				2023			
	0–5 cm	5–15 cm	15–30 cm	31–45 cm	0–5 cm	5–15 cm	15–30 cm	31–45 cm
<i>Soil PH</i>								
Fallow	6.40 ^b	5.92 ^b	6.27 ^c	6.70	6.67	6.22	6.52 ^{ab}	6.95
LM	6.45 ^b	6.50 ^a	6.82 ^a	6.87	6.87	6.40	6.77 ^a	7.07
GM	6.62 ^a	6.17 ^{ab}	6.52 ^{bc}	6.85	6.97	6.17	6.37 ^b	6.80
LM + GM	6.47 ^{ab}	6.17 ^{ab}	6.62 ^{ab}	6.82	6.60	6.27	6.47 ^{ab}	6.75
<i>Cation exchange capacity (CEC) cmolc kg⁻¹</i>								
Fallow	8.53	8.84	11.97	8.84	9.05	10.46	13.41	15.45 ^{ab}
LM	8.59	9.17	10.95	9.17	9.67	9.57	13.80	17.76 ^{ab}
GM	8.59	8.69	11.14	8.69	9.49	10.13	13.15	18.20 ^a
LM + GM	8.64	9.62	12.08	9.62	9.28	10.27	13.43	14.62 ^b
<i>Organic carbon (g kg⁻¹)</i>								
Fallow	6.09	3.66 ^b	3.29 ^a	3.50	6.77	4.05	3.21	2.97 ^b
LM	7.01	4.28 ^a	3.06 ^b	3.36	7.58	4.50	3.18	3.38 ^{ab}
GM	6.88	4.17 ^{ab}	3.21 ^{ab}	3.48	7.57	4.57	3.26	3.17 ^a
LM + GM – CC	7.37	4.36 ^a	3.17 ^{ab}	3.58	6.49	4.41	2.95	2.85 ^{ab}
<i>Total nitrogen (mg kg⁻¹)</i>								
Fallow	845	600	555 ^a	560	1050 ^{ab}	870	730	760
LM	905	650	525 ^b	545	1130 ^a	840	670	670
GM	923	655	545 ^{ab}	550	1060 ^{ab}	810	720	720
LM + GM	96	667	537 ^{ab}	570	960 ^b	834	650	610
<i>Phosphorus (mg kg⁻¹)</i>								
Fallow	36	32 ^b	34 ^{ab}	37	36	38	38	41
LM	34	36 ^{ab}	30 ^b	34	34	35	38	39
GM	33	36 ^{ab}	33 ^{ab}	35	34	37	37	36
LM + GM	36	38 ^a	37 ^a	36	40	36	41	41
<i>Potassium (mg kg⁻¹)</i>								
Fallow	130	94	91 ^a	99	127	85	79	119 ^{ab}
LM	130	89	73 ^b	86	129	83	81	132 ^a
GM	128	79	76 ^{ab}	88	126	87	85	124 ^{ab}
LM + GM	129	98	86 ^{ab}	102	122	87	83	108 ^b
<i>Calcium (mg kg⁻¹)</i>								
Fallow	1183	1181	1732	249	129	145	193	233 ^{ab}
LM	1204	1343	1654	230	141	136	205	266 ^a
GM	1244	1214	1656	256	138	139	188	266 ^a
LM + GM	123.6	1342	1796	249	132	143	193	217 ^b
<i>Zinc (mg kg⁻¹)</i>								
Fallow	0.97	0.61	0.63	0.63	1.0 ^{ab}	0.68	1.12	0.76
LM	1.07	0.62	0.56	0.62	1.1 ^{ab}	0.68	0.63	0.65
GM	1.07	0.60	0.51	0.68	1.2 ^a	0.72	0.63	0.72
LM + GM	1.03	0.71	0.52	0.74	0.9 ^b	0.68	0.69	0.62
<i>Magnesium (mg kg⁻¹)</i>								
Fallow	232	216 ^b	291	46	24	25	32	41 ^{ab}
LM	242	252 ^a	296	44	27	24	35	50 ^a
GM	236	226 ^{ab}	284	44	25	25	32	49 ^a

Table 1 (continued)

Treatments	2022				2023			
	0–5 cm	5–15 cm	15–30 cm	31–45 cm	0–5 cm	5–15 cm	15–30 cm	31–45 cm
LM + GM	236	242 ^{ab}	318	49	24	25	33	38 ^b

LM = Austrian winter pea + clover + vetch; GM = wheat + rye + oats; Means within the column with different letters indicate significant difference ($p \leq 0.10$) due to cover crop treatments

Table 2 Analysis of variance (ANOVA) results for cover crop biomass, soybean yield, protein, fiber and oil content as affected by fixed effect year (Y), cover crops (CC) and their interactions (CC*Y) in 2022 and 2023

Parameter	P value			Overall mean with standard error	
	CC	Y	CC*Y	2022	2023
Cover crops biomass (MT ha ⁻¹)	0.312	0.001*	0.307	1.95 ± 0.29	3.72 ± 0.22
Soybean yield (Y) (kg ha ⁻¹)	0.008*	0.117	0.941	4096 ± 163	4231 ± 170
Fiber content in soybean seed (%)	0.26	0.005*	0.556	6.25 ± 0.14	6.41 ± 0.14
Oil content in soybean seed (%)	0.917	<0.001*	0.655	23.68 ± 0.13	22.60 ± 0.15
Protein content in soybean seed (%)	0.988	<0.001*	0.52	36.38 ± 0.26	38.58 ± 0.28

N.S. = nonsignificant at the 0.05 probability level

*Significant at the 0.05 probability level

Fig. 4 Soybean yield as affected by CC treatments (LM + GM = Legume-mix + grass-mix cover crops; LM = Legume-mix cover crops, GM = grass-mix cover crops, fallow = no cover crops) in 2022 and 2023 growing season. Different letters within each year indicate statistical differences ($p < 0.10$) of means. Error bars represent standard error

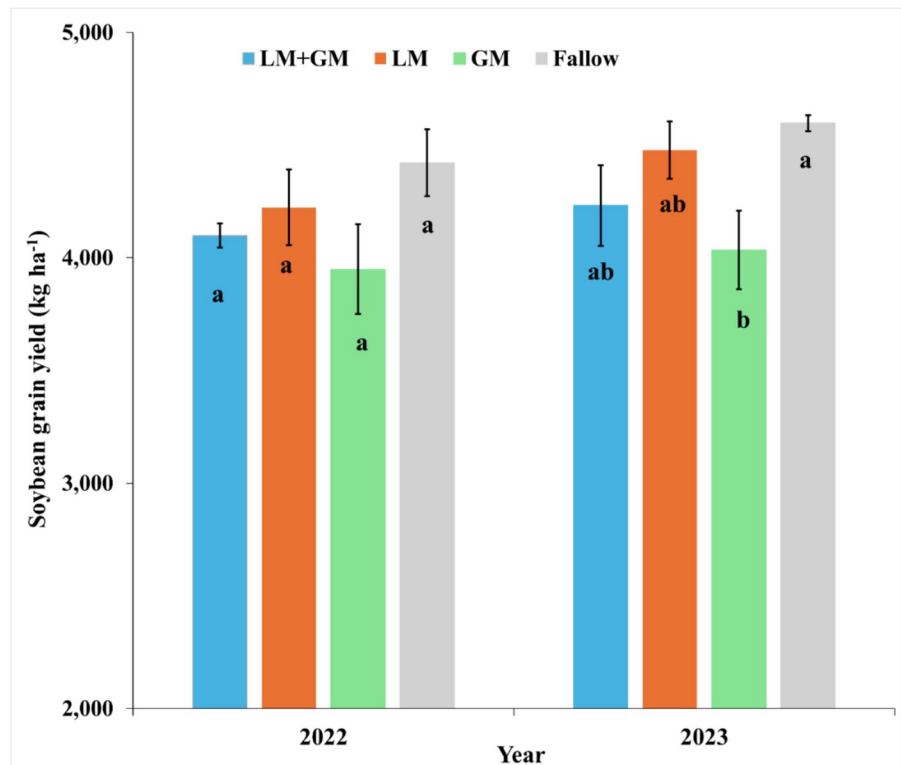


Table 3 Effect of winter cover crops on soybean fiber, protein, oil, fatty acids and sugars (g kg⁻¹)

Tested parameters	2022				2023			
	Fallow	LM	GM	LM + GM	Fallow	LM	GM	LM + GM
Fiber	62.55	60.22	60.17	61.11	63.55	65.60	63.0	63.85
Protein	363.8	364.0	365.7	237.1	388.9 ^a	383.1 ^b	387.0 ^{ab}	382.9 ^b
Oil	236.8	237.3	236.6	237.1	225.1	226.3	224.6	225.3
Palmitic	106.5 ^b	107.5 ^a	107.1 ^{ab}	107.1 ^{ab}	105.3 ^b	105.7 ^{ab}	105.9 ^a	106.0 ^a
Oleic	212.2 ^b	215.3 ^{ab}	218.4 ^a	215.1 ^{ab}	217.3	214.4	216.6	215.8
Linoleic	475.9 ^a	471.9 ^b	475.9 ^a	475.0 ^{ab}	486.1	484.6 ^a	485.0	483.4
Linolenic	87.08	85.93	82.20	85.78	84.63 ^b	88.83 ^a	85.18 ^b	85.68 ^{ab}
Glucose (mg g ⁻¹)	2.30	2.33	2.23	2.23	1.48 ^b	1.73 ^a	1.55 ^{ab}	1.65 ^{ab}
Sucrose (mg g ⁻¹)	56.28 ^a	54.25 ^{ab}	53.95 ^b	54.50 ^{ab}	56.30	57.25	55.73	57.03
Fructose (mg g ⁻¹)	0.500 ^a	0.425 ^{ab}	0.38 ^b	0.425 ^{ab}	0.200 ^b	0.250 ^{ab}	0.250 ^{ab}	0.275 ^a
Raffinose (mg g ⁻¹)	18.30 ^a	14.25 ^b	13.25 ^b	14.70 ^{ab}	12.48	13.75	13.70	14.45
Stachyose (mg g ⁻¹)	28.95 ^b	34.60 ^a	34.25 ^{ab}	32.68 ^{ab}	32.05	31.30	32.13	32.60

LM = Austrian winter pea + clover + vetch;
GM = wheat + rye + oats.
Different letters within rows for each year indicate significant differences ($p \leq 0.10$) by cover crop treatments

exhibited a weak, non-significant positive association (Pearson $r = 0.33$, $p = 0.43$), indicating no strong overall relationship.

Effect of winter cover crops mix on soybean seed fatty acids, sugars and amino acids

In 2022, the results indicated that LM increased palmitic acid, and GM increased the oleic acid in soybean seeds compared to fallow (Table 3). However, linoleic acid was greater in fallow compared to LM and the difference was not observed in LM and LM + GM (Table 3). The sugars (sucrose, fructose) were greater in fallow compared to the GM treatment. Likewise, raffinose was greater in fallow compared to LM and GM.

In 2023, the fallow had greater protein content ($p = 0.03$) compared to the LM and GM (Table 3).

The GM treatment exhibited greater palmitic acid, whereas LM had significantly greater linoleic acid compared to fallow and GM (Table 3).

In 2022, the LM CC had greater palmitic acid and stachyose compared to fallow. The linoleic acid was greater in GM and fallow than other treatments (Table 3). Interestingly, the other sugars (sucrose, fructose and raffinose) were significantly greater with the fallow treatment compared to the CC treatments in 2022 ($p \leq 0.05$), but these differences were not statistically significant in 2023. In 2023, the glucose content was greater with LM ($p \leq 0.076$) and fructose was significantly increased with LM + GM compared to control (Table 3).

In 2022, arginine, isoleucine, leucine, threonine, tryptophan, and valine, were significantly higher in the GM treatment compared to the fallow treatments (Table 4). Although these amino acids trended to be

Table 4 Cover crops effect on soybean seeds essential amino acids content (g kg⁻¹)

Amino acids	2022				2023			
	Fallow	LM	GM	LM + GM	Fallow	LM	GM	LM + GM
Arginine	25.65 ^b	26.23 ^{ab}	26.63 ^a	26.23 ^{ab}	27.73	27.18	27.75	27.33
Isoleucine	16.73 ^b	17.10 ^{ab}	17.20 ^a	16.90 ^{ab}	17.68	17.42	17.85	17.70
Leucine	26.53 ^b	27.17 ^{ab}	27.55 ^a	26.98 ^{ab}	27.95	27.50	28.18	27.90
Lysine	24.98	25.23	25.45	25.23	25.88	25.70	25.70	25.65
Methionine	4.83 ^b	4.95 ^a	4.85 ^{ab}	4.88 ^{ab}	4.90	4.80	4.98	4.95
Cysteine	4.00	4.10	4.22	4.08	4.35	4.18	4.65	4.55
Threonine	13.13 ^b	13.40 ^{ab}	13.60 ^a	13.28 ^{ab}	13.75	13.58	13.85	13.70
Tryptophan	3.25 ^b	3.40 ^{ab}	3.48 ^a	3.40 ^{ab}	3.63 ^a	3.58 ^{ab}	3.53 ^b	3.50 ^b
Valine	16.58 ^b	17.13 ^{ab}	17.35 ^a	16.80 ^{ab}	17.70 ^{ab}	17.40 ^b	18.03 ^a	17.78 ^{ab}

LM = Austrian winter pea + clover + vetch;
GM = wheat + rye + oats.
Different letters within rows for each year indicate significant differences ($p \leq 0.10$) by cover crop treatments

higher in GM compared to other CC treatments, they were statistically non-significant. In 2023, only valine was significantly higher in the GM treatment compared to the fallow treatment. Conversely, tryptophan was significantly higher in the fallow compared to the GM treatment (Table 4).

Effect of winter cover crops mix on soybean seeds nutrient composition

In 2022, phosphorus (P) content in soybean seeds was higher ($p \leq 0.01$) in the GM compared to both fallow and LM treatments (Table 5). Similarly, Mg, Fe and Mn content in soybean seed were significantly greater in the GM treatment compared to the fallow treatment. In 2023, Zn content was significantly higher in the GM compared to both fallow and LM treatments (Table 5). The Mn content was also significantly greater in the LM+GM compared to the fallow treatment.

Discussion

Cover crops biomass and nutrient cycling

Cover crops biomass was greater in 2023 than in 2022, with legume-based CCs (LM and LM+GM) consistently producing greater biomass than grass-mix CCs (GM). The increased CC biomass, together with higher concentrations of N, K, Zn, B, Fe, and S, suggests that legumes may contribute to soil fertility through both the quantity and quality

of biomass produced, potentially due to their rapid growth and efficient nutrient uptake. These findings are consistent with previous studies showing that legume CCs decrease N requirements for subsequent crops compared to non-legumes (Adetunji et al. 2020; Williams and Weil 2018), which could be related to the higher N concentration observed in legume CCs and possible N immobilization with non-legume CCs. Recent work also confirms that CC biomass production and nutrient composition vary by species and environment (Patel et al. 2024; Brito et al. 2025). Meta-analyses and field studies show that legume CCs enhance soil nutrient availability, fertility, and ecosystem services across diverse agroecosystems (Ma et al. 2024; Perrone et al. 2022; Chen et al. 2024a, b; Akchaya et al. 2025), highlighting their role in improving soil fertility and sustainable crop production.

The results from the study indicate that grass CCs with higher C:N ratios were associated with greater soybean yield reductions compared to legume cover crops, which aligns with previous reports linking high C:N ratios to nutrient immobilization during critical crop growth stages (Chu et al. 2017; Peng et al. 2024). The higher nutrient content in LM and its lower C:N ratio compared to GM is consistent with conditions that favor faster nutrient mineralization and recycling into soil systems, which could improve nutrient availability for subsequent crops. Corroborating these findings, Euteneuer et al. (2022) reported lower soybean seed yield with cereal grass cover crops (ryegrass and black oat) compared to the legume (*Lathyrus sativus* L.). Similarly, Acharya et al. (2019) found 8% and

Table 5 Mean comparisons of soybean grain nutrient compositions as affected by winter cover crops mix (g kg^{-1})

Treatments	2022				2023			
	Fallow	LM	GM	LM+GM	Fallow	LM	GM	LM+GM
Nitrogen (N)	57.66	58.23	58.36	57.33	58.51	57.83	58.71	58.10
Phosphors (P)	5.90 ^b	5.90 ^b	6.41 ^a	6.09 ^{ab}	5.03	5.12	5.10	5.30
Potassium (K)	18.63	18.72	20.09	19.29	17.18	17.81	17.58	18.25
Calcium (Ca)	3.75	3.89	4.00	3.96	3.42	3.54	3.44	3.71
Magnesium (Mg)	2.46 ^b	2.52 ^{ab}	2.69 ^a	2.57 ^{ab}	2.16	2.18	2.19	2.28
Sulfur (S)	0.86	0.88	0.97	0.915	1.13	1.12	1.16	1.15
Iron (Fe)	0.079 ^b	0.085 ^{ab}	0.096 ^a	0.079 ^b	0.066	0.670	0.070	0.076
Zinc (Zn)	0.038	0.037	0.040	0.038	0.039 ^b	0.039 ^b	0.041 ^a	0.039 ^{ab}
Boron (B)	0.028	0.029	0.028	0.0292	0.032	0.034	0.035	0.036
Manganese (Mn)	0.028 ^b	0.029 ^{ab}	0.030 ^a	0.029 ^{ab}	0.028 ^b	0.029 ^{ab}	0.030 ^{ab}	0.031 ^a
Copper (Cu)	0.004	0.004	0.005	0.004	0.010	0.009	0.009	0.010

LM = Austrian winter pea + clover + vetch;
GM = wheat + rye + oats.
Different letters within rows for each year indicate significant differences ($p \leq 0.10$) by cover crop treatments

6.7% higher average soybean yield in 2017 and 2018, respectively, with hairy vetch CC compared to control in a three-year study conducted in Louisiana under conventional tillage production systems.

Soil nutrients and organic matter dynamics

The higher TN content in fallow soils at deeper depths in 2022 could be related to the nutrient depleting effect of CCs, which may utilize more N from the soil, thereby reducing inorganic N levels (Mubvumba et al. 2023; Engedal et al. 2023). In 2023, the greater TN content in the LM at the 0–5 cm depth suggest increased N turnover, while the presence of grass in the LM+GM treatment could have contributed to N uptake by the grass CC, potentially resulting in slower decomposition and nutrient turnover. Similar findings by Mubvumba and Tyler (2024) indicated that legumes contributed to increased TN content in topsoil due to faster decomposition and mineralization, releasing N from legume cover crop biomass into the soil. These observations are consistent with broader evidence that legume-based cover crops enhance N mineralization and turnover compared to non-legumes (De Notaris et al. 2020; Nyabami et al. 2023). Moreover, CC biomass has been reported to influence inorganic N, soil nitrate, and phosphorus (P) content in the soil (Blesh and Drinkwater 2014; Christopher et al. 2021).

The significantly higher P content in LM+GM soils at deeper depths could be associated with greater biomass production compared to LM and fallow, contributing to increased P after decomposition. The higher K content in fallow soils at deeper depths may reflect the absence of CCs to extract K from the soil. Increased SOM in LM and LM+GM at the 5–15 cm depth in 2022 is consistent with previous findings in the Mississippi Delta (Mubvumba and Tyler 2024) and with reports of greater SOM accumulation under legumes compared to non-legumes (Hu et al. 2023). The higher SOM in fallow soils at deeper depths could be due to reduced soil disturbance, limited microbial activity, and slower decomposition, which may enhance SOM stabilization (Nielsen and Vigil 2010). Recent studies further confirm that CCs improve soil structure and organic carbon distribution in macroaggregate fractions (Gentsch et al. 2024) and systematically enhance soil organic carbon, a key component of SOM (Salisu et al. 2025). The 2023

results further indicate that SOM dynamics vary with CC species, biomass production, decay rates, and soil depth (Cordeiro et al. 2022; Kuo et al. 1997; Mubvumba and Tyler 2024).

Soybean yield

The typical precipitation during the CC growing season in the Mississippi Delta was adequate (Fig. 1a) and the soybean was irrigated, alleviating concerns regarding CC water usage affecting main crop yields, as Kramberger et al. (2009) reported. Thus, the observed decrease in soybean yield with CC use could be linked to the excessive uptake of soil nutrients by CCs, as suggested by the higher nutrient concentrations in CC biomass compared to the fallow. Additionally, delayed decomposition of CCs biomass might result in nutrient immobilization, increased root density, and altered soil properties, all of which could adversely affect soybean yield (Alonso-Ayuso et al. 2014; Euteneuer et al. 2022). These findings underscore the potential risk of reduced soybean yields during the initial years of CC adoption in the Mississippi Delta region.

Our results indicated a 3%–14% soybean yield loss with CC adoption compared to the fallow treatment. As emphasized by Reddy et al. (2003), these findings highlight the need to evaluate the economic implications of short-term CC adoption alongside soil and environmental benefits. The findings of this study are consistent with those of Reddy et al. (2003), who found no yield advantage from seven different cover crops (CCs) compared to a no CC control in soybean systems without herbicide application in the Mississippi Delta. Similarly, using the causal forest analysis machine learning technique, Deines et al. (2023) reported an average 3.5% reduction in soybean yield associated with CC adoption across six states. Alvarez et al. (2017) documented a ~2% yield reduction with CCs compared to no CCs in Argentina. Several studies have also indicated no significant yield response in the initial years of CC adoption (Chu et al. 2017; Finney and Kaye 2016; Smith et al. 2014). Contrarily, Chu et al. (2017) reported significantly higher yields with multispecies CCs compared to single species and no cover crop control. Chalise et al. (2019) observed a 14% yield increase due to CC treatments compared to no CC treatments in the Northern Great Plains,

a region with differing weather conditions from the Mississippi Delta. More recently, Kirkpatrick et al. (2023) found that CCs influenced soybean yield and partial economic returns in Arkansas, further highlighting the variability of CC impacts across environments and management systems.

Soybean seed quality and nutrient composition

Euteneuer et al. (2022) supported the results on seed composition, indicating that yearly weather conditions influence protein and oil content. In accordance with the results, Assefa et al. (2018) reported an increase in protein content corresponding to the increase in soybean yield. Conversely, other studies have documented an inverse relationship between protein content and yield (Mello Filho et al. 2004; Singh et al. 2016). Winter CC mixtures were associated with changes in soybean seed composition, with LM linked to higher palmitic acid and sugars, and GM linked to oleic acid and amino acids compared to fallow. These changes suggest that CC type can influence soybean quality traits through nutrient cycling and decomposition. Euteneuer et al. (2022) reported no effect of cover crops on protein, oil, and sucrose content in Australia, while Darby and Krezinski (2025) showed that winter rye termination timing affected soybean seed quality traits, and Kassem (2021) emphasized broader genetic and environmental drivers of fatty acid, sugar, and amino acid variation.

Despite reduced yield, the GM treatment increased soybean seed P, Mg, Fe, Mn, and Zn, while LM + GM also increased Mn compared to the fallow. This pattern of higher nutrient concentration in seeds under reduced yield conditions is consistent with the mechanism described by Bender et al. (2015), where fewer seeds accumulate proportionally more nutrients. Consistent with our findings, Gonzalez et al. (2024) reported that gypsum applied with CCs interacted with soil amendments to influence seed nutrient profiles. In contrast, Acharya et al. (2019) observed no significant effects on seed nutrients except for boron, and Rice et al. (2024), reported only limited impacts on soybean seed quality. Further research is needed, as genetic and environmental factors strongly influence soybean seed nutrient composition (Kassem 2021).

Conclusions

Cover cropping has been regarded as a viable management intervention in crop-fallow systems for enhancing soil and environmental quality, as well as crop production. However, the known risks associated with cover crops, including depleting soil water and nutrients for subsequent cash production in certain crop species, climates, and soils, limit their adoption by the farming community. Results of this investigation over two years in the humid climate of the Mississippi Delta showed that legume and grass cover crop mixes as well as their combinations were found to reduce soybean seed yield. Among the cover crop treatments, the legume mix outperformed the grass mix in terms of yield, though the difference was not significant. Seed quality and nutrient composition responded variably to the different cover crop treatments. The legume mix resulted in higher oil content, palmitic acid, and methionine levels compared to the grass mix and fallow. In contrast, the grass species mix enhanced the concentrations of several amino acids (arginine, isoleucine, leucine) and nutrients (phosphorus, magnesium, iron, and manganese) in soybean seeds relative to both the fallow and legume treatments. Further long-term studies involving novel cover crop species mixes across various soils and climates of the Mississippi Delta region can lead to the identification and adaptation of an optimal cover cropping strategy for soybean seed yield and quality, as well as for maintaining soil environmental quality.

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