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## ORIGINAL ARTICLE

## Agricultural Soil and Food Systems

# Soil health responses to cover crop functional group and richness in semiarid Montana

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## Abstract

Despite a plethora of studies on the effects of cover crops on soil health, few published papers have reported the effects of plant functional group and richness on soil health, especially in semiarid regions. We initiated a no-till study in semiarid Montana in 2012 that consisted of Pea (*Pisum sativum* L.), four two-spp single functional groups (Brassica, Fibrous-rooted, Legumes, and Tap-rooted), four three-functional group mixes, a four-functional group mix (Full), and a summer fallow control (Fallow). Cover crops were terminated with herbicide when pea was at first flower stage, and wheat (*Triticum aestivum* L.) was grown after each cover crop at three nitrogen (N) rates. After four cover crop cycles, soil in both Pea and Full had greater soil organic carbon (SOC), soil total nitrogen (STN), and potentially mineralizable nitrogen (PMN) than Fallow, but Pea, Full, and Fallow treatments did not differ in infiltration rate, penetration resistance, or soil enzyme activity. There were few differences in soil health parameters between Pea and Full, and among functional groups. Soil in the three-functional group treatments had 20%–35% greater PMN than in the one-functional group treatments ( $p < 0.05$ ), yet SOC and STN were not affected by functional richness. Nitrogen rate did not affect SOC, STN, or PMN. Concentrations of SOC were weakly related ( $R^2 = 0.05$ – $0.14$ ,  $p < 0.05$ ) with 7-year aboveground biomass returned, suggesting practices that increase residue amounts might be more important to SOC and other soil health parameters than functional group or richness.

## Plain Language Summary

Growing cover crops in place of summer fallow (no crop grown for a year) has the potential to improve soil health. The goal of this study was to determine if more diverse cover crop mixtures improved soil health more than single species or simple mixtures in Montana where there are low amounts of precipitation. Cover crops generally improved soil health compared to not growing any crop but cover crops

**Abbreviations:** IC, inorganic carbon; LTA, long-term average; MAOM, mineral-associated organic matter; PMN, potentially mineralizable nitrogen; POM, particulate organic matter; PR, penetration resistance; RLD, root length density; SOC, soil organic carbon; STN, soil total nitrogen; TC, total carbon.

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with six or eight species in the mixture generally did not improve soil health compared to cover crops with one or two species. Farmers should select cover crops based on seed cost, their ability to produce ample biomass, and ability to “fix” nitrogen, but should not feel the need to plant numerous species if their primary goal is to improve soil health.

## 1 | INTRODUCTION

Soil health is increasingly recognized as a key component of agroecosystem sustainability and resilience. Reducing tillage, incorporating perennials into cropping systems, and replacing fallow are among numerous management practices that can positively influence soil health parameters (Engel et al., 2017; Kelly et al., 2020; Sprunger et al., 2020). In semiarid regions where no-till has been widely adopted, and relatively low abundance of livestock reduces the need for perennial hay, summer fallow replacement likely provides the largest opportunity to improve soil health. Replacing summer fallow with cover crops, instead of an annual grain crop, has the advantage of conserving soil water for the subsequent cash crop (Miller et al., 2006), especially when the cover crop is terminated well before maturity (Miller et al., 2011).

Measured changes in soil health parameters from cover crops depend on study duration (Blanco-Canqui, 2022), soil texture (Blanco-Canqui & Ruis, 2020; Hao et al., 2023; Wood & Bowman, 2021), initial soil organic carbon (SOC) (Blanco-Canqui, 2022), and climate (Hao et al., 2023; Kim et al., 2020), among other variables. Cover crops generally improve or have no effect on soil physical properties (Blanco-Canqui & Ruis, 2020; Hao et al., 2023), biological parameters (Domnariu et al., 2024; Housman et al., 2021; Kim et al., 2020; Nevins et al., 2018), and chemical properties, including SOC (Domnariu et al., 2024; Engel et al., 2017; Simon et al., 2022).

Despite a plethora of published studies on cover crop effects on soil health combined with a growing interest in cover crop diversity, there are relatively few published papers that address the role of cover crop functional group and functional group or species richness on soil health. In a large review that summarized the effects of cover crops on soil physical properties, legume cover crops reduced PR more than brassicas and mixes, but legumes had less of a benefit on infiltration rate than grasses and mixes (Blanco-Canqui & Ruis, 2020). Otherwise, there were few effects of cover crop composition on soil physical parameters. Hacker et al. (2015) found that phosphatase activity was not related to cover crop functional group or species richness. In semiarid western Kansas, levels of most soil health parameters, including SOC pool, bulk density, water-stable aggregates, and infiltration rates, were the same between herbicide-terminated spring trit-

icale (*Triticum* × *Secale*) and three- and six-species mixtures after 12 years (Simon et al., 2022). It is important to stress that in comparisons between a single species cover crop and cover crop mixtures, that very often one well-adapted, large biomass-producing single species such as annual rye (*Lolium multiflorum*), triticale, or common vetch (*Vicia sativa*) is compared with a mixture, producing a potential bias toward the single species with a dominant effect. For statistical reasons, treatments should consist of each different single species or functional group from the pool of species or functional groups used in the diverse mix (Huston, 1997; Schappert et al., 2019). In a greenhouse study with legume and brassica cover crops, where individual species were each included in the mixtures, cover crop species richness was positively correlated with SOC and meso-aggregates (250 to 500 μm) in the top 5 cm, but not lower in the profile (Saleem et al., 2020). In a 7-year study in a humid region, functional type (grass, brassica, and legume) affected C inputs, particulate organic matter (POM), and mineral-associated organic matter (MAOM), but not the SOC pool (Zhang et al., 2022). In the same study, a mixture with each functional type had higher MAOM than the brassica or grass, but POM and SOC were not different between the mixture and each functional type. Because there was only one species within each functional type, it is not possible to know if the results reflected the effects of species or functional richness on C fractions.

Given the dearth of studies that investigated the effects of functional group and functional richness on soil health parameters, especially in semiarid regions, we initiated a study in 2012 to fill this research void. We asked three key questions specific to our semiarid, no-till agroecosystem when cover crops were grown in a 2-year rotation with wheat for four cover crop cycles: (1) Are there differences in soil health parameters among a single species well-adapted legume cover (Pea), a four-functional group/eight-species mixture, and summer fallow? (2) Does functional group of the cover crop affect soil health parameters? (3) Does functional richness (one- vs. three-functional groups) affect soil health parameters? Subsequently, we asked an additional question to address the mechanisms behind our results: (4) Is SOC related to shoot biomass of both cover crop and cash crop residue?

Cover crops were selected to represent four plant functional groups based on their abilities to affect soil health:

(1) Brassica for glucosinolate-based root exudates and early, rapid growth; (2) fibrous root species for SOC building; (3) legumes, which contribute to organic and inorganic nitrogen (N) pools; and (4) tap-rooted plants to increase infiltration and reduce compaction. We hypothesized that cover crops would increase most soil health parameters compared to summer fallow, that functional groups would vary in their effects on different aspects of soil health, and that increased functional group richness would increase soil health parameters, following hypotheses related to biological diversity and ecosystem function (Hooper et al., 2005).

## 2 | METHODS

### 2.1 | Study design and management

Each of the four functional groups included two species, and a Full eight-spp mix was formed by combining the four two-spp mixes (Table 1). In addition, four six-spp “Minus” treatments were included that consisted of the Full treatment minus each functional group. Finally, two controls were added: Pea, because of substantial research on sole pea cover crops in Montana (Miller et al., 2011, 2015), and summer fallow (Fallow) because of its prevalence in the region, for a total of 11 treatments. Grasses are well known to have fibrous root structures, while safflower (*Carthamus tinctorius* L.) and turnip (*Brassica rapa* L.) have long taproots based on water use or observation (Miller & Holmes, 2012; Weaver & Bruner, 1927). Species that performed poorly in their first year of use were replaced as detailed in Miller et al. (2023). The experiment was a randomized complete block design with 11 treatments and four replicate blocks.

Cover crops were seeded directly into standing wheat stubble on fields that had been under no-till management for at least 4 years prior to 2012. Seeding rates targeted typical grain seeding rates in 2012 but resulted in greatly favoring certain cover crops with high seeding rates and high emergence rates like oat. Therefore, in subsequent years we used an equal target plant density of 120 plants  $m^{-1}$  for all cover crop treatments (Miller et al., 2023). Seeding (23-cm row, no-till disk) occurred in mid-April 2012 and in early to mid-May in subsequent years. Cover crops were terminated with glyphosate (630–840 g a.e.  $ha^{-1}$ ) with adjuvants when 50% of pea plants had at least one open flower; this termination timing was based on previous research demonstrating substantial water use and often large cereal grain losses the subsequent year when terminating later, especially at pod (Miller et al., 2006, 2011). Termination dates ranged from June 30 to July 11. No fertilizer was added to cover crops based on most producers in the region expressing a desire to minimize input costs when growing cover crops.

### Core Ideas

- Soil carbon and nitrogen concentrations were greater with cover crops than summer fallow after 7 years.
- Levels of most measured soil health parameters did not differ between a pea cover crop and an eight-spp mixture.
- Plant functional group generally did not affect soil health parameters.
- Functional richness affected potentially mineralizable nitrogen, but not soil organic carbon (SOC) or soil total nitrogen.
- Total study aboveground biomass (cover crop, weeds, and stubble) was positively, though weakly, related with SOC.

Winter wheat was seeded in September 2016, and spring wheat was seeded in April 2013, 2015, and 2019. Wheat was seeded perpendicular to cover crop rows, fertilized at 0, 67, or 135 kg N  $ha^{-1}$  (subplots) with mid-row banded urea so that the 2-year crop rotation was cover crop (or fallow)–wheat. No phosphorus (P) or potassium (K) was applied based on soil tests that showed P was close to adequate at Amsterdam and more than adequate at Conrad, and K was above critical levels at both sites (Jacobsen et al., 2005). Other details of cover crop management and subsequent wheat management are described by Miller et al. (2023).

### 2.2 | Sites

The study was conducted at Amsterdam, located in southwest Montana, and Conrad, located in northcentral Montana (Figure 1). The sites were selected to represent typical precipitation amounts for dryland crop production in Montana, with Conrad receiving 303 mm (Western Regional Climate Center [WRCC] 241974) and Amsterdam 358 mm (WRCC 240622) mean annual precipitation (1981–2010). Approximately one-third of the annual precipitation at each site was received during the May–June period, typical for this region. Mean annual temperatures were 7.4°C at Amsterdam and 6.2°C at Conrad. Soil at Amsterdam was a coarse-silty, mixed, superactive frigid Typic Calcistoll, and at Conrad was a fine, smectitic frigid Aridic Argiustoll (Table 2). The sites had circumneutral to high pH, somewhat typical SOC for Montana, Olsen P generally near, or above, Montana’s critical level of 16 mg  $kg^{-1}$  and exchangeable K levels above Montana’s 250 mg  $kg^{-1}$  critical level.

TABLE 1 Cover crop treatments, composition, and years used.

Treatment	Composition	Years
Fallow	–	2012–2018
Pea	Pea ( <i>Pisum sativum</i> L.)	2012–2018
Full Mix	Brassica, fibrous root, legume, tap root	2012–2018
Brassica	Camelina [ <i>Camelina sativa</i> spp. <i>sativa</i> (L.) Crantz] and daikon radish [ <i>Raphanus sativus</i> var. <i>longipinnatus</i> (L.) G. Beck] Radish and winter canola ( <i>Brassica napus</i> L.)	2012 2014–2018
Fibrous root	Oat ( <i>Avena sativa</i> L.) and perennial ryegrass ( <i>Lolium multiflorum</i> L.) Oat and canaryseed ( <i>Phalaris canariensis</i> L.)	2012 2014–2018
Legume	Common vetch ( <i>Vicia sativa</i> L.) and pea lentil ( <i>Lens culinaris</i> Medik) and pea	2012 2014–2018
Tap root	Safflower ( <i>Carthamus tinctorius</i> L.) and turnip ( <i>Brassica rapa</i> L.)	2012–2018
Minus Brassica	Fibrous root, legume, safflower <sup>a</sup>	2012–2018
Minus Fibrous	Brassica, legume, tap root	2012–2018
Minus Legume	Brassica, fibrous root, tap root	2012–2018
Minus Tap Root	Brassica, fibrous root, legume	2012–2018

<sup>a</sup>Turnip, a taproot species, was excluded from Minus Brassica because turnip is also a brassica.

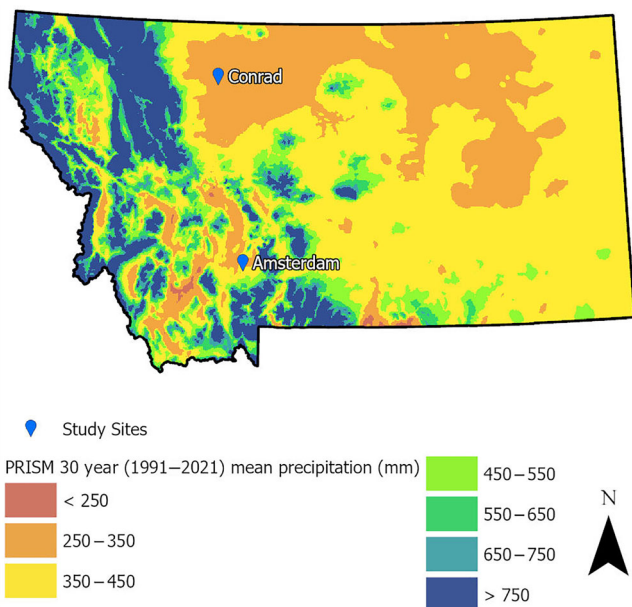


FIGURE 1 Locations of cover crop sites in Montana, including 30-year mean annual precipitation (PRISM Climate Group, Oregon State University, <https://prism.oregonstate.edu>, US average total precipitation, 1991–2020 [80 m; Band Interleaved by Line]). Data created October 29, 2021.

## 2.3 | Data collection and analysis

This paper focuses on selected biological, chemical, and physical soil parameters. Cover crop biomass, wheat yield and protein, soil water, and soil nitrate methods and results are described in Miller et al. (2023). Soil was characterized in early April 2019, 9 months after cover crop termination and just prior to spring wheat seeding, to evaluate the soil health

TABLE 2 Pre-study site characteristics of the two cover crop study sites in Montana.

	Amsterdam	Conrad
Sample date	March 26, 2012	April 11, 2012
Location	45.72° N, 111.37° W	48.21° N, 111.50° W
Elevation (m)	1446	1039
Texture	Silt loam	Clay loam
pH	8.2	6.5
Soil organic carbon (g kg <sup>-1</sup> )	14	14
NO <sub>3</sub> -N (mg kg <sup>-1</sup> )	6.0	8.5
Olsen P (mg kg <sup>-1</sup> )	13	28
Exchangeable K (mg kg <sup>-1</sup> )	359	498

Note: All samples analyzed by AgVise Laboratories, Northwood, ND, are from the 0- to 15-cm depth; values are means of 12 soil samples per site. Laboratory methods used include texture, Bouyoucos hydrometer; pH and salinity, 1:1 soil-to-water method; soil organic carbon, loss on ignition (LOI); NO<sub>3</sub>-N, 1 M KCl extraction, Cd reduction, spectrophotometer determination; Olsen P, 0.5 M NaHCO<sub>3</sub> extraction, colorimetric determination; extractable K, NH<sub>4</sub>OAc extraction, atomic absorption spectrometry.

conditions that actively growing spring wheat roots would experience.

### 2.3.1 | Biomass

Biomass collection and processing are detailed in Miller et al. (2023). Briefly, biomass sampling involved collecting shoot biomass of cover crops and weeds, by cutting plants at the soil surface from four locations (ca. 1 m<sup>2</sup> total) within each

plot in 2012 (prior to N treatments applied in 2013), the 0 and high N rate subplots in 2014 and 2016, and the medium N rate treatments for all treatments plus low and high N rates for Fallow, Full, and Pea in 2018. In 2014 and 2016, the medium N biomass was estimated by interpolating between 0 and high N rate biomass amounts. Biomass was dried (60°C) and weighed, and the mass was converted to  $\text{Mg ha}^{-1}$ . Wheat straw biomass was estimated using grain harvest index (GHI) by applying GHI values collected in 2019 at both sites and from similar studies with similar growing conditions by the same authors in dryland Montana (data not shown). The GHI values often had to be adjusted upward for the Fallow control treatment and occasionally downward for the Pea and Legume treatments and higher N rate treatments, especially at the drier Conrad location, where N-induced “haying off” was most severe. Unkovich et al. (2010) reported that both N fertilizer and legumes occasionally influenced GHI downward. Weed biomass was negligible in wheat years and was not collected. Aboveground biomass for the first 7 years of the study was summed to produce total study biomass at both sites; the 7-year timeframe was selected to compare total biomass returned with soil health parameters collected immediately prior to the 8th year wheat crop. For logistical reasons, root biomass was not quantified in these fine-textured soils, and given effects of soil texture, pot size, and management conditions on root/shoot ratios (Ahmadi et al., 2025), we felt we could introduce substantial error by using published root/shoot ratios to calculate root biomass and thus opted to focus on shoot biomass only.

### 2.3.2 | Soil organic carbon and soil total nitrogen

Soils were sampled in the upper 10 cm at both sites in each medium N rate ( $67 \text{ kg N ha}^{-1}$ ) subplot (all treatments) and in subplots of all three N rates in Fallow, Full, and Pea treatments. Two subsamples were collected with a 3.0-cm diameter core using a hydraulic probe truck, and the subsamples were composited. Samples were dried, weighed, ground ( $<2 \text{ mm}$ ), and analyzed for total carbon (TC) and soil total nitrogen (STN) on a LECO CN combustion analyzer (LECO Corporation). Inorganic carbon (IC) was measured with a modified pressure-calimeter method (Sherrod et al., 2002) on all soils with  $\text{pH} > 7.5$  (based on likelihood for presence of  $\text{CaCO}_3$ ), and SOC was calculated as the difference between TC and IC. All Amsterdam soils had  $\text{pH} > 7.5$ , where the minimum pH was 8.2, whereas only seven samples at Conrad met the  $\text{pH} > 7.5$  criterion. The pH 7.5 cutoff appeared reasonable based on IC representing only 2.6% and 3.2% of TC on the two Conrad samples with pH levels between 7.5 and 8.0, (compared to 6%–25% on the five samples with  $\text{pH} > 8.0$ ), meaning there was very little IC between pH 7.5 and 8.0 suggesting even less below pH 7.5.

### 2.3.3 | Infiltration rate

In April 2019, infiltration rate was measured at both sites in each Fallow, Full, and Pea treatment (medium N rate subplots only) using a double ring (15-cm inner diameter, 30-cm outer diameter, and 10-cm tall) infiltrometer (AMS Inc.). The falling head method was used with minor modifications (ASTM, 2003). Four rings per subplot were pounded 5 cm into the soil and pre-equilibrated by pouring water gently onto a sponge to avoid soil disturbance until the water surface was at the top of each ring. After the water had infiltrated, the procedure was repeated, and both time and water surface distance from the top of the ring were recorded periodically in the inner ring only. The targeted minimum number of readings was three for statistical analysis, which was attained in 45 of 48 rings at Conrad and 37 of 48 rings at Amsterdam. Water height versus time plots demonstrated a high degree of linearity for infiltration rate, with  $R^2$  values averaging 0.96 per plot or greater. Rates from the four rings per plot were averaged to produce a mean infiltration rate per plot.

### 2.3.4 | Penetration resistance

Penetration resistance (PR) was measured with a static cone penetrometer (Model S-215) in the same subplots as infiltration was measured. Given the strong, inverse effect of soil water content on PR (Lardy et al., 2022), all measurements were taken within the areas moistened by the infiltration tests, approximately 24 h after the infiltration rate test was completed. Volumetric water content at sampling time should have been near field capacity to at least 30 cm regardless of cover crop treatment based on the 10 cm of water used in the infiltration test plus typical field capacities of silt and clay loams. Measurements were taken at 0–7.5 cm, 7.5–15 cm, 15–22.5 cm, and 22.5–30 cm in each of the four locations. The highest pressure was recorded for each depth and then averaged across the four locations to obtain one pressure per depth per subplot.

### 2.3.5 | Biological parameters

Soil sampling for biological parameters was done with a push core sampler that allowed us to collect composited smaller samples from within the plots to better capture heterogeneity at a fine scale. Six cores (10-cm depth, 2-cm diameter) were composited from each of the 11 treatments at the medium N fertilization rate ( $67 \text{ kg N ha}^{-1}$ ). For potentially mineralizable nitrogen (PMN), we collected soils for all three N fertilizer rates in the Fallow, Full and Pea treatment, to know whether N fertilization was affecting PMN measures. Field-moist soils were sieved to 2 mm and stored at 4°C for less than 30

days before lab analyses were performed. Soil extracellular enzyme activity was measured in only the Fallow, Full, and Pea treatment and in the single functional group treatments. To do this, we used 1 g of field-moist soil in duplicate with lab controls as outlined by Dick et al. (1996), Dick (2011), and Parham and Deng (2000). Enzymes analyzed include:  $\beta$ -1,4-glucosidase (EC 3.2.1.21),  $\beta$ -1,4-N-acetyl glucosaminidase (EC 3.2.1.30), arylsulfatase (EC 3.1.6.1), and acid and alkaline phosphatases (EC 3.1.3.1/2). As adapted from Keeney and Nelson (1982), PMN was calculated as the difference between plant-available N ( $\text{NH}_4^+$  only) at time 0 and after a 14-day lab incubation (D'Agati, 2020).

## 2.4 | Statistical analysis

Statistical procedures were conducted using R (R Core Team, 2022) and Statistical Package for the Social Sciences (SPSS 28.0; IBM 28.0). To evaluate differences among all treatments, a linear fixed model for each response variable (e.g., SOC) was fit using cover crop treatment as a fixed effect and block as a random effect in the “lme4” package of R (Bates et al., 2015) or the Generalized Linear Model (GLM) analysis in SPSS. For SOC, PMN, and STN, the N rate was included as a second fixed effect. Differences in response variables among cover crop treatments or N rates applied to wheat were further investigated using Tukey–Kramer honestly significant difference familywise comparisons of means ( $\alpha = 0.1$ ) with the “multcomp” package (Hothorn et al., 2008). Sites were analyzed independently, since they differed in soil types and weather patterns, and our interest was in testing for treatment differences. In addition, analyzing by site allowed us to compare and contrast biomass and nitrate results from Miller et al. (2023), which were analyzed by site, with soil health. Tests of differences between one and three functional groups on soil parameters were conducted using SPSS Univariate GLM, with functional group number as the fixed factor.

## 3 | RESULTS

### 3.1 | Climate context

Precipitation for the August–April period prior to each growing season ranged from as much as 81 mm below average (Amsterdam, 2013) to 84 mm above average (Conrad, 2017) but means were similar to the long-term averages (LTA; Table 3). The important May–June growing period for cover crops and May–July for wheat varied somewhat more on a relative scale, with precipitation ranging from 66 mm above the LTA (Amsterdam, 2014) to 81 mm below the LTA (Conrad, 2017), although again study means were similar to LTAs. Average annual precipitation during the study period was very close to LTAs at both sites.

### 3.2 | Biomass returned

At Amsterdam, 7-year aboveground cover crop, weeds (in cover year), plus wheat straw biomass (“total biomass”) analyzed across the three N rates was greater in Pea, Brassica, and Minus Taproot than Fallow, by 4.8, 3.2, and 4.2 Mg ha<sup>-1</sup>, respectively (Table 4). Pea total biomass was also greater than Minus Fibrous by 3.5 Mg ha<sup>-1</sup> at Amsterdam. Total biomass for the other six treatments was not different than total biomass for any other treatment, and notably there were no biomass differences among any of the four functional groups. At Conrad, total biomass was approximately 3 Mg ha<sup>-1</sup> greater for Pea, Minus Brassica, Fibrous, and Minus Taproot than both Fallow and Taproot. The only difference at either site between a functional group biomass and its paired three-functional group mixture without that functional group was at Conrad, where the Minus Taproot yielded 3.5 Mg ha<sup>-1</sup> more than Taproot. There were no differences between one- and three-functional group treatments at Amsterdam, but the three-functional group treatments yielded about 6% more biomass at Conrad ( $p = 0.03$ ). Biomass was strongly affected by N rate with ca. 4.5 Mg ha<sup>-1</sup> more biomass returned at the high N rate than low N rate at both sites (data not shown), yet there was no cover crop  $\times$  N rate effect ( $p = 0.42$ – $0.43$ ). For the medium N rate only, there were no differences between three- and one-functional group treatments at Amsterdam (16.5 vs. 16.3 Mg ha<sup>-1</sup>, respectively,  $p = 0.69$ , data not shown), but at Conrad the three-functional group treatments yielded more biomass (19.6 vs. 18.5 Mg ha<sup>-1</sup>,  $p = 0.038$ ). Functional group biomass amounts averaged over years were relatively evenly distributed in the Full mix, ranging from 17% for Taproot to 30% for Legumes at Amsterdam, and 18% for Taproot to 29% for Fibrous at Conrad (data not shown). Individual year cover crop biomass amounts are tabulated in Miller et al. (2023).

### 3.3 | Soil organic carbon and soil total nitrogen

Concentrations of SOC and STN in the upper 10 cm were greater with cover crops (Full and Pea) than with Fallow at both sites (Table 5). The concentrations of SOC were approximately 10% greater with cover crops than with Fallow at both sites, whereas STN was approximately 12%–18% greater with cover crops. There were no SOC and STN differences between the one-sp Pea and eight-spp Full treatments at either site. There was no N rate effect, or crop  $\times$  N rate effect, for either SOC or STN at either site for the Fallow, Full, and Pea dataset. There were no SOC differences among the four two-spp functional groups at Amsterdam ( $p = 0.72$ ) or Conrad ( $p = 0.11$ ), nor were there STN differences at Amsterdam ( $p = 0.53$ ), although there were STN differences at Conrad

**TABLE 3** Precipitation (mm) for pre-crop (August–April), crop growth periods (May–June for cover crops and May–July for wheat), and year at both sites in Montana.

Period	2012	2013	2014	2015	2016	2017	2018	2019	Average	LTA <sup>a</sup>
Precipitation (mm)										
<b>Amsterdam</b>										
Aug.–Apr.	152	124	191	279	172	227	260	221	171	205
May–June	87	–	191	–	69	–	160	–	127	125
May–July	–	164	–	148	–	107	–	–	140	153
Annual total	237	300	395	329	336	331	396	381	338	358
<b>Conrad</b>										
Aug.–Apr.	101	98	229	179	184	244	190	178	175	160
May–June	131	–	113	–	116	–	119	–	120	114
May–July	–	177	–	102	–	68	–	–	116	149
Annual total	260	252	335	267	324	359	332	371	312	303

<sup>a</sup>Long-term average (1981–2010) for Amsterdam calculated from Western Regional Climate Center (WRCC) station 240622, located 20 km from field site, and for Conrad calculated for WRCC station 241974, located 34 km from field site (WRCC, Reno, NV). August to April and annual data were collected from same two stations. Growing season precipitation was collected on-site with Hobo data logger (Onset Computer Corp.).

( $p = 0.02$ ). Specifically, STN for Brassica was 12%–20% greater than STN in the other three treatments. There were no SOC or STN differences between one- and three-functional group treatments at either site ( $p$ -values of 0.40 and 0.98).

### 3.4 | Infiltration rate

At Amsterdam there were no infiltration rate differences ( $p = 0.37$ ) among Fallow (3.7 cm h<sup>-1</sup>), Full (4.1 cm h<sup>-1</sup>), and Pea (5.5 cm h<sup>-1</sup>), at the medium N rate after four cover crops and three wheat growing seasons (data not shown). Similarly, infiltration rates were not different at Conrad ( $p = 0.14$ ) among Fallow (5.3 cm h<sup>-1</sup>), Full (3.5 cm h<sup>-1</sup>), and Pea (5.1 cm h<sup>-1</sup>).

### 3.5 | Penetration resistance

PR was greater for the Full eight-spp mix in the 15- to 22.5-cm depth at Amsterdam than either Fallow or Pea, yet there were no other differences (Table 6). This one exception was unexpected and not observed at Conrad.

### 3.6 | Soil enzyme activity

At Amsterdam, alkaline phosphatase activity was ca. 10% higher in Fallow and Full soil than in Pea soil ( $p = 0.02$ ), with no other differences in individual enzymes (Table 7). No differences among Fallow, Full, and Pea treatments were found for any enzyme at Conrad. Soil enzyme activity did not differ in soils among the single functional groups for any of the indi-

vidual enzymes or the geometric means at either site (Table 8). Arylsulfatase data were extremely variable among treatments, leading to large numerical variation within cover crop treatments without significant differences at Conrad. Arylsulfatase variability was also high in 2015 at Conrad (Housman et al., 2021).

### 3.7 | Potentially mineralizable nitrogen

Because N fertilizer rate did not affect PMN values at either site ( $p = 0.42$  at Amsterdam and  $p = 0.78$  at Conrad), we averaged PMN across the three N rates. PMN was affected by cover crop treatment at both Conrad ( $p = 0.02$ ) and Amsterdam ( $p < 0.01$ ; Table 9). At Conrad, soils following Pea had 1.6 times greater PMN values than those following Fallow ( $p = 0.02$ ). There were no differences between Pea and Full or Full and Fallow. At Amsterdam, PMN was approximately 1.6 times higher in soils following Pea and Full than soils following Fallow ( $p < 0.01$ ). There were no differences in PMN between Pea and Full.

PMN was also analyzed for only the medium nitrogen rate in all 11 treatments. At Amsterdam, medium N rate PMN values were 68% greater in Full than Fallow ( $p < 0.01$ ) and Pea had 56% greater PMN values than Fallow ( $p < 0.01$ ), while there were no differences among the Full, Pea, and Fallow treatments at Conrad in the medium N rate (D'Agati, 2020). There was no difference in PMN between the four functional group treatments at either site (Question 2; Table 9). A comparison of one- versus three-functional group treatments (Question 3) found 21% greater PMN at Amsterdam with three functional groups than a single functional group ( $p = 0.01$ ), and 34% greater PMN at Conrad with three functional groups ( $p = 0.02$ ; Table 9).

**TABLE 4** A total of 7-year aboveground dry biomass returned at Amsterdam and Conrad, MT, summed for four cover crop and three wheat growing seasons across the three N rates.

Treatment	Amsterdam	Conrad
Total 7-year biomass (Mg ha <sup>-1</sup> )		
Fallow	13.6c	16.2c
Full	16.4abc	18.4abc
Pea	18.4a	19.5a
Brassica	16.8ab	16.8bc
Minus Brassica <sup>a</sup>	16.0abc	19.0ab
Fibrous	15.3abc	19.3ab
Minus Fibrous	14.9bc	17.7abc
Legumes	15.7abc	17.8abc
Minus Legumes	16.1abc	18.3abc
Taproot	15.5abc	16.2c
Minus Taproot	17.8ab	19.7a
ANOVA		
Source of variation		
Crop (C)	_***	_***
N rate (N)	_***	_***
C × N	0.42	0.43
Number of functional groups		
One-functional group	15.8	17.6
Three-functional group	16.2	18.7
<i>p</i> -values		
One- versus three-functional group	0.43	0.03*

Note: Values within a column with no matching letters indicate different means (Tukey HSD,  $p < 0.1$ ).

Abbreviations: ANOVA, analysis of variance; HSD, honestly significant difference.

<sup>a</sup>“Minus” indicates full mix minus indicated functional group to form a six-species, three-functional group mix.

\*Significant at the 0.05 probability level; \*\*\*significant at the 0.001 probability level.

## 4 | DISCUSSION

### 4.1 | Question 1: Do soil health parameters differ among Fallow, a well-adapted monoculture cover (Pea), and an eight-spp mixture (Full)?

#### 4.1.1 | Soil organic carbon, soil total nitrogen, and potentially mineralizable nitrogen

Having a cover crop, whether it was a one-sp Pea or eight-spp Full mixture, resulted in 8%–14% greater SOC and 12%–18% greater STN in the upper 10 cm than Fallow. At a somewhat wetter site in Montana (annual precipitation 411 mm), a pea cover crop resulted in 23% more SOC and 19% more STN in the upper 10 cm after 10 years (Engel et al., 2017), or some-

what more than we found. After 7 years, in that same study, PMN was approximately 50% greater in a soil of a pea cover crop–wheat than fallow–wheat rotation (O’Dea et al., 2015), similar to our finding here of approximately 60% greater PMN in Pea than Fallow at both sites. Comparing soil health between soils with cover crops or summer fallow in semi-arid Kansas (Garden City, 489 mm annual precipitation), SOC stocks (top 15 cm) were greater with cover crops than fallow after 5 years, yet there was no difference after 11 years (Simon et al., 2022). The different responses were attributed to drought conditions later in the study, minimizing cover crop biomass and apparently reversing earlier trends. Although the generally positive response of cover crops on SOC in this study and elsewhere in the Great Plains might not seem unexpected, only in 29% of 35 cover crop studies largely in the US Midwest did cover crops increase SOC (Blanco-Canqui, 2022). There was a higher likelihood of an SOC response from cover crops when studies were >5 year in duration, initial SOC was less than 10 g C kg<sup>-1</sup> (depth not stated), and biomass production was >2 Mg ha<sup>-1</sup> year<sup>-1</sup>. Our study was slightly longer, with slightly greater SOC, and very similar annual cover crop biomass (Miller et al., 2023). Given generally low biomass production in cool semiarid regions, these combined results demonstrate the challenge of building SOC by growing cover crops, especially in the short term.

The lack of SOC, STN, or PMN differences between Pea and Full suggests that a well-adapted, high biomass producing cover can produce similar soil health outcomes as more diverse mixtures, at least in a semiarid environment. Lack of an SOC benefit from diverse mixtures compared to a single species has been previously observed in semiarid Kansas, where the SOC pool after 6–12 years was no different among triticale, an oat triticale/pea mix, and a six-species mix (Simon et al., 2022). Our finding also matches results from a review in the United States where mixes accumulated no more SOC than single species (Blanco-Canqui, 2022). The lack of an SOC difference in that review was attributed to similar aboveground and belowground biomass production between a high biomass producing single species and mixes (Blanco-Canqui, 2022). Similarly, based on a review of 27 studies, comprising 243 comparisons of the best-performing monoculture and best-performing mixture, there were no differences in biomass for 88% of the comparisons (Florence & McGuire, 2020), with mixtures outcompeting monocultures only 2% of the time. Given strong correlations between biomass returned and SOC in the Great Plains (Engel et al., 2017; Ruis et al., 2020; Shrestha et al., 2013; Simon et al., 2022), combined with the lack of differences in biomass between Pea and Full at either Amsterdam or Conrad, the lack of SOC differences between the two treatments was somewhat expected. Despite the lack of an STN or PMN difference between Pea and Full, soil nitrate-N, measured immediately prior to spring wheat seeding, after 7 years was an impressive 73 kg N ha<sup>-1</sup> greater after

**TABLE 5** Soil organic carbon (SOC) and soil total nitrogen (STN) concentrations in upper 10 cm at Amsterdam and Conrad, MT, in April 2019 after four cover crop and three wheat growing seasons.

Question/treatment	Amsterdam		Conrad	
	SOC (g kg <sup>-1</sup> )	STN (g kg <sup>-1</sup> )	SOC (g kg <sup>-1</sup> )	STN (g kg <sup>-1</sup> )
Question 1; Summer fallow vs. eight-species Full vs. Pea				
Fallow	12.5b	1.19b	10.7b	1.08b
Full	13.5a	1.32a	12.2a	1.21a
Pea	13.5a	1.33a	11.9a	1.27a
ANOVA				
Crop (C)	0.035*	0.004**	0.008**	0.007**
N rate (N)	0.44	0.18	0.47	0.78
C × N	0.80	0.72	0.92	0.95
Question 2; Functional group				
Brassica	13.4	1.28	12.0	1.32a
Fibrous	13.8	1.32	11.4	1.11b
Legumes	13.3	1.36	11.9	1.18b
Taproot	13.0	1.23	10.6	1.09b
<i>p</i> -values				
	0.72	0.53	0.103	0.021*
Question 3; Functional richness				
One-functional group <sup>a</sup>	13.3	1.30	11.4	1.17
Three-functional group <sup>b</sup>	13.3	1.31	11.8	1.18
<i>p</i> -values <sup>c</sup>				
	0.98	0.86	0.40	0.95

Note: Values are averages across the three N rates applied to wheat for Fallow, Pea, and Full and represent the medium N rate treatment concentrations for the other treatment comparisons. Values with no matching letters within a column indicate different means (LSD,  $p < 0.1$ ).

Abbreviations: ANOVA, analysis of variance; LSD, least significant difference.

<sup>a</sup>One-functional group indicates the four single functional group treatments.

<sup>b</sup>Three-functional group indicates the four three functional group treatments (minus treatments).

<sup>c</sup>*p*-value for functional group number effect.

\*Significant at the 0.05 probability level; \*\*significant at the 0.01 probability level.

Pea than Full, whereas there was no nitrate difference between the two treatments at cover crop termination the previous summer (Miller et al., 2023). This large increase in nitrate after Pea, combined with relatively low PMN in the spring after termination, is consistent with findings in Alberta, Canada, that found that more than 80% of N from legume cover crops was released in the first 9 months of a 3-year study (Lupwayi & Soon, 2015). The combined PMN and nitrate results indicate that a substantial portion of Pea residue N had mineralized prior to spring soil sampling and points out that while PMN is a useful soil health measure, it should be evaluated in conjunction with other available N measurements.

#### 4.1.2 | Infiltration rate and PR

Among the three treatments, there were no infiltration rate differences and only one PR difference (Full > Fallow = Pea, Amsterdam, 15.0–22.5 cm). In addition, the presence of tap-

rooted species in the Full mix was expected to produce root channels, and hence more preferential flow paths than Fallow or Pea, increasing infiltration rates. Infiltration is notoriously noisy due to high spatial variability in soil physical properties (Haws et al., 2004), which would decrease the potential for treatment differences. Yet using identical methodology at an 18-year cropping systems study near Bozeman, MT, infiltration rates were strongly affected by cropping system ( $p < 0.01$ ), and notably a no-till pea cover–wheat system had a 12-fold greater infiltration rate than a no-till fallow–wheat system (Jones et al., 2022). Crop yields and residue returned in this longer study in a wetter environment were 2- to 2.5-fold larger after 10 years than in the present study (Engel et al., 2017; Miller et al., 2023), likely increasing potential for aggregate formation from increased aboveground and belowground biomass and root exudates, increasing preferential flow from established root channels, and decreasing raindrop splash and resulting soil pore restrictions, in cover crop–wheat than fallow–wheat.

**TABLE 6** Penetration resistance measured with a static cone penetrometer after four cover crop and three wheat growing seasons for Amsterdam and Conrad for Full, Pea, and Fallow (to address Question 1) in four equally spaced depths between 0 and 30 cm.

Treatment	Amsterdam				Conrad			
	0–7.5 <sup>a</sup>	7.5–15.0	15.0–22.5	22.5–30.0	0–7.5	7.5–15.0	15.0–22.5	22.5–30.0
	Penetration resistance (MPa)							
Fallow	1.36	1.51	1.08b	0.92	0.69	0.74	0.93	1.05
Full	1.46	1.54	1.28a	1.01	0.65	0.83	0.91	0.94
Pea	1.42	1.48	1.04b	0.99	0.66	0.77	0.88	0.84
	<i>p</i> -values							
	0.30	0.80	0.02*	0.17	0.63	0.16	0.80	0.25

Note: Four replicated measurements were made per plot and averaged. Values with no matching letters indicate different means ( $p < 0.1$ ).

<sup>a</sup>All depths in cm.

\*Significant at the 0.05 probability level.

In the western Kansas cover crop study discussed previously (Simon et al., 2022), saturated infiltration rates were not different between fallow and both a chemically terminated triticale cover and a three-spp oat/triticale/pea cover, but infiltration rate of the three-spp mixture was greater than infiltration rates of both the triticale and a six-spp oat/triticale/pea/buckwheat (*Fagopyrum esculentum*)/radish/turnip. Therefore, despite that study's ability to detect infiltration rate differences, there was not a clear effect of richness on infiltration rate. In a meta-analysis of 81 comparisons between cover crops and a control representing 23 studies, infiltration rates were greater with cover crops than without by an average of 35% (Basche & DeLonge, 2019). In another review, cover crops increased infiltration rate or cumulative infiltration in 14 of 17 studies (Blanco-Canqui & Ruis, 2020), different than our findings, though only one study evaluated a cover crop mix versus single species, finding no difference in infiltration rate after 10 years, similar to our findings.

In a review of the effects of cover crops on soil physical parameters within the United States, cover crops decreased PR in 11 of 17 studies and had no effect in the other six (Blanco-Canqui & Ruis, 2020). In a global review, PR was 12% lower with cover crops but with a standard deviation of 20% (Hao et al., 2023), indicating high variability among studies. Interestingly, within silt loams (texture at Amsterdam), which represented 40% of the comparisons, there was no meaningful PR decrease from cover crops (Hao et al., 2023). The general lack of differences herein suggests that at least for silt and clay loams in this semiarid environment where biomass returns are low, PR is not a very dynamic property. PR is likely much more dependent on pre-existing factors such as SOC, soil texture, equipment compaction, and historical biogeochemical processes than cover crop treatment in the medium term in a semiarid region. In the only studies on PR in the Blanco-Canqui and Ruis (2020) review where a single species (rye) was compared with a mix, the mix had

greater PR in one study, lower PR in another, and no difference in a third in the top 5 cm (all in Nebraska on fine-textured soil). Based on this small group of studies, species richness does not appear to be as important at decreasing PR as simply the presence of a cover crop.

### 4.1.3 | Enzyme activity

There was only one difference in enzyme concentration (alkaline phosphatase, Full = Fallow > Pea, Amsterdam) after 7 years. Given known positive relationships between SOC and enzymes (Zhu et al., 2024), the lack of differences was unexpected. After only 3 years, there were differences in acid phosphatase (Full = Pea > Fallow at Amsterdam),  $\beta$ -glucosaminidase (Full > Pea = Fallow at Conrad), and geometric mean (Full = Pea > Fallow at Amsterdam: Full > Pea = Fallow at Amsterdam) in this same study (Housman et al., 2021). It is possible that the loss of differences at Conrad was the result of an invasion of glyphosate-resistant *Bassia scoparia*, especially in Fallow (D'Agati, 2020); the presence of a weedy species, or any plant species, could have enhanced soil enzymatic activity in the Fallow plots relative to the previous sampling time, making it less likely cover crops would affect enzyme activity compared to Fallow. Also, there was no correlation at the plot level between 2018 biomass and the geometric mean of enzyme activity or activity of individual enzymes (D'Agati, 2020), consistent with findings from four of six site-years measured earlier in this study (Housman et al., 2021). The lack of response, observed particularly in low precipitation years, suggests that higher biomass return is necessary to increase soil enzyme activity.

### 4.1.4 | Question 1 summary

In summary, cover crops (Full and Pea) increased concentrations of chemical (SOC and STN) parameters, had

**TABLE 7** Enzymatic activity (mg PNP g soil<sup>-1</sup> h<sup>-1</sup>) of five soil enzymes ( $\beta$ -glucosidase,  $\beta$ -glucosaminidase, acid and alkaline phosphatases, and arylsulfatase) for treatments at the medium N rate, following four rotations of cover crops with wheat at Amsterdam (A) and Conrad (C) to address Question 1.

Treatment	Acid phosphatase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		Alkaline phosphatase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		Arylsulfatase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		$\beta$ -Glucosidase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		$\beta$ -Glucosaminidase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		Geometric mean (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )	
	A	C	A	C	A	C	A	C	A	C	A	C
Fallow	90.6	193	330a	73.4	327	48.5	84.4	81.5	12.5	17.7	99.7	60.4
Full	88.1	218	336a	72.9	260	70.9	78.8	92.0	10.3	17.2	89.6	69.6
Pea	89.1	170	296b	70.7	251	82.6	76.3	87.9	10.4	19.2	85.6	64.5
<i>p</i> -values												
	0.94	0.27	0.02*	0.99	0.45	0.51	0.61	0.63	0.42	0.77	0.34	0.42

Note: Values with no matching letters indicate different means ( $p < 0.1$ ).

\*Significant at the 0.05 probability level.

**TABLE 8** Enzymatic activity (mg PNP g soil<sup>-1</sup> h<sup>-1</sup>) of five soil enzymes ( $\beta$ -glucosidase,  $\beta$ -glucosaminidase, acid and alkaline phosphatases, and arylsulfatase) for four functional group treatments at the medium N rate, following four rotations of cover crops with wheat at Amsterdam (A) and Conrad (C) to address Question 2.

Treatment	Acid phosphatase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		Alkaline phosphatase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		Arylsulfatase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		$\beta$ -glucosidase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		$\beta$ -glucosaminidase (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )		Geometric mean (mg PNP g soil <sup>-1</sup> h <sup>-1</sup> )	
	A	C	A	C	A	C	A	C	A	C	A	C
Brassica	92.8	169	304	79.5	318	84.4	85.3	75.5	11.1	17.2	95.8	62.7
Fibrous	84.0	203	333	37.2	303	42.8	82.7	68.4	11.1	19.0	94.6	51.5
Legumes	95.3	199	348	54.0	303	51.5	84.6	86.1	11.0	21.6	98.0	62.5
Taproot	82.1	189	299	59.6	316	52.4	76.3	70.0	10.5	18.9	89.6	56.4
<i>p</i> -value												
	0.51	0.60	0.36	0.19	0.50	0.53	0.56	0.43	0.98	0.50	0.65	0.53

**TABLE 9** Potentially mineralizable nitrogen (mg N kg<sup>-1</sup>) in upper 10 cm at Amsterdam and Conrad, MT, in April 2019 after four cover crop and three wheat growing seasons.

Question and treatment	Amsterdam	Conrad
	Potentially mineralizable nitrogen (mg N kg <sup>-1</sup> )	
Fallow	25.4b	18.2b
Full	42.5a	22.5ab
Pea	40.9a	29.9a
	<i>p</i> -values	
	<0.01**	0.02*
<b>Question 2</b>		
Brassica	33.8	15.4
Fibrous	31.2	16.4
Legumes	37.0	20.9
Taproot	28.3	22.6
	<i>p</i> -values	
	0.39	0.55
<b>Question 3</b>		
One-functional group <sup>a</sup>	32.5b	18.8b
Three-functional group <sup>b</sup>	39.3a	25.3a
	<i>p</i> -values <sup>c</sup>	
	0.012*	0.02

Note: Values are averages across the three N rates for Fallow, Pea, and Full, and represent the medium N rate treatment concentrations for the other treatment comparisons. Values with no matching letters within a column indicate different means ( $p < 0.1$ ).

<sup>a</sup>One-functional group indicates the four single functional group treatments.

<sup>b</sup>Three-functional group indicates the four three functional group treatments (minus treatments).

<sup>c</sup>*p*-value for functional group number effect.

\*Significant at the 0.05 probability level; \*\*significant at the 0.01 probability level.

mixed effects on one biological parameter (PMN), but no effect on other biological parameters (five enzymes) or physical parameters (infiltration rate and PR) compared to Fallow. High spatial variability in physical parameters, especially in no-till systems where a few preferential flow paths can alter infiltration rates, combined with low biomass returned in this semiarid region, might explain lack of differences in physical parameters, but the lack of differences in enzyme concentrations remains a mystery. The general lack of soil health differences between a one-sp and eight-spp mix suggests that mixtures might not improve soil health compared to a high biomass producing single species, but by itself does not indicate that species richness or functional richness is unimportant to soil health (addressed in Question 3).

## 4.2 | Question 2: Does functional group affect soil health parameters?

### 4.2.1 | Soil organic carbon, potentially mineralizable nitrogen, and enzyme activity

There were no differences in concentrations of SOC, the five enzymes, or PMN among the four functional group treatments in soil at either site after 7 years. This was counter to our hypothesis, especially given generally positive effects of legumes on enzyme activities (Maltais-Landry, 2015; Mubumba & Tyler, 2024) and PMN (O'Dea et al., 2015). The 9-month gap between cover crop termination and soil collection might have been sufficient to mineralize most of the cover crop residue, muting PMN differences, yet enzymes can persist for years within the soil (Nannipieri et al., 2018). Soil after Legumes had ca. 20 kg N ha<sup>-1</sup> greater soil nitrate-N stocks (top 0.9 m) than after Fibrous and Taproots, with intermediate nitrate stocks after Brassica at the time of soil sampling for PMN (Miller et al., 2023). This supports our contention that PMN should not be evaluated in isolation, but that nitrate-N should be included when the goal is to determine effects of cover crops on N availability. Greater nitrate after Legumes translated into greater wheat yield in one of six site-years and greater protein in five of six site-years (Miller et al., 2023).

The lack of SOC differences was anticipated given that there was only one total biomass difference among the four functional group treatments at either site (Fibrous > Taproot at Conrad), and SOC is correlated with biomass returned in Great Plains cover crop studies (Simon et al., 2022) and cropping system studies (Engel et al., 2017; Shrestha et al., 2013). Zhang et al. (2022) also found no SOC differences after 7 years among a brassica, legume, and grass cover crop. In a global meta-analysis, SOC gains from cover cropping were not different between legumes and nonlegumes (Joshi et al., 2023). Our findings and these literature findings are somewhat unforeseen because legume cover crops often produce more biomass than nonlegumes in low N soils (Miller et al., 2011) and would be expected to result in greater SOC; however, lower C:N ratios in legume cover crops could result in faster and more complete decomposition, counteracting the biomass benefit. In addition, root biomass has been found to be 2.4 times more important than shoot biomass at building SOC (Rasse et al., 2005), while certain root characteristics that vary among functional groups are more strongly tied to SOC accrual (Pisarčik et al., 2024). Although root biomass was not collected for logistical reasons, we recognize that some of the lack of SOC differences could have been due to differences in root/shoot ratios and root recalcitrance

among functional groups, perhaps counteracting aboveground biomass differences. In this study, biomass produced from each functional group was generally similar (Table 4). Early termination, low precipitation, and hence reduced biomass production may have limited the potential for soil enzyme activity differences among cover crops, after both two rotations (Housman et al., 2021) and four rotations, as reported here.

#### 4.2.2 | Soil total nitrogen

The only measured difference in parameters that we analyzed in soil for each functional group treatment was STN at Conrad where soil in the Brassica treatment had 15%–23% greater STN than soil in the other three functional groups. We expected greater STN in Legume treatment soils; the lack of a Legume benefit on STN, despite fixing N, suggests greater losses of N from the Legume treatment. This could have occurred due to lower C:N than in the other three functional groups (D'Agati, 2020), resulting in greater N mineralization rates, more soil nitrate (Miller et al., 2023), and subsequently more nitrate leaching and denitrification, rather than organic N accumulation. Brassica, on the other hand, are known to scavenge soil nitrate (Thorup-Kristensen et al., 2003); notably, radish has been found to reduce nitrate leaching by twofold compared to a legume (Elhakeem et al., 2023). Scavenging of deep nitrate would result in a redistribution of N from deeper layers to near the surface where STN was measured in our study, although this would have also occurred with Taproots and Fibrous. However, biomass N was not different between Brassica and Taproots in 2012 (Tallman, 2014) or 2014 (Housman, 2016), and C:N or biomass was not different between the two groups in 2018 (D'Agati, 2020; Miller et al., 2023), suggesting that deep N scavenging alone cannot explain why STN was greater in Brassica than Taproots. In semiarid New Mexico, STN and SOC were greater after an oat cover crop than both pea and canola cover crops in a winter wheat system (Ghimire et al., 2019); this result was partially attributed to 2.4-fold greater biomass of oat than pea or canola residue. In our study, there were generally no 7-year total biomass differences among functional group treatments at either site, suggesting a different cause of STN differences between Brassica and the other functional groups.

#### 4.2.3 | Aboveground and belowground residue effects on soil health

Despite known differences in shoot C:N ratios (D'Agati, 2020), rooting characteristics (Pisarčík et al., 2024), and root exudates (Sietz et al., 2023) among different cover crops and functional groups, there were almost no soil health differences

among functional group treatments. A potential reason for this finding is that more durable, higher C:N wheat residue (stubble and roots) could have tempered any functional group effect. Notably, wheat stubble biomass represented approximately 60% of the total 7-year total aboveground biomass averaged across N rate at both sites (data not shown). Therefore, the soil health “signature” was likely strongly influenced by wheat residue, making cover crop treatment differences less probable. This would be especially true considering that C:N ratios of early-terminated cover crops were generally below 30:1 (D'Agati, 2020; Housman, 2016; Tallman, 2014), likely resulting in relatively fast and somewhat consistent decomposition rates among functional groups. While we and others have documented that replacing fallow with cover crops benefits many soil health parameters (Blanco-Canqui & Ruis, 2020; Simon et al., 2022), within a cover crop-wheat rotation, it is interesting to consider that wheat residue could benefit soil health more than the cover crop or other crops in rotation.

For functional groups to affect soil health parameters, they would need to vary in either aboveground or belowground parameters that affect soil health. In a review of cover crop rooting characteristics that affect SOC, Pisarčík et al. (2024) reported that larger levels of root mass density, root length density (RLD), and cellulose/lignin ratios increased SOC accrual. The authors reported that RLD was generally greater in grasses and brassica than legumes, but there was tremendous variability among species within those groups. Selecting cover crop functional groups and species that enhance specific root characteristics such as RLD and cellulose should, in theory, improve soil health.

#### 4.2.4 | Question 2 summary

In summary for Question 2, there were essentially no soil health differences among functional groups. These findings suggest that in semiarid environments producers should consider selecting cover crop species and functional groups based on how well adapted they are, seed cost, effects on the subsequent crop, and so on, rather than possible soil health benefits of certain functional groups.

#### 4.3 | Question 3: Does functional richness (1 vs. 3 functional groups) affect soil health parameters?

While the relationship between functional richness and ecosystem services is well-documented in experimental grassland and natural systems (Finn et al., 2013; Hooper et al., 2005; Tilman et al., 1997), the application of those findings to semiarid agricultural systems is less clear. Even with the use of cover crops, semiarid agriculture includes

large portions of the year with bare soils. Consistent with the hypothesis of increased functionality with increased diversity, concentrations of PMN were 20% and 35% greater in the three-functional group treatments than one-functional group treatments at Amsterdam ( $p = 0.01$ ) and Conrad ( $p = 0.02$ ), respectively. Conversely, SOC and STN were not affected by functional group richness. The difference in results is possibly because “conservative” properties like SOC are largely controlled by biomass returned (Engel et al., 2017; Simon et al., 2022), which was not affected herein in the medium N treatments by numbers of functional groups at Amsterdam ( $p = 0.69$ ) and was only slightly increased (6%) in the three-functional group treatments at Conrad ( $p = 0.04$ ). Conversely, PMN is likely more controlled by shoot and root C:N and other root characteristics that are likely affected by functional richness (Pisarčík et al., 2024; Saleem et al., 2020). In a greenhouse study, root length and root area were greater in mixtures than from soil within the mixture’s individual species, resulting in improved SOC with mixtures (Saleem et al., 2020), contrary to our results. There are very few papers that have assessed the effects of functional group richness on soil health, although functional richness has been found to increase cover crop biomass (Florence et al., 2019), biomass N (Finney & Kaye, 2017), and several ecosystem services (Finney et al., 2017). More papers have assessed effects of single species versus mixtures, often summarized in review papers that have a SOC focus (Blanco-Canqui et al., 2013; Jian et al., 2020; Lavergne et al., 2021), with generally no or inconsistent SOC differences between monoculture cover crops and mixtures. In humid Tennessee, PMN was greater in a six-spp mix than wheat-only cover, but there were no differences in PMN between one-spp, two-spp/two-functional group, and the six-spp/four-functional group treatments (Chu et al., 2017). Concentrations of PMN in our study reflect mineralizable N immediately prior to wheat seeding, 9 months after cover crop termination; therefore, greater PMN in more diverse mixtures could reflect slower decomposition over the 9 months, perhaps due to more mesoaggregates and SOC caused by more root area and length in mixtures, at least based on a greenhouse study (Saleem et al., 2020). The lack of a PMN difference between Pea and Full (Question 1) appears to contrast with our finding here that functional richness increased PMN; however, four cycles of Pea likely increased levels of labile organic N that counteracted any benefit of richness on PMN, demonstrating the risk of comparing a well-adapted high biomass producing legume to a mixture.

The lack of large biomass benefits of richer mixes likely decreased the potential for a functional richness effect on soil properties including SOC and STN. The reduced competition for resources in mixtures due to different shoot and root morphologies, compared to single functional groups with more intragroup competition, is possibly less important in semiarid regions where one resource, water, perennially limits cover

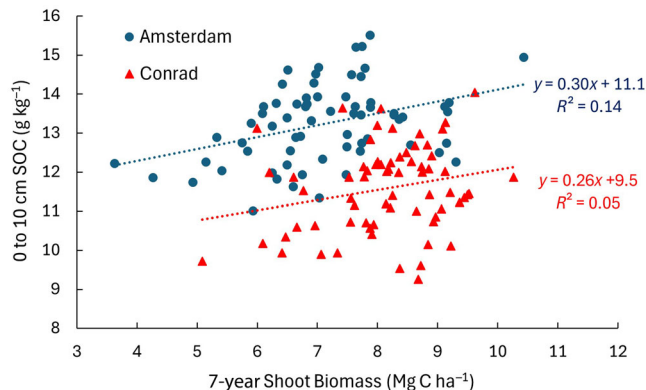
crop biomass. Specifically, in our study, cover crop biomass across site-years was highly related ( $p < 0.01$ ;  $R^2 = 0.72$ ) with growing season (May–June) precipitation (Miller et al., 2023). It is interesting that despite no meaningfully large functional richness effect on 7-year total biomass, that cover crop biomass was greater in the three-functional group treatments at Amsterdam in the 3 years analyzed (2014, 2016, and 2018), and at Conrad in 1 year (Miller et al., 2023), averaging about 15% more in those four site-years. No functional richness response in 7-year total biomass at Amsterdam and only a 6% benefit at Conrad likely reflected greater water use or N uptake when cover crop biomass was greater, reducing soil water and hence subsequent wheat growth and stubble remaining.

In summary, for Question 3, functional richness benefited PMN but had no effect on SOC and STN after 7 years. These are relatively novel findings because most studies of cover crop diversity compare soil health parameters after one monoculture cover crop with one or more mixtures, which is not an assessment of species or functional richness. The lack of a large total 7-year shoot biomass benefit from mixtures likely decreased the potential that more diverse mixtures would increase SOC. The benefit of diverse mixtures on PMN is noteworthy and warrants further study.

#### 4.4 | Question 4: Does biomass returned relate with SOC?

Functional group and functional richness had no effect on SOC and most other soil health parameters in this study, whereas just the presence of a cover crop, whether a monoculture or eight-spp mix, increased SOC and STN compared with summer fallow. These findings beg the question: “If functional group and functional richness do not influence soil health, what does vary among treatments that controls SOC and likely other variables often correlated with SOC?”. To determine if SOC could be related with total biomass C returned (biomass  $\times$  0.44 based on combustion) as found in other Great Plains studies (Engel et al., 2017; Simon et al., 2022; Shrestha et al., 2013), we included all plots where SOC was analyzed (all medium N rate treatments, plus low and high N rates for Fallow, Pea, and Full: Figure 2). There were relatively weak relationships between SOC (top 10 cm) and total 7-year biomass C returned at Amsterdam ( $R^2 = 0.14$ ;  $p < 0.001$ ) and Conrad ( $R^2 = 0.05$ ;  $p = 0.056$ ).

In previous long-term research studies in somewhat wetter portions of the northern Great Plains (NGP; ca. 400–500 mm annual precipitation), the change in SOC pool was positively related ( $p < 0.05$ ) to total biomass C (shoot + estimated root + estimated rhizodeposits) returned ( $R^2 = 0.86$ , Engel et al., 2017;  $R^2 = 0.18$ , Shrestha et al., 2013). Much lower mean shoot biomass C returned (2- to 2.5-fold lower) in our study



**FIGURE 2** Relationship between upper 10 cm soil organic carbon (SOC) in April 2019 and 7-year total shoot biomass (cover crop, weeds, and wheat stubble) from 2012 to 2018 at both Amsterdam and Conrad.

than in Engel et al. (2017), combined with a narrower range of shoot biomass C returned for both sites herein by treatment (3.0 and 4.6 Mg C ha<sup>-1</sup> vs. 13 Mg C ha<sup>-1</sup> in Engel et al. [2017]), would have reduced the potential for shoot biomass returned to be strongly related to SOC concentrations. In addition, both previous studies evaluated changes in SOC pool rather than final SOC concentration as was evaluated herein. Any pre-existing SOC differences would have been accounted for by evaluating changes, whereas pre-existing SOC differences could have weakened the SOC-biomass relationship. It is notable that despite different soil textures and precipitation amounts between Amsterdam and Conrad, the slope of the two SOC-biomass regressions was nearly identical (0.30 for Amsterdam; 0.26 for Conrad). The slope is relevant because it should approximate the proportion of shoot C that was converted to SOC in the top 10 cm. Specifically, each additional 1 Mg C ha<sup>-1</sup> of total shoot biomass returned should increase SOC by 0.26–0.30 g kg<sup>-1</sup> in the top 10 cm or prevent SOC declines by the same amount if SOC is not steady or increasing. By comparison, the average shoot biomass C input at both Amsterdam and Conrad was only ca. 1 Mg C ha<sup>-1</sup> per year, demonstrating why it is challenging to meaningfully build SOC in a semiarid environment due to low residue amounts.

In semiarid western Kansas (annual precipitation ca. 490 mm), aboveground crop residue over a 12-year period was positively related to the upper 15 cm SOC pool ( $R^2 = 0.17$ ;  $p < 0.01$ ; Simon et al., 2022) when a range of hayed and chemically-terminated cover crops (largely cool-season grasses and legumes) replaced fallow in wheat–fallow (2007–2012) or wheat–sorghum (*Sorghum bicolor* Moench)–fallow (2013–2018) rotations. Interestingly, SOC in 2012 was not related to wheat residue amounts but was related to cover crop residue amounts ( $p < 0.01$ ) from 2007 to 2012, whereas SOC in 2018 was related to sorghum residue ( $p < 0.01$ ) but not related to cover crop or wheat residue from 2013 to 2018.

The authors attributed the differences to a shift in precipitation timing from early to late in the growing season; too late to benefit covers, decreasing their biomass and reducing the potential they would influence SOC compared to sorghum, a warm-season crop. The benefit of residue to SOC is likely highly dependent on edaphic characteristics such as initial SOC (Blanco-Canqui, 2022), texture, and climate. While the conversion rate of total residue C to SOC in the NGP has been found to be between 34% (Shrestha et al., 2013) and 40% (Engel et al., 2017) over 10–11 years, in temperate sub-humid Italy, only 4.7% of residue C inputs were converted to SOC in a 55-year study (Piccoli et al., 2024). The difference in conversion rates could be due to lower C respiration rates in the NGP's cooler, drier climate, combined with different study lengths. Determining C conversion rates is critical to cover crop work because they allow producers to know the relative value of growing cover crops to SOC and other soil health parameters associated with SOC. Piccoli et al. (2024) pointed out that with such low conversion rates in their study, the land might better be used to grow bioenergy crops, whereas the much greater rates in Great Plains studies suggest there is potential to build SOC in the region despite relatively low biomass returns.

## 5 | CONCLUSIONS

Cover crops generally resulted in greater SOC, STN, and PMN than summer fallow, but did not benefit biological or physical parameters in this semiarid region. Soil health parameters were generally not different among different functional groups, possibly because biomass returned of those groups was similar. Notably, functional richness did not affect SOC or STN, but PMN was positively related to richness. Finally, SOC was positively, though weakly, related to shoot biomass. The combined findings from our studies and previous Great Plains studies suggest that maximizing biomass returned, across the rotation, should increase SOC and possibly other associated soil health parameters, regardless of functional group or richness.

## AUTHOR CONTRIBUTIONS

**Claim A. Jones:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; writing—original draft; writing—review and editing. **Catherine A. Zabinski:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; writing—original draft; writing—review and editing. **Perry R. Miller:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; writing—original draft; writing—review and editing. **Kristen D'Agati:** Data curation; formal analysis; investigation; methodology; writing—review and

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