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Cool-season cover crop effects on forage productivity and short-term soil health in a semi-arid environment

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Research Paper

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Abstract

There have been no systematic experiments conducted in Nevada's water-limited environment that examined the simultaneous benefits of soil health and feed value derived from cool-season cover cropping systems. The objective of this study was to determine the influence of different annual cool-season cover crop systems on above and belowground biomass production, plant tissue carbon and nitrogen, forage nutritive value (crude protein, acid, and neutral detergent fiber), relative feed value (RFV), and short-term soil health indicators under irrigation in a semi-arid environment. Treatments (cover crop systems) were a fallow (no cover crop), five monocultures of rye (*Secale cereale* L.), winter lentil (*Lens culinaris* Medik.), arrowleaf clover (*Trifolium vesiculosum* Savi), white sweetclover (*Melilotus alba*), forage kale (*Brassica oleracea* L.), and two three-species mixtures in 50-25-25 seeding ratios (CCM 1: rye, winter lentil, arrowleaf clover; CCM 2: rye, white sweetclover, forage kale). Cover crop systems were arranged in an RCBD with three replications. Plots were fall seeded in Reno, NV early October of each year (2020 and 2021) and terminated at the end of July of 2021 and 2022, respectively. Averaged across years, aboveground biomass production was lowest for the monoculture of winter lentil (4104 kg DM ha^{-1}; SE = 1551) compared to all other cover crop systems (average = 7593 kg DM ha^{-1}; SE = 1551). Biomass carbon produced was lowest for winter lentil (1717 kg ha^{-1}; SE = 675) relative to all other cover crop systems (average = 3227 kg ha^{-1}; SE = 675). The CCM 1 system had a greater C/N ratio (36.3) than CCM 2 and the monocultures of winter lentil, arrowleaf clover, and white sweetclover (average = 24.9). Belowground biomass did not differ among cover crop systems (average = 3161 kg DM ha^{-1}; SE = 962). Crude protein concentration was similar among cover crop systems but the RFV was greatest for forage kale (RFV = 165; SE = 4.0) among all cover crop systems. Soil total N and organic carbon concentration did not differ among cover crop systems but soil K concentration was greatest under fallow (428 mg kg^{-1} soil; SE = 26) relative to all other systems (average = 345.6 mg kg^{-1} soil; SE = 26). Soil microbial community biomass was not altered by cover crop system or its interaction with year. While the short-term impact of the cover crop systems on soil health indicators was minimal relative to the fallow system, the overall results suggested that there is potential to integrate cover crops in Nevada's semi-arid environment under irrigation.

Introduction

Dryland regions of the world account for approximately 41% of the earth's terrestrial surface and accommodate 38% of the global population (Reynolds *et al.*, 2007; Zika and Erb, 2009). Apart from the main constraint of water limitation for crop production in dryland regions (Peterson *et al.*, 2020), agricultural intensification to meet the food demand of the fast-growing world population has created a myriad of problems in these agriculturally challenge landscapes (Rockström *et al.*, 2016; Hoover *et al.*, 2020). For example, intensified agriculture has brought about soil degradation linked primarily to poor physical and chemical properties, microbial community composition, and functions necessary for sustainable agricultural production (Squire *et al.*, 2015; Kopittke *et al.*, 2019; Xue *et al.*, 2022).

The concept of a productivity-first approach in crop production agriculture has been a major contributor to the estimated 24 billion tons of fertile soil loss annually (Rockström *et al.*, 2016; Dudley *et al.*, 2017). The current primary issue is managing agricultural lands in fragile ecosystems to help strengthen long-term environmental sustainability and resilience without jeopardizing productivity (Norris *et al.*, 2020). To attain agricultural sustainability, it is pertinent to employ systematic approaches that tackle both biophysical and social factors

targeted at the human–environment systems (Reynolds *et al.*, 2007). For example, many dryland soils have inherently low organic matter, poor aggregate stability, low water-holding capacity, and low overall nutrient availability (Reynolds *et al.*, 2007). This condition has been exacerbated by agricultural intensification in an unsustainable manner coupled with climate change (Hamidov *et al.*, 2018; Kopittke *et al.*, 2019). There have been several agronomic interventions employed in dryland cropping systems to safeguard sustainable crop production. For example, several soil conservation approaches have been undertaken, such as the no-till system, biodiversity inclusion, reduced tillage, and cover cropping to help restore soil health and sustainability of dryland agroecosystems (Blanco-Canqui *et al.*, 2013; Nielsen *et al.*, 2016). In more recent times, cover cropping has been the subject of widespread scientific studies across varying agroecosystems at both temporal and spatial scales to elucidate their benefits compared to the traditional fallow systems (e.g., Nair and Ngouajio, 2012; Reese *et al.*, 2014; Liebig *et al.*, 2015; Amsili and Kaye, 2020; Obour *et al.*, 2021; Thapa *et al.*, 2021).

The integration of cover cropping into traditional crop rotations offers multiple ecosystem services on agricultural landscapes. These include reducing soil susceptibility to erosion, conserving and enhancing soil quality by increasing soil organic matter, reducing fertilizer use, disrupting crop pests and disease cycles, increasing biodiversity, and enhancing crop weed suppression (Sainju *et al.*, 2003; Snapp *et al.*, 2005; Blanco-Canqui *et al.*, 2013, 2015; Obour *et al.*, 2021). Several studies have reported positive effects of cover cropping on soil microbial biomass and diversity, and total soil carbon and nitrogen relative to the fallow system (Sainju *et al.*, 2003; Blanco-Canqui *et al.*, 2013; Chavarría *et al.*, 2016; Kim *et al.*, 2020; Thapa *et al.*, 2021). Further, cover crops have been reported to enhance soil structure and water retention, reduce N leaching, and reduce greenhouse gas emissions (Tonitto *et al.*, 2006; Blanco-Canqui *et al.*, 2013; Behnke and Villamil, 2019; Reicks *et al.*, 2021).

In Nevada, field crop production is dominated by alfalfa hay as the leading cash crop followed by grass hay production with smaller-scale production of small grains (e.g., winter and spring wheat, barley, oats), corn, potatoes, onions, garlic, mint, and vegetables. Alfalfa is grown on expansive acreage as a monoculture that is rotated on a 4–5-year interval and typically followed by annual cool-season cereal grains, corn, or fallow. The common water usage for alfalfa in Nevada ranges from 0.25 to 0.37 ha m for each growing season and this would be substantially greater than the quantity required to grow cover crops. Producers of all cropping systems in Nevada are interested in adopting cover crop practices on their farms. However, this initial cover crop research in Nevada focuses on filling the gap between the summer termination of alfalfa and the replanting of alfalfa in late summer or early fall of the succeeding year. This traditional rest period (fallow) has also been used to avoid alfalfa autotoxicity during establishment in the field (e.g., Jennings and Nelson, 2002). This practice offers an opportunity to integrate cover crops in the alfalfa cropping systems sequence.

In Nevada's semi-arid environment, irrigation is required to successfully cultivate cover crops. Even with all the aforementioned benefits of cover crops, producers are generally still skeptical to integrate cover cropping systems in their operation based solely on soil health improvement. This is partly because soil health benefits are accrued over a long period without any immediate economic benefits. To overcome this skepticism, there is a need to examine multiple simultaneous uses of cover crops in these resource-limited agroecosystems. The focus on soil health benefits combined with forage production and quality potential for ruminant livestock use through grazing or haying is critical (e.g., Obour *et al.*, 2020). This approach can help offset the cost associated with cover crops seeds and the additional agronomic inputs required for success and ultimately increase the adoption of cover cropping in water-scarce environments like Nevada.

Studies on cover cropping systems are constantly increasing across varying agricultural landscapes. However, more experimental data are needed to address the challenges of incorporating cover crops in irrigated semi-arid environments. The critical areas such as cover crops' species resilience, compatibility (functional group mixtures), productivity, feed value, and contributions to the restoration of soil health under irrigation in semi-arid environments should also be addressed. There have been no systematic experiments conducted in Nevada's water-limited environment that examined the simultaneous benefits of soil health and feed value derived from cool-season cover cropping systems. Hence, this research aims to understand the differential benefits of annual cool-season cover cropping systems under irrigation in semi-arid environments like Nevada. The objective of this study was to determine the influence of different annual cool-season cover crop systems on above and belowground biomass production, tissue carbon and nitrogen, forage nutritive value, and short-term soil health indicators under irrigation in a semi-arid environment. For this initial experiment in Nevada, understanding the impact of cover crops cultivated as mixtures or monocultures of different species (grass, legume, or non-legume forb) is invaluable. Research results in the literature have shown that cover crop mixtures offer excellent complimentary effects such as greater resilience to extreme weather (flexibility to survive extreme environmental conditions), improved soil health benefits, greater stability in biomass production, balanced carbon-to-nitrogen ratio, and improved weed suppression (e.g., Khan and McVay, 2019; St Aime *et al.*, 2023). We hypothesized that annual cool-season cover crop systems that include grass, legume, and non-legume forb (Brassicaceae) in mixtures will increase forage biomass and nutritive value, and lower tissue carbon-to-nitrogen (C/N) ratio but will have similar short-term soil physical, chemical, and microbial properties as grass-only systems. The different cover crop systems used will improve soil health indicators over the fallow system (no cover crop).

Materials and methods

Site description: winter cover crop study

This study was conducted over a 2-year duration (2020–2022) at the University of Nevada, Reno Valley Road Field Laboratory, Reno, NV. The experimental area was previously cropped with alfalfa for 4 years. The soil at the experimental site is an Orr gravelly sandy loam, a fine-loamy, mixed, superactive, mesic Aridic Argixerolls with a 0–15 cm slope. Soil samples were collected and composited from the experimental site before seeding the cover crops for initial soil health indicators. Before the cover crop experiment was seeded, soil test analysis indicated that the pH was 7.5, 28 g kg^{-1} organic matter, 16.9 g kg^{-1} organic carbon, cation exchange capacity of 20.2 meq 100 g^{-1}, extractable P (Olsen), K, Mg, Ca, Na, NO_3-N, and S concentrations 21, 281, 571, 2729, 24, 14, and 7 mg kg^{-1} respectively. The initial total microbial biomass, total bacterial, Gram-positive, and

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Table 1. Monthly total precipitation, mean air temperature, solar radiation, and evapotranspiration at the University of Nevada, Reno Valley Road Field Laboratory, Reno, NV, during 2020–2022 growing seasons and 20-year average (2001–2020)

	Total precipitation			Mean air temperature			Mean solar radiation			Mean evapotranspiration		
	mm			°C			kWh m^{-2}			mm		
Month	2020–2021	2021–2022	20-yr	2020–2021	2021–2022	20-yr	2020–2021	2021–2022	20-yr	2020–2021	2021–2022	20-yr
October	0.0	87.1	13.6	13.3	10.9	11.7	142	116	118	113.3	97.0	99.8
November	11.2	2.3	12.3	5.0	7.8	5.6	95	88	79	60.2	62.7	55.6
December	6.9	76.7	28.7	1.6	1.7	1.6	73	63	59	41.4	35.6	35.1
January	29.5	0.0	36.8	2.7	2.2	2.0	75	89	71	46.7	46.5	40.4
February	2.3	2.5	20.9	3.8	2.8	3.6	103	111	92	62.7	63.5	61.0
March	1.5	0.8	13.3	5.9	8.5	7.1	159	157	145	109.7	118.62	104.4
April	0.3	7.6	10.3	12.1	10.1	10.2	208	209	184	174.5	146.3	147.1
May	3.8	0.0	13.7	16.1	14.1	15.1	249	231	221	221.7	196.9	192.0
June	4.3	0.3	6.5	24.1	20.4	20.6	255	213	244	274.6	229.4	243.1
July	3.1	0.0	5.4	27.2	25.8	24.7	233	250	236	278.1	280.9	263.7
Total	62.7	177.3	161.4							1383.0	1277.4	1242.1

negative bacteria, fungi, actinomycetes, arbuscular mycorrhizal fungi, and saprophytes were 443.6, 208.1, 107.9, 101.8, 0, 50.3, 0, 0 ng g soil^{-1} respectively. The soil microbial diversity index was 1.04 and the soil respiration was 22.3 μg CO_2-C g soil$^{-1\ h-1}$. In this semi-arid environment, monthly and seasonal total precipitation and evapotranspiration differed between the study years and the 20-year average (Table 1). Differences in monthly mean air temperature and solar radiation were marginal between the two study years and the 20-year average (Table 1).

Cover crop systems (treatments) and experimental design

Treatments hereafter called cover crop system included five monocultures of rye (*Secale cereale* L.), arrowleaf clover (*Trifolium vesiculosum* Savi), white sweetclover (*Melilotus alba* Medik.), forage kale (*Brassica oleracea* L.), and winter lentil (*Lens culinaris* L.) and two three-species mixtures (cover crop mixture 1 [CCM 1]: rye + arrowleaf clover + winter lentil; cover crop mixture 2 [CCM 2]: rye + white sweetclover + forage kale) and a fallow (no cover crop). Cover crop mixtures were blended in a 50–25–25% seeding mixture ratio for the grass, legume, and legume/broadleaf, respectively, based on each species' seeding rate (Table 2). The cover crop systems were arranged in a randomized complete block design with three replications of each system.

Plot establishment and management

The experimental area was sprayed with glyphosate [N-(phosphonomethyl) glycine] herbicide at a rate of 1.12 kg a.i. ha^{-1} to control both grass and broadleaf weeds before sowing. The plot area was minimum tilled with a rototiller and leveled to create a uniform seedbed. The plot size was 6.1 m long × 1.5 m wide (9.15 m^2) separated by 0.6 m between plots and 1.5 m alleys between blocks. All cover crop systems were seeded in early October of each year using an XL Plotseed cone seeder (Wintersteiger AG., Salt Lake City, UT, USA). However, due to extensive damage of seedlings by marmots (*Marmota* spp.) in the first year (2020), the plots were reseeded in the spring (mid-March) of 2021. The plots were seeded using the seeding rate for each cover crop system (Table 2) in 20 cm row spacing to a depth of 1 cm. Phosphorus was applied using triple superphosphate at a rate of 60 kg P_2O_5 ha^{-1} before sowing on each plot based on soil test recommendations. Fertilizer nitrogen was applied at 60 kg ha^{-1} to the grass and mixed systems in late April of each year. Supplemental irrigation was carried out for the first 3 weeks after sowing (end of October), and thereafter from the end of March to June of each year. Irrigation was applied uniformly through a K-Line irrigation system (St Joseph, MI, USA) set at a pressure of 262 KPa to replace the total grass reference Penman evapotranspiration every 7 days during the first month of the active growth period (March–April) and thereafter, every 14 days based on data collected from the UNR Valley Road Weather Station. Irrigation was terminated 2 weeks before the last harvest. Because of the challenge in finding appropriate herbicides to use in mixed cover crop systems, hand weeding was carried out intermittently to remove weeds from plots. For the fallow plots, glyphosate [N-(phosphonomethyl) glycine] was applied to control weeds (chemical fallow).

Data collection

Aboveground biomass

Cover crop aboveground biomass was randomly sampled twice at the end of June (early termination) and the end of July (late termination) from an undisturbed area of 1.9 m^2 at the center of each plot using a 36A RCI engineering plot harvester (Mayville, WI, USA) set to 12.7 cm residual stubble height. The fresh sample for each cover crop system was weighed and recorded before a subsample of approximately 500 g was collected from each cover crop system for dry matter, tissue carbon and nitrogen, and forage nutritive value determination. The subsamples were oven-dried using a forced-air oven set at 60°C for 72 h or beyond until a constant dry weight was achieved and recorded. Cover crop biomass production

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Table 2. Winter annual cover crop systems and seeding rate used

Cover crop system	Family	System	Variety	Seeding rate
Monocultures				(kg PLS ha^{-1})
Rye	Poaceae	Monoculture	Rymin	100
Arrowleaf clover	Fabaceae	Monoculture	Apache	10
White sweetclover	Fabaceae	Monoculture	Hubam	15
Forage kale	Brassicaceae	Monoculture	Dwarf Siberian	8
Winter lentil	Fabaceae	Monoculture	Morton	40
Mixtures				
CCM 1[a]		Mixture		63
CCM 2[b]		Mixture		56
Fallow		Control		No seeding

[a]Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil).
[b]Cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

was calculated on a dry matter basis. The dry subsamples were ground separately using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1 mm screen and stored in Whirl-Pak (Nasco, Fort Atkinson, WI, USA) sample bags before tissue carbon and nitrogen and forage nutritive value analysis.

Cover crop system tissue carbon and nitrogen concentrations and nutritive value

Cover crop system ground tissue samples were analyzed separately to determine carbon (C) and nitrogen (N) concentration by dry combustion using a Leco 928 CN analyzer (St. Joseph, MI, USA). The C/N ratio of all cover crop systems was determined by dividing the concentration of C by that of N for each experimental unit. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations were determined using the filter bag technique with an Ankom Fiber Analyzer (ANKOM Technology, Macedon, NY, USA), modified detergent procedures of Van Soest *et al.* (1991). Cover crop system crude protein (CP) concentration was calculated by multiplying the tissue N concentration by 6.25. The relative feed value (RFV) for each cover crop system was estimated based on digestible dry matter (DDM) and dry matter intake (DMI) using the following equations below (Undersander *et al.*, 1993).

$$DDM = 88.9 - (0.779 \times \%ADF),$$

$$DMI = 120/\%NDF,$$

$$RFV = (\%DDM \times \%DMI)/1.29,$$

Root biomass and soil sampling

Cover crop system root biomass was quantified to a depth of 15.24 cm × 5.08 cm diameter using a soil core bulk density sampler in two randomly sampled areas in the center of each plot following the final harvest (late sampling). The core samples collected for each experimental unit were dried at 60°C for 72 h using a forced-air oven for bulk density determination. Thereafter, the soil cores were washed separately in a 425 μm sieve to remove soil and other unwanted materials. Root samples were dried in separate bags following the same drying protocol above. Cover crop root biomass was estimated per hectare based on the 0–12.7 cm soil depth and soil bulk density (Ghimire *et al.*, 2014; Santos *et al.*, 2018). A day after the final harvest, six random soil samples from each experimental unit were collected from the 0–15.24 cm soil, depth using a 1.43 cm inner diameter AMS Gator soil probe (AMS, American Falls, ID, USA), and composited for analysis of soil health parameters. Soil samples were analyzed at a commercial soil testing laboratory (Ward Laboratories, Inc., Kearney, NE, USA). Phospholipid fatty acid analysis to determine microbial biomass and community composition in the soil was done using the method described by Buyer and Sasser (2012). Soil chemical composition, that is, pH (1:1 soil: water ratio method), organic matter (loss-on-ignition method), phosphorus (Olsen sodium bicarbonate method), exchange cations K, Ca, Mg, and Na (ammonium acetate [NH_4OAc] extraction method), NO_3-N (KCL extraction method), SO_4 (Mehlich III extraction method), and total carbon (sulfurous acid treated to remove inorganic carbon) and nitrogen by dry combustion (LECO CN 928 analyzer) were analyzed based on the reference methods. Soil CO_2 respiration (Haney test) was analyzed using closed containers incubated at 24°C for 24 h. Soil penetration resistance (SPR) was collected after the final harvest of cover crop systems. A day after the final harvest, the entire experimental area was irrigated uniformly for 8 h and allowed to drain for 48 h (field capacity). Thereafter, SPR was measured at three randomly selected sites within each plot from 0 to 15.24 cm depth using a digital compaction meter (Field Scout SC-900, Spectrum Technologies, Inc., Aurora, IL, USA). Soil volumetric moisture content was measured at the same time and depth as SPR measurements using a Delta T HH2 soil moisture meter with ML3 Theta Probe soil moisture sensor (Dynamax Inc., Houston, TX, USA). SPR (cone index) was adjusted with the moisture data using the approach of Busscher and Bauer (2003).

Statistical analyses

The data were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) in SAS (version 9.4, SAS Institute, Inc. 2016, Cary, NC, USA). The degrees of freedom were adjusted using the Kenward–Roger method. The cover crop system plant characteristics response variables were aboveground biomass, tissue C and N concentrations, C/N ratio, biomass C and N, nutritive value (CP, ADF, and NDF), and the forage quality estimate RFV. Because of the difference in planting date in the first year relative to the second year of the study, the data were analyzed

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to present the average across the 2 years and for each year separately. For the average across years, the fixed effects were cover crop system and sampling date while the random effects were replication (block), year, and their interactions. For each year separately, the random effect was replication. The fallow system (no cover crop) was only included for the analysis of the measured soil parameters. For the soil data (physical, chemical, and microbial properties), the cover crop system and year were considered fixed. Since samples were collected on the same plot each year, the year was analyzed as a repeated measurement, and the covariance structure used was based on the lowest Bayesian information criteria value. Cover crop system effects were considered significant when F test P values were ≤0.05. Cover crop system means separation was done using the lines option of LSMEANS procedure.

Results

Above- and belowground biomass

Averaged across years, aboveground biomass was affected by the main effects of cover crop system ($P = 0.003$) and sampling date ($P < 0.001$). However, there was no interaction of cover crop system × sampling date ($P = 0.748$) on aboveground biomass production. Among cover crop systems, biomass production of the monoculture winter lentil was least but no other systems differ in biomass production (Table 3). Aboveground biomass at early sampling (5171 kg DM ha^{-1}; SE = 1449) was lower than that at late sampling (9018 kg DM ha^{-1}; SE = 1449). In year 1, there was only a main effect of sampling date ($P < 0.001$) as neither cover crop system independently ($P = 0.145$) nor its interaction with sampling date ($P = 0.932$) affected aboveground biomass production (Table 4). Aboveground biomass production was less at the early sampling date (2877 kg DM ha^{-1}; SE = 242) compared to the late sampling date (5029 kg DM ha^{-1}; SE = 242). In year 2, aboveground biomass production was altered by cover crop system ($P < 0.001$) and sampling date ($P < 0.001$) but there was no interaction between the two experimental variables ($P = 0.280$). The monoculture of rye produced greater aboveground biomass than the monocultures of forage kale, arrowleaf clover, and winter lentil (Table 4). The monoculture winter lentil produced the lowest aboveground biomass among cover crop systems (Table 4). Aboveground biomass production was greater for the late sampling date (13,007 kg DM ha^{-1}; SE = 633) compared to the early sampling date (7464 kg DM ha^{-1}; SE = 633).

The average root biomass across the 2 years was not influenced by cover crop system ($P = 0.339$; Table 3). Also, in year 1, cover crop system did not alter root biomass production ($P = 0.736$; Table 4). However, in year 2, there was a trend for a main effect of cover crop system ($P = 0.092$) on root biomass production (Table 4). In year 2, CCM 2 tended to produce greater root biomass than monocultures of winter lentil, arrowleaf clover, and white sweetclover (Table 4).

Cover crop system tissue carbon and nitrogen characteristics

The tissue carbon concentration averaged across the 2 years was affected by the main effect of cover crop system ($P < 0.001$; Table 3) but not by sampling date ($P = 0.182$) or the interaction between the two variables ($P = 0.831$). The monoculture of white sweetclover had the greatest tissue carbon concentration while forage kale, apart from being similar to winter lentil, ranked lowest among the other cover crop systems (Table 3). In year 1, tissue carbon concentration was different among cover crop systems ($P < 0.001$) but neither the sampling date ($P = 0.334$) nor the interaction of the two variables ($P = 0.362$) altered tissue carbon concentration. The white sweetclover monoculture had greater tissue carbon concentration than the monocultures of rye, forage kale, and winter lentil, and the mixed system CCM 1 (Table 4). While the monoculture forage kale had the lowest tissue carbon concentration among cover crop systems in year 1 (Table 4). In year 2, there were main effects of cover crop system ($P = 0.002$) and sampling date ($P = 0.003$) but only a trend for the interaction between the two variables ($P = 0.075$; not discussed).

Table 3. Average aboveground biomass, root biomass, carbon and nitrogen concentrations, biomass carbon and nitrogen, and carbon-to-nitrogen (C/N) ratio of different cover crops systems across 2 years (2020–2022) in Reno, Nevada, USA

	kg DM ha^{-1}		g kg^{-1} DM		kg ha^{-1}		
Cover crop system	Aboveground biomass	Root biomass	Carbon	Nitrogen	Biomass carbon	Biomass nitrogen	C/N ratio
Monocultures							
Rye	8226^{a†}	3781	418.6^{bc}	17.5	3462^{a}	93.3^{bc}	34.8^{ab}
Arrowleaf clover	6487^{a}	3361	422.7^{bc}	19.7	2758^{a}	115.8^{b}	22.8^{cd}
White sweetclover	7959^{a}	1680	432.1^{a}	21.6	3472^{a}	158.8^{a}	20.8^{d}
Forage kale	7014^{a}	3128	411.2^{d}	16.6	2927^{a}	96.4^{b}	31.1^{ab}
Winter lentil	4104^{b}	2474	417.3^{dc}	18.3	1717^{b}	66.3^{c}	28.8^{bc}
Mixtures‡							
CCM 1	7885^{a}	2708	420.9^{bc}	15.9	3334^{a}	92.3^{bc}	36.3^{a}
CCM 2	7987^{a}	4996	424.4^{b}	17.8	3410^{a}	119.9^{b}	27.3^{bcd}
P-value	0.003	0.339	<0.001	0.122	0.002	<0.001	0.005
SE	1551	962	3.5	2.9	675	13.0	5.3

SE, standard error.
†Means within columns that are followed by the same lowercase letter superscripts are not different among cover crop systems ($P > 0.05$).
‡Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil) and cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

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Table 4. Aboveground biomass, root biomass, tissue carbon and nitrogen concentrations, biomass carbon and nitrogen, and carbon-to-nitrogen (C/N) ratio of different cover crop systems in Reno, Nevada, USA in separate years

	Aboveground biomass		Root biomass		C		N	Biomass C		Biomass N		C/N
	kg DM ha^{-1}				g kg^{-1} DM			kg ha^{-1}				
Cover crop system	2021	2022	2021	2022	2021	2022	2021	2021	2022	2021	2022	2021
Monocultures												
Rye	3302	13149^{a}	4014	3548^{ab}	415.4^{bc}	421.9^{c}	26.9	1370	5554^{a}	81.8	104.7^{cd}	16.7
Arrowleaf clover	3439	9534^{b}	4575	2147^{b}	419.8^{ab}	425.5^{bc}	23.0	1446	4070^{b}	74.2	157.3^{b}	19.2
White sweetclover	4732	11186^{ab}	1214	2147^{b}	425.4^{a}	438.8^{a}	24.1	2017	4926^{ab}	110.3	207.3^{a}	18.0
Forage kale	4288	9739^{b}	2894	3361a^{b}	398.5^{d}	424.0^{bc}	20.8	1702	4152^{b}	83.3	109.5^{cd}	20.2
Winter lentil	3404	4803^{c}	2614	2334^{b}	409.6^{c}	424.9^{bc}	25.6	1391	2043^{c}	80.0	53.0^{e}	18.0
Mixtures												
CCM 1	3872	11898^{ab}	2708	2707^{ab}	416.1^{bc}	425.8^{bc}	23.8	1608	5060^{ab}	90.2	94.3^{d}	18.5
CCM 2	4633	11341^{ab}	5882	4108^{a}	418.5^{ab}	430.3^{b}	23.0	1936	4885^{ab}	105.0	134.8^{bc}	18.2
P-value	0.145	< 0.001	0.736	0.092	< 0.001	0.002	0.580	0.114	< 0.001	0.155	< 0.001	0.652
SE	453	936	1999	508	2.6	2.6	3.3	188	399	10.8	14.7	1.5

SE, standard error.
[†]Means within columns that are followed by the same lowercase letter superscripts are not different among cover crop systems ($P > 0.05$).
[‡]Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil) and cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

The white sweet clover had the greatest tissue carbon concentration among cover crop systems (Table 4).

Averaged across years, tissue nitrogen concentration did not differ among cover crop systems ($P = 0.122$; Table 3). However, the sampling date ($P < 0.001$) but not the interaction of the two variables ($P = 0.931$) affected tissue nitrogen concentration averaged across years. Sampling early resulted in greater tissue N concentration (20.7 g kg^{-1} DM; SE = 2.7) compared to late sampling (15.7 g kg^{-1} DM; SE = 2.7). In year 1, tissue nitrogen concentration was only altered by sampling date ($P = 0.002$) but not by cover crop system ($P = 0.580$; Table 4) or the interaction between the two variables ($P = 0.784$). Again, sampling early produced greater tissue nitrogen concentration (27.5 g kg^{-1} DM; SE = 1.5) compared to late sampling (20.3 g kg^{-1} DM; SE = 1.5) in year 1. In year 2, tissue nitrogen concentration was influenced by a cover crop system × sampling date ($P = 0.006$; Fig. 1A). At the early sampling date, tissue nitrogen concentration was greatest for white sweetclover monoculture among cover crop systems (Fig. 1A). At the late sampling date, tissue nitrogen concentration was greatest for the monocultures of arrowleaf clover and white sweetclover among cover crop systems (Fig. 1A). For each cover crop system, only the monoculture of forage kale sampling date altered tissue nitrogen concentration and it was greater for early sampling date compared to the late sampling date (Fig. 1A).

Biomass carbon averaged across years was influenced by the main effects of cover crop system ($P = 0.002$) and sampling date ($P < 0.001$) but not by the interaction of the two variables ($P = 0.709$). Similar to biomass production, the monoculture system winter lentil had the lowest biomass carbon but all other systems were similar in biomass carbon produced (Table 3). The early sampling date produced lower biomass carbon (2181 kg C ha^{-1}; SE = 632) compared to the late sampling date (3842 kg C ha^{-1}; SE = 632). Analyzed separately by year, in year 1, there was only a main effect of sampling date ($P < 0.001$) on biomass carbon but neither cover crop system independently ($P = 0.114$) nor the interaction between the two variables ($P = 0.934$) alter biomass carbon (Table 4). Again, the late sampling produced greater biomass carbon (2080 kg C ha^{-1}; SE = 100) than early sampling (1197 kg C ha^{-1}; SE = 100). In year 2, there were main effects of the cover crop system ($P < 0.001$) and sampling date ($P < 0.001$) but no interaction ($P = 0.258$) between the two variables to alter the quantity of biomass carbon produced (Table 4). In year 2, biomass carbon produced was greater for the rye monoculture compared to forage kale, arrowleaf clover, and winter lentil monocultures (Table 4). The winter lentil system produced the lowest biomass carbon among the cover crop systems (Table 4). In year 2, late sampling produced greater biomass carbon (5603 kg C ha^{-1}; SE = 270) than early sampling date (3165 kg C ha^{-1}; SE = 270).

Biomass N averaged across the 2 years was influenced by the main effects of cover crop system ($P < 0.001$) and sampling date ($P = 0.002$) but not by the interaction of the two variables ($P = 0.427$). The monoculture white sweetclover produced the greatest quantity of biomass N among cover crop systems (Table 3). The monocultures of arrowleaf clover and forage kale and the mixture CCM 2 produced greater biomass N than winter lentil (Table 3). Biomass N was greater for the late sampling date (122.2 kg N ha^{-1}; SE = 9.4) compared to early sampling (90.0 kg N ha^{-1}; SE = 9.4). In year 1, biomass N produced was affected by sampling date ($P = 0.004$) but not by the main effect of cover crop system ($P = 0.155$) nor the interaction of the two variables ($P = 0.705$). Biomass N in year 1 was greater for late sampling date (101.7 kg N ha^{-1}; SE = 6.3) compared to the early sampling date (76.7 kg N ha^{-1}; SE = 6.3). In year 2, biomass N produced was affected by the main effects of cover crop system ($P < 0.001$) and sampling date ($P = 0.001$) but not by the interaction of the two variables ($P = 0.118$). The monoculture white sweetclover produced the greatest quantity of biomass N among cover crop systems (Table 4). The quantity of biomass N produced by arrowleaf clover was greater than the monocultures of forage kale, rye, and winter lentil and the mixed system CCM 1 (Table 4). The cover crop system CCM 2 produced greater biomass N than CCM 1 (Table 4). The monoculture winter lentil produced the lowest quantity of biomass N among cover crop systems in year 2 (Table 4). Similar to the previous responses, sampling late produced greater biomass N (142.8 kg N ha^{-1}; SE = 9.1) compared to early sampling (103.2 kg N ha^{-1}; SE = 9.1).

The average C/N ratio across the 2 years was altered by the main effects of cover crop system ($P = 0.005$) and sampling date ($P = 0.005$) but no interaction ($P = 0.311$) between the two variables (Table 3). The CCM 1 system produced a greater C/N ratio than CCM 2 and the monocultures of winter lentil, arrowleaf clover, and white sweetclover but did not differ from rye and forage kale monocultures (Table 3). The monocultures of rye and forage kale produced a greater C/N ratio than the monocultures of arrowleaf clover and white sweetclover (Table 3). Further, winter lentil monoculture produced a greater C/N ratio than white sweetclover monoculture (Table 3). The late sampling date

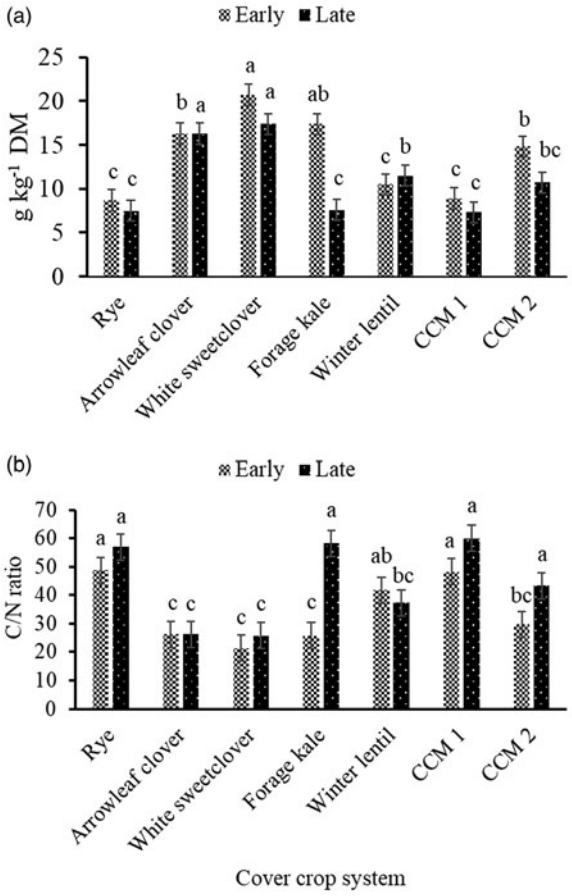

Figure 1. Cover crop system × sampling date interaction of (a) plant tissue nitrogen concentration and (b) carbon-to-nitrogen (C/N) ratio in Reno, Nevada, USA. Bars within each sampling date with the same lowercase letter are not different ($P > 0.05$).

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produced a greater C/N ratio (32.5; SE = 4.8) compared to the early sampling date (25.0; SE = 4.8). In year 1, the C/N ratio was influenced by sampling date ($P < 0.001$) but not by cover crop system ($P = 0.652$), nor the interaction of the two variables ($P = 0.710$). In year 1, late sampling produced a greater C/N ratio (21.0; SE = 1.1) than the early sampling date (15.8; SE = 1.1). In year 2, there was a cover crop system × sampling date interaction ($P = 0.013$) on the C/N ratio (Fig. 1b). At the early sampling date in year 2, the rye monoculture and the mixed system CCM 1 had a greater C/N ratio than CCM 2, arrowleaf clover, forage kale, and white sweetclover (Fig. 1b). The monoculture winter lentil had a greater C/N ratio than arrowleaf clover, forage kale, and white sweet clover (Fig. 1b). At the late sampling date, the cover crop systems CCM 1, forage kale, and rye all had a greater C/N ratio than CCM 2, winter lentil, arrowleaf clover, and white sweetclover (Fig. 1b). Also, the system CCM 2 had a greater C/N ratio than arrowleaf clover and white sweetclover monocultures (Fig. 1b). The cover crop systems forage kale, CCM 1, and 2 had a greater C/N ratio at late sampling compared to early sampling date (Fig. 1b).

Nutritive value and quality index of cover crop systems

Averaged across the 2 years, the CP concentration was affected by the sampling date ($P < 0.001$) but not by the main effect cover crop system ($P = 0.122$; Table 5) nor the interaction of the two variables ($P = 0.930$). CP concentration was greater for the early sampling date (129.3 g kg^{-1} DM; SE = 5.4) compared to late sampling (98.4 g kg^{-1} DM; SE = 5.4). In year 1, only the main effect of sampling date ($P = 0.002$) influenced CP concentration (Table 6). CP concentration was greater at the early sampling date (171.7 g kg^{-1} DM; SE = 9.4) relative to the late sampling date (126.7 g kg^{-1} DM; SE = 9.4). In year 2, the CP concentration was influenced by a cover crop system × sampling date interaction ($P = 0.006$; Table 7). At the early sampling date, the monocultures of white sweetclover and forage kale had similar CP concentrations (Table 7). However, the monoculture white sweetclover had greater CP concentration than the mixtures CCM 1 and 2, and the monocultures of arrowleaf clover, winter lentil, and rye (Table 7). The monocultures of forage kale and arrowleaf clover and the mixed system CCM 2 had greater CP concentrations than the monocultures of winter lentil and rye and the mixed system CCM 1 (Table 7). At the late sampling date, the monocultures of white sweetclover and arrowleaf clover had greater CP concentration than all other cover crop systems (Table 7). The monoculture winter lentil had greater CP concentration than forage kale and rye monocultures and the mixed system CCM 1 (Table 7). For each cover crop system, sampling date only affected the CP concentration of CCM 2 and it was greater at early compared to late sampling date (Table 7).

In this experiment, the ADF concentration averaged across the 2 years was affected by the main effects of cover crop system ($P < 0.001$) and sampling date ($P < 0.001$) but not by the interaction of the two variables ($P = 0.944$). The ADF concentration was greatest for the monoculture of white sweetclover among the cover crop systems (Table 5). Sampling early resulted in greater ADF concentration than late sampling (Table 5). Analyzed by separate years, ADF concentration was altered by the main effects of cover crop system and sampling date in year 1 ($P < 0.001$; <0.001) and year 2 ($P = 0.002$; <0.001) respectively (Table 6). There was no interaction of cover crop system × sampling date ($P > 0.05$) in each year of the experiment to alter ADF concentration. In year 1, the monoculture white sweetclover had the greatest ADF concentration while forage kale had the lowest among cover crop systems (Table 6). In year 2, the monocultures of forage kale and white sweetclover had greater ADF concentrations than all other systems (Table 6). Sampling early in both years resulted in lower ADF concentrations than sampling late (Table 6).

Table 5. The main effects of cover crop system and sampling date on crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations, and relative feed value (RFV) averaged across 2 years in Reno, Nevada, USA

	g kg^{-1} DM			
Cover crop system	CP	ADF	NDF	RFV
Monocultures				
Rye	109.3	384^{b†}	581^{a}	96^{c}
Arrowleaf clover	122.9	390^{b}	442^{c}	132^{b}
White sweetclover	135.0	464^{a}	527^{b}	96^{c}
Forage kale	103.4	381^{b}	431^{c}	165^{a}
Winter lentil	114.4	388^{b}	514^{b}	115^{bc}
Mixtures				
CCM 1‡	99.7	401^{b}	597^{a}	92^{c}
CCM 2	111.5	399^{b}	529^{b}	107^{bc}
P-value	0.122	<0.001	<0.001	<0.001
SE	18.3	34.2	36.0	16.6
Sampling date				
Early	129.3^{a}	369^{b}	477^{b}	131^{a}
Late	98.4^{b}	433^{a}	558^{a}	98^{b}
SE	5.4	32.9	32.8	14.3
P-value	<0.001	<0.001	<0.001	<0.001

SE, standard error.
†Means within columns that are followed by the same lowercase letter superscripts are not different among cover crop systems ($P > 0.05$).
‡Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil) and cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

The NDF concentration averaged across years was affected by the main effects of cover crop system ($P < 0.00$) and sampling date ($P < 0.001$) but not by their interaction ($P = 0.623$). Averaged across the years, NDF concentration was greatest for rye and CCM 1, lowest for arrowleaf clover and forage kale, and intermediate for white sweetclover and winter lentil (Table 5). Sampling late resulted in greater NDF concentration than early sampling (Table 5). Analyzed by separate years, NDF concentration was affected by the main effects of cover crop system in year 1 ($P < 0.001$) and year 2 ($P < 0.001$) and sampling date in both years (Table 6). However, there was no cover crop system × sampling date interaction that affected NDF concentration in both years of this experiment ($P > 0.05$). In year 1, the monoculture forage kale had the lowest NDF concentration while the monoculture rye and the mixed system CCM 1 were ranked among the greatest in NDF concentration (Table 6). In year 2, the mixed system CCM 1 had the greatest NDF concentration (Table 6). In each year, NDF concentration was greater late compared to the early sampling date (Table 6).

The RFV averaged across the 2 years was altered by the main effects of cover crop system ($P < 0.001$) and sampling date ($P <$

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Table 6. The main effects of cover crop system and sampling date on crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations, and relative feed value (RFV) in separate years in Reno, Nevada, USA

	Year					
	CP (g kg^{-1} DM)	ADF (g kg^{-1} DM)		NDF (g kg^{-1} DM)		RFV
Cover crop system	2021	2021	2022	2021	2022	2022
Monoculture						
Rye	167.9	328.4^{bc†}	439.0^{b}	525.2^{a}	636.6^{ab}	81.0^{b}
Arrowleaf clover	143.9	316.2^{c}	464.5^{b}	377.0^{d}	507.3^{d}	98.0^{a}
White sweetclover	150.9	402.3^{a}	525.8^{a}	469.0^{bc}	584.5^{c}	77.0^{b}
Forage kale	129.8	251.4^{d}	511.0^{a}	269.2^{e}	592.5^{c}	80.0^{b}
Winter lentil	160.1	328.5^{bc}	446.7^{b}	491.0^{abc}	539.0^{d}	94.0^{a}
Mixtures						
CCM 1‡	148.5	344.0^{b}	457.2^{b}	540.3^{a}	654.5^{a}	77.0^{b}
CCM 2	143.5	324.1^{bc}	473.3^{b}	448.5^{c}	610.2^{bc}	80.0^{b}
P-value	*0.581*	<0.001	0.002	<0.001	<0.001	0.003
SE	15	8.3	12.6	19.4	14.5	4.0
Sampling date						
Early	171.7^{a}	299.8^{b}	437.2^{a}	405.2^{b}	548.3^{b}	95.0^{a}
Late	126.7^{b}	355.9^{b}	510.7^{b}	486.3^{a}	630.1^{a}	73.0^{b}
SE	9.4	4.5	6.8	10.4	7.7	2.0
P-value	*0.002*	<0.001	<0.001	<0.001	<0.001	<0.001

SE, standard error.
†Means within columns that are followed by the same lowercase letter superscripts are not different among cover crop systems ($P > 0.05$).
‡Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil) and cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

Table 7. Cover crop system × sampling date interaction for crude protein (CP) concentrations in the second year (year 2) and relative feed value (RFV) in the first year (year 1) in Reno, Nevada, USA

	Sampling date (year 2)			Sampling date (year 1)		
	CP (g kg^{-1} DM)			RFV		
Cover crop system	Early	Late	*P-value*	Early	Late	*P-value*
Monocultures						
Rye	54.3^{c†}	47.1^{c}	0.527	119^{d}	105^{b}	0.535
Arrowleaf clover	101.8^{b}	102^{a}	0.988	204^{b}	130^{b}	0.003
White sweetclover	129.2^{a}	108.9^{a}	0.079	125^{d}	105^{b}	0.385
Forage kale	108.5^{b}	47.4^{c}	<0.001	303^{a}	195^{a}	<0.001
Winter lentil	65.6^{c}	72.0^{b}	<0.001	173^{bc}	98^{b}	0.003
Mixtures						
CCM 1‡	55.9^{c}	45.9^{c}	0.376	114^{d}	101^{b}	0.545
CCM 2	92.4^{b}	66.7^{bc}	0.029	143^{cd}	123^{b}	0.385
SE	7.9	7.9		15.7	15.7	

SE, standard error.
†Means within columns that are followed by the same lowercase letter superscripts are not different among cover crop systems ($P > 0.05$).
‡Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil) and cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

0.001; Table 5) but no interaction of the two variables ($P = 0.289$). The RFV was greatest for forage kale among cover crop systems and lower at late compared to early sampling date (Table 5). In year 1, RFV was affected by a cover crop system × sampling date interaction ($P = 0.023$; Table 7) but in year 2, only by the main effects of cover crop system ($P = 0.003$), and sampling date ($P < 0.001$; Table 6). In year 1, at both early and late sampling dates, the monoculture of forage kale had the greatest RFV among

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cover crop systems (Table 7). For each cover crop system, the sampling date only affected the RFV of monocultures of arrowleaf clover, forage kale, and winter lentil and it was greater at early compared to late sampling for these systems (Table 7). In year 2, the RFV was greatest for arrowleaf clover and winter lentil among cover crop systems and lower at late compared to the early sampling date (Table 6).

Soil physical, chemical, and microbial properties

Soil bulk density was not affected by cover crop system ($P = 0.893$; Table 8), nor the interaction with year ($P = 0.285$). However, soil bulk density was greater in year 2 (1.54 Mg m^{-3}; SE = 0.04) compared to year 1 (1.35 Mg m^{-3}; SE = 0.04) of the experiment.

There was a trend for a main effect of cover crop system ($P = 0.089$) on soil cone index (Table 8). Soil cone index tended to be greater for the fallow system compared to the monocultures of rye, arrowleaf clover, winter lentil, CCM 1, and 2 (Table 8). Soil volumetric water content was affected by cover crop system × year interaction ($P < 0.001$; Fig. 2). In year 1, soil volumetric water content was greater for the fallow system compared to all other systems. However, in year 2, the fallow system had lower soil water content than all other systems except the monoculture of white sweetclover (Fig. 2). For each cover crop system, only the fallow system soil moisture content was not different between the 2 years (Fig. 2).

Soil pH, total N, nitrate-N, phosphorus, magnesium, calcium, sulfur, sodium, copper, iron, zinc, boron, cation exchange capacity, and total organic carbon concentrations were not influenced by cover crop systems nor its interaction with year ($P > 0.05$; data not shown) in this experiment. However, there was a main effect of cover crop system on soil potassium concentration ($P = 0.044$; Table 8). Soil potassium concentration was greater for the fallow system compared to all other systems (Table 8). There was a main effect of year on soil chemical characteristics ($P < 0.05$). Soil organic matter, nitrate-N, potassium, sulfur, calcium, magnesium, and total organic carbon concentrations decreased in the second year compared to the first (data not shown). However, soil phosphorus increased in year 2 compared to year 1 of the experiment (data not shown) but year did not affect soil total nitrogen concentration ($P = 0.678$).

Table 8. Soil bulk density, cone index, and soil exchangeable potassium (K) concentration among cover crop system in Reno, Nevada, USA

Cover crop systems	Soil bulk density	Cone index	Soil K
	Mg cm^{-3}	Mpa	mg kg^{-1} soil
Monocultures			
Rye	1.44	1.05^{bc†}	344^{b}
Arrowleaf clover	1.46	0.98^{bc}	322^{b}
White sweetclover	1.40	1.26^{ab}	367^{b}
Forage kale	1.38	1.13^{abc}	334^{b}
Winter lentil	1.45	1.10^{bc}	359^{b}
Mixtures			
CCM 1‡	1.48	0.90^{c}	353^{b}
CCM 2	1.46	1.07^{bc}	340^{b}
Control			
Fallow	1.48	1.46^{a}	428^{a}
P-value	0.908	0.089	0.044
SE	0.07	0.14	26.0

SE, standard error.
†Means within columns that are followed by the same lowercase letter superscripts are not different among cover crop systems ($P > 0.05$).
‡Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil) and cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

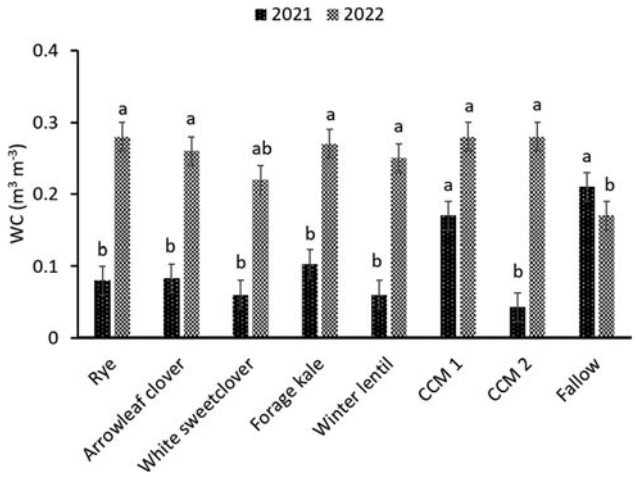

Figure 2. Soil volumetric water content of cover crop system × year interaction in Reno, Nevada, USA. Bars within each year with the same lowercase letter are not different ($P > 0.05$).

Soil microbial community biomass was not altered by cover crop system ($P > 0.05$) or its interaction with year ($P > 0.05$; Table 9). Only a trend for a main effect of cover crop system on microbial diversity index (Table 9). The microbial diversity index tended to be greater for CCM 2 than the fallow, rye, and white sweet clover systems (Table 9). Forage kale, winter lentil, and CCM 1 all had a greater microbial diversity index than the fallow system (Table 9). However, there was a main effect of year on soil microbial biomass ($P > 0.05$). All microbial community structure biomass increased in year 2 compared to year 1 of the experiment. The Gram-positive and negative bacteria and the saturated–unsaturated ratios were lower in year 2 compared to year 1 of the experiment but the opposite occurred for the fungi–bacteria ratio (Table 9). Soil CO_2 respiration was not affected by the main effects of cover system ($P = 0.608$) and year ($P = 0.340$) nor the interaction between the two variables ($P = 0.327$; Table 9).

Discussion

The systematic and strategic use of cover cropping systems is vital for sustainable agricultural production as this practice can help alleviate soil stress as a result of agricultural intensification. The results from this experiment conjure a partial agreement with the hypothesis concerning the studied parameters of the cover crop systems. In this experiment, there was a 58–100% greater aboveground biomass averaged across the 2 years of all other cover crop systems over the legume monoculture of winter lentil. However, the two mixtures overall or in separate years had similar biomass to the monocultures except for winter lentil. Based on the results, winter lentil may not be a suitable standalone cover crop for this semi-arid environment when multiple agronomic roles are the focus. There were temporal trends in biomass production

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Table 9. Soil microbial community biomass and indices determined by phospholipid fatty acid analysis (PLFA) and soil respiration for cover crop systems and years in Reno, Nevada, USA

	ng g soil^{-1}													μg CO_2-C g soil^{-1} h^{-1}
Cover crop systems	TMB	TBB	AB	GPB	GNB	TFB	AMB	SB	UB	FBR	GPNR	SUR	DI	Soil respiration
Monocultures														
Rye	1656	606	164	414	192	144	40	104	905	0.17	2.9	3.7	1.33bc	19.0
Arrowleaf clover	1989	660.0	166	439	220	156	42	114	1173	0.19	2.8	5.0	1.37abc	21.7
White sweetclover	1747	701	157	423	278	183	43	140	863	0.18	2.1	3.7	1.33bc	31.3
Forage kale	1774	665	165	431	234	180	47	133	929	0.21	2.2	3.1	1.41ab	25.0
Winter lentil	1795	579.0	140	386	193	153	40	113	1063	0.23	2.3	3.8	1.4ab	23.5
Mixtures														
CCM 1[†]	1478	538	128	354	184	137	38	99	802	0.21	1.9	2.8	1.41ab	22.0
CCM 2	1971	742	184	482	260	188	53	135	1041	0.22	2.0	3.0	1.43a	24.9
Control														
Fallow	1846	682	153	436	246	200	54	146	964	0.22	2.6	4.7	1.32c	20.6
P-value	0.753	0.783	0.788	0.82	0.638	0.807	0.85	0.789	0.433	0.675	0.129	0.183	0.052	0.571
SE	216	96	23.5	55.00	43.8	33.7	9.6	25	121	0.03	0.3	0.64	0.03	4.2
Year														
2021	1268^{b‡}	324^{b}	66^{a}	235^{b}	88.8^{a}	34^{b}	13^{b}	21^{b}	910	0.1^{b}	2.9^{a}	5.5^{a}	1.3^{b}	24.9
2022	2296^{a}	970^{a}	248^{a}	607^{a}	362.8^{a}	301^{a}	76^{a}	225^{a}	1025	0.31^{a}	1.8^{b}	2.0^{b}	1.5^{a}	22.0
P-value	<0.001	0.002	0.005	0.002	0.004	0.001	0.002	0.001	0.301	0.003	0.015	0.002	0.004	0.462
SE	111	65.1	12.6	34	32.1	23	6.2	17	68	0.01	0.2	0.4	0.02	2.5

TMB, total microbial biomass; TBB, total bacteria biomass; AB, actinomycetes biomass; GPB, Gram-positive bacteria biomass; GNB, Gram-negative bacteria biomass; TFB, total fungi biomass; AMB, arbuscular mycorrhizal fungi biomass; SB, saprophytes biomass; UB, undifferentiated biomass; FBR, fungi-bacteria ratio; GPNR, gram positive-negative bacteria ratio; SUR, saturated-unsaturated ratio; DI, diversity index; SE, standard error.

[†]Cover crop mixture 1 [CCM 1] (rye + arrowleaf clover + winter lentil) and cover crop mixture 2 [CCM 2]: (rye + white sweetclover + forage kale).

[‡]Means within columns that are followed by the same lowercase letter superscripts are not different among cover crop systems ($P > 0.05$).

among the cover crop systems used due to the lack of difference in the first compared to the second year of the experiment. This trend can be attributed to the growing environment (site–year variations). Our sampling date was used as a reference to the termination date and its potential impact on the measured aboveground parameters of cover cropping systems used in this experiment. The late sampling (late-termination date) resulted in a 74.4% increase in aboveground biomass production. This will be a beneficial response to consider when cover cropping systems serve the dual role of animal feed production and soil health improvement. The results from an experiment by Obour *et al.* (2022) examining biomass responses of single and multispecies mixtures of dual-purpose cover crops in Kansas showed that the single species (Triticale; *Tritcosecale* Wittm.) produced similar biomass to the mixtures across 3 years. The biomass production response in their experiment was similar to the rye monoculture compared to the mixtures in our experiment. The temporal trend in biomass production observed by Obour *et al.* (2022) was similar to the results in this experiment among cover crop systems. Under rainfed and irrigated practices in different environments, biomass production for different cover crop species and systems ranges from 438 to 12,000 kg DM ha^{-1} (Duiker, 2014; Nielsen *et al.*, 2015; Holman *et al.*, 2018; Haramoto, 2019; Decker *et al.*, 2022; Obour *et al.*, 2022). The results from our experiment under irrigation fall well within this range and indicate that with irrigation there is great potential for integrating cover crops in Nevada's semi-arid environment. In a systematic review by Florence and McGuire (2020), they examined 243 full comparisons of cover crop mixtures *vs* monocultures and found 88% of the comparisons were similar, 10% favors monoculture, and 2% favors mixtures in biomass production. The results of biomass production among cover crops systems in our experiment reflected the reported trends by Florence and McGuire (2020).

Understanding cover crop root biomass response in semi-arid environments like Nevada is an important tool for selecting cover crop systems that will perform well and also contribute to ecosystem services such as organic matter stock, and carbon and nutrient cycling in the soils (Ruis *et al.*, 2020; Xu *et al.*, 2020). Unlike our experiment, temporal differences in root biomass between a monoculture and a mixed cover crop system were reported by Faé *et al.* (2009). In this experiment, only a trend occurred for differences in root biomass in the second year that favors the mixed cover crop system CCM 2 relative to the monocultures of legumes (arrowleaf clover, white sweetclover, and winter lentil) or broadleaf (forage kale). This observation may dictate microbial community structure, soil carbon, and other important soil-related characteristics necessary for soil health improvement under these cover crop systems, especially when aboveground biomass is removed for animal feeding (Faé *et al.*, 2009).

Carbon and nitrogen are key elements in plant tissue and integral in nutrient cycling in the soil ecosystem. The quality of residue returning to the soil is dictated by its carbon and nitrogen content which can influence soil health indicators (Sainju *et al.*, 2021). In our experiment, the monoculture of white sweetclover had the greatest carbon concentration among cover crop systems but overall biomass carbon produced was similar among all systems except winter lentil. This was attributed to the lower biomass of winter lentil. While cover crop systems' nitrogen concentrations were all similar, the biomass nitrogen produced was greatest for white sweetclover monoculture. The C/N ratio is a critical factor in the decomposition and release of nutrients during cycling (Cotrufo *et al.*, 2013). The range of the C/N ratio from the cover crop systems studied was 20.8–36.3 when averaged across both years in this experiment. The potential for rapid decomposition and release of nutrients based on the C/N ratio among cover crop systems used in this experiment favor the monocultures of arrowleaf clover and white sweetclover, slowest for the mixed system CCM 1 and monoculture rye while the mixed system CCM 2 was intermediate. The sampling date had a marked effect on these parameters and thus offers a guide to the appropriate date for cover crop termination when these parameters are considered at the forefront. The average across years lack of difference in C/N ratio among the systems was quite unusual as typically, non-legume cover crops tend to have greater carbon concentration, and lower nitrogen concentrations than legumes and broadleaf species (Finney *et al.*, 2016; Sainju *et al.*, 2021).

The increased adoption and integration of cover cropping systems in crop production agriculture will increase with its role expanded beyond soil health (Simon *et al.*, 2021). Forage quality parameters can also be a suitable guide to producers in selecting cover cropping systems for their farming operations. Except for the lack of difference in CP concentration averaged across years, differences were evident for the other measured or estimated quality parameters among cover crop systems. For example, the RFV ranked highest for forage kale overall, and temporal differences were evident for the quality parameters in years 1 and 2 separately. Similar to the results reported by Sadeghpour *et al.* (2021), our experiment revealed that the sampling date had a consistent impact on the forage quality parameters. The early sampling provided greater CP, lower ADF, and NDF concentrations, and greater RFV across all cover crop systems relative to late sampling. In our experiment, apart from forage kale, mixtures were generally similar in forage quality parameters compared to the single species cover crop. However, in contrast to our experiment, Obour *et al.* (2022) reported greater overall forage quality for mixtures (grass-legume-broadleaf) relative to single species based on available energy, digestibility, DMI, CP, ADF and NDF, and digestibility compared to single species of grass-only (Oat; *Avena sativa* L. and rye). These trends in forage quality indices among cover crop systems will allow producers to focus on yield-quality trade-offs and economic value to be had when animal productivity is a core objective or hay quality for sales is the principal focus.

Our experiment revealed very few changes in the short-term impact of the cover cropping systems on the physical, chemical, and biological properties of the soil. When differences occurred in soil volumetric water content, the results were mixed as planting no cover crop (fallow) had greater water content among cover crops systems in year 1 but the reverse occurred in year 2. The fallow system did not favor the microbial diversity index (year 2) and greater cone index (penetrating resistance) but contained greater soil exchangeable potassium concentrations. This means that when harvesting cover crops for hay production, monitoring soil potassium levels will be important since a great proportion of the potassium will not be recycled in the soil. Unlike the mixed response in soil volumetric water content in our experiment, results from an experiment by Ghimire *et al.* (2019) showed a consistently higher soil water content for the fallow relative to the cover crop treatments. Similar to the results of this experiment, Liebig *et al.* (2015) observed very few short-term changes in the measured soil properties in their experiment.

For soil microbial community structure, the mixed systems (CCM 1 and 2) and monocultures of forage kale and winter lentil are more favorable to a greater microbial diversity index than planting no cover crop (fallow system). Unlike our experiment,

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Thapa *et al.* (2021) reported a clear advantage of growing a diverse cover crop mixture for microbial community size, arbuscular mycorrhizal fungi, saprophytic fungi, total fungi, Gram-positive bacteria, and total bacteria over the fallow system (no cover crop). While the fallow system had similar microbial biomass in our experiment, the different cover crop systems used (excluding the fallow system) increased microbial biomass from the first to the second year of this experiment which is a positive indicator for a positive soil health progression on a long-term basis. Additionally, in contrast to our experiment, Rankoth *et al.* (2019) reported greater total microbial biomass, total bacterial biomass, and total fungi biomass, which were all greater under cover crops compared to no cover crops (fallow) in their 3-year experiment. The differences in microbial population biomass observed in the studies of Rankoth *et al.* (2019) and Thapa *et al.* (2021) compared to ours may be a result of the longer experiment period (3-year) and more conducive environments that allowed for greater biomass build-up (microbial substrate) under cover crops relative to the fallow system.

Conclusion

This experiment provided valuable information that covers the dual role cover cropping systems can provide under irrigation in a semi-arid environment. Based on aboveground biomass, winter lentil will not be a suitable standalone cover crop in Nevada's semi-arid environment. Based on the measured parameters, the mixtures used in this experiment may offer greater reliability under this semi-arid environment compared to the monocultures. Forage kale grown as a monoculture had an overall superior RFV than all other cover crop systems. Terminating cover crop systems early (early sampling date) favors greater forage quality and lower C/N ratio but substantially lower biomass than late termination (late sampling date). Cover cropping studies of substantially longer duration will be required to assess the full effectiveness of these cover crop systems on positively altering soil health relative to the fallow system. While the short-term impact of the cover crop systems on soil health indicators was minimal relative to the fallow system, the overall results suggested that there is potential to integrate cover crops using irrigation in Nevada's semi-arid environment with a focus on dual utilization (forage and soil health improvement).

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