

Research Paper

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Abstract

Cover crops are an important management tool for organic vegetable farmers, but selection of individual cover crop species can lead to a tradeoff between agroecosystem services provisioned. In contrast, cover crop mixtures may provide multiple ecosystem services simultaneously, known as multifunctionality. This study evaluated the performance of browntop millet (*Urochloa ramosa*; BTM), buckwheat (*Fagopyrum esculentum*; BW), cowpea (*Vigna unguiculata*; CP), and sunnhemp (*Crotalaria juncea*; SH) in monocultures, two three-way mixtures (3-CP = browntop millet, buckwheat, and cowpea; 3-SH = browntop millet, buckwheat, and sunnhemp), and a four-way mixture containing all evaluated cover crop species (4-W). An autumn cabbage vegetable crop (*Brassica oleracea* var. Caraflex) was grown following cover crop termination. To evaluate the cover crop treatments and explore the applicability of some tenets of biodiversity theory to cover crop mixtures, we monitored the ecosystem services of weed suppression, inorganic nitrogen provisioning, vegetable yield, and habitat for microorganisms. Overall, the cover crop mixtures evaluated were able to combine the benefits of the individual species in the mixture and provision the sought ecosystem services, although they did not exceed the performance of the best monocultures. Weed suppression was similar between mixtures and the top performing monoculture, BTM in 2022 and BW in 2023. The high productivity of browntop millet in mixtures, accounting for on average 74% of 3-CP biomass and 56% of 4-W biomass, when seeded at 20% and 25% its full rate, respectively, likely drove weed suppression in mixtures. In 2022, cabbage yield following 3-SH and 4-W was similar to the legume monocultures. Due to the suppression of cowpea in 3-CP from interspecific competition, 3-CP plots had a lower cabbage yield than the legume monocultures and were similar to BTM and Control. Soil microbial biomass, used to measure habitat for microorganisms, was 18% higher following mixtures compared to monocultures in the first year, although no differences were found in the second year. The seeding rate proportions used in the three-way mixtures, 60% of the legume full rate and 20% of the full rate of both browntop millet and buckwheat, achieved the target of a C:N ratio $\leq 30:1$ and can be recommended when based on appropriate seeding rates for a given area. Tailoring future cover crop mixture research to questions of seeding rate thresholds and interspecific competition will improve complementarity and the provisioning of multiple ecosystem services in mixtures, offering valuable, practical guidance to growers.

Introduction

Cover crops are an important management tool for vegetable farmers exploring sustainable practices to maintain organic certification. Cover crops can increase soil health and crop production by reducing soil erosion, increasing soil organic matter, providing nitrogen and other essential plant nutrients, and suppressing weeds (Cherr et al., 2006a; Blanco-Canqui et al., 2013; Baraibar et al., 2018). Distinct windows exist throughout the season when cover crops may be established, including the short, summer fallow period following the harvest of a spring crop and preceding an autumn crop. Cover crop species selection for this summer window require quick-growing, heat-loving species. It is important to target specific farm management goals when selecting cover crop species to avoid potential pitfalls, such as soil nitrogen immobilization and cash crop yield drag (Finney et al., 2016). Emerging research points to an opportunity for cover crop mixtures containing three or more species to increase the number of ecosystem services provided, enhancing multifunctionality (Finney and Kaye, 2017). Often referred to as cover crop “cocktails,” cover crop mixtures have grown in popularity and use on organic vegetable farms (Silva and Moore, 2017). Yet, questions remain concerning the performance of individual species in a mixture and the ability of such mixtures to provide multifunctionality in an organic vegetable production system.

According to the ecological theories of niche complementarity and diversity-productivity, growing a diversity of cover crop functional groups, with varying root morphology, canopy structure, and resource requirements should more efficiently utilize limiting resources, like water and plant-available nutrients, and increase overall plant productivity (Tilman et al., 1996; Hooper et al., 2005; Florence and McGuire, 2020). As a result, insufficient resources remain for weeds to

become established, known as the diversity-invasibility hypothesis (Hooper et al., 2005; Florence and McGuire, 2020). Although cover crop biomass can be an indicator of weed suppression (Finney et al., 2016; Bilenky et al., 2022), in a review of published research on cover crop mixtures, Florence and McGuire (2020) found only 2 of 95 studies reported a significant increase in biomass in mixtures compared to monocultures. Additional research has suggested weed suppression is instead provided by a highly productive individual species in the mixture, such as sorghum sudangrass (*Sorghum bicolor* ssp. *Drummondii*), cereal rye (*Secale cereale*), buckwheat (*Fagopyrum esculentum*), and mustard (*Brassica juncea*) (Holmes et al., 2017; Baraibar et al., 2018; MacLaren et al., 2019; McKenzie-Gopsill et al., 2022), rather than an increase in diversity. Farmers use cover crops for weed suppression (Clark, 2012); thus, discerning the relative weed suppression benefits of cover crop mixtures compared with single species counterparts seeded at comparable rates is critical to inform farm practices.

A tradeoff in providing weed suppression and contributing plant-available N among cover crop species is often recognized. Legumes, although generally performing poorly as weed suppressors (Bilenky et al., 2022; Baraibar et al., 2018), can biologically fix nitrogen, contributing inorganic N when decomposed in the soil. Nitrogen contributions are dependent on the amount and quality of the biomass, such as the carbon to nitrogen (C:N) ratio, and environmental conditions which mediate microbial mineralization (Gaskin et al., 2020; O'Connell et al., 2015). For example, cowpea contributes an estimated 29 mg N g⁻¹ of dry matter (DM) residue (Clark, 2012), but DM yield can range from 2,800 to 5,000 kg DM ha⁻¹ in Iowa (USDA, 2024), resulting in a difference of almost 64 kg N ha⁻¹ supplied. On the other hand, grass and nonlegume broadleaf cover crops, with their high productivity and fibrous root systems, provide superior weed suppression, but are characterized as having a high C:N ratio, which may decrease crop yields (Bilenky et al., 2022; Creamer and Baldwin, 2000; Finney et al., 2016). The threshold C:N ratio between N mineralization and immobilization, or supporting and inhibiting crop yields, is broadly recognized between 20 and 40:1 (Creamer et al., 1997; O'Connell et al., 2015; Finney et al., 2016). Research comparing crop yield responses following cover crop monocultures and mixtures supports the widely recognized negative relationship between cover crop C:N ratio and crop yields, regardless of number of species in the mixture (Finney et al., 2016; White et al., 2017).

Balancing the species in a cover crop mixture to provide weed suppression, plant-available N, and support crop yields is a challenge. For example, highly productive grasses and nonlegume broadleaves are known to exert interspecific competition on the other species in a mixture, limiting the growth of legumes and increasing biomass C:N ratio (Creamer and Baldwin, 2000; O'Connell et al., 2015; White et al., 2017). Seeding rates of individual species can be adjusted to balance plant productivity, but familiarity with the growth characteristics and interspecific competitiveness of each species is needed (Bybee-Finley et al., 2022). Models and calculators are being developed to help build cover crop mixtures with complementarity and multifunctionality but currently are either not tailored to vegetable production (Green Cover Smart-Mix® Calculator; Chapagain et al., 2020) or remain as a framework for future incorporation into a grower-friendly tool (Bybee-Finley et al., 2022). Furthermore, cover crop mixture research exploring multifunctionality has often been conducted for field crop production (Wortman et al., 2012; Smith et al., 2014; O'Connell et al., 2015; Finney et al., 2016; White et al., 2017; Baraibar et al., 2018) or did

not directly measure the impact on vegetable production by growing a vegetable crop following the cover crop (Creamer and Baldwin, 2000; Blesh et al., 2019; McKenzie-Gopsill et al., 2022).

Cover crops are widely known to enhance soil biological activity and increase microbial biomass, by increasing labile carbon or altering the soil microclimate (Finney et al., 2017; Hu et al., 2023; Zuber and Villamil, 2016). As many soil functions are microbially driven, including nutrient cycling, aggregate stability, and disease suppression, cover crops can improve overall soil health and productivity (Lehman et al., 2015; Finney et al., 2017). A cover crop mixture may offer an opportunity to further enhance these benefits by increasing plant diversity. Each plant species releases distinct exudates into the soil and supports specific microbial communities (Wardle et al., 2004), therefore, as the diversity aboveground increases, belowground diversity may similarly increase. While this aboveground-belowground relationship is supported in ecological research (Tilman et al., 1997; Wardle et al., 2004), a debate remains over its validity in an agroecosystem. For example, research has shown differences in microbial biomass C and N between cover crop monocultures and mixtures to be heavily influenced by seasonality (De Souza et al., 2023). Additionally, in a meta-analysis, Florence and McGuire (2020) found that mixtures did not promote biology better than the best monocultures. On the other hand, some studies have found evidence to support the aboveground-belowground diversity link in cover crop mixtures, including higher enzyme activity (Thapa et al., 2021), increased total phospholipid fatty acids (PLFA) concentrations (Finney et al., 2017), and an increase in the diversity of carbon food sources consumed in a microbial incubation (Drost et al., 2020). Such studies, however, have not explored the soil biological impact of summer grown cover crop mixtures on an organic vegetable production system, warranting additional research under these conditions.

This study was designed to address the gap in research exploring the multifunctional impact of summer cover crop mixtures on organic vegetable crop production in the Upper Midwest. Utilizing summer cover crop species with known performance under Upper Midwest growing conditions (Bilenky et al., 2022), the project objectives were to i) compare the performance of four summer cover crop species in mixtures and monocultures and ii) assess the ability of cover crop mixtures to provide the ecosystem services of weed suppression, N contribution, enhanced crop yield, and increased habitat for soil microbes in an organic vegetable production system.

Materials and methods

Site description and experimental design

The study was conducted on certified organic land at the Iowa State University Horticulture Research Station in Ames, IA, USA. The experimental site was different in the first and second years of the experiment. The soil in the first year, 2022, was Clarion loam (fine-loamy, mixed, superactive, mesic Typic Hapludoll) and in the second year, 2023, was Lester loam (fine-loamy, mixed, superactive, mesic Mollic Hapludalfs). In both years, no cash crop was grown prior to cover crop establishment. The fields were previously cover cropped with oats (*Avena sativa*) in 2022 and with red clover (*Trifolium pratense*) in 2023. During the growing season the mean annual temperature was 18.9°C and 19.3°C in 2022 and 2023, respectively, and total precipitation was 351.9 and 427.8 mm in 2022 and 2023, respectively (Table 1).

Table 1. Mean air temperature and cumulative precipitation by month of growing season^x in 2022 and 2023 in Ames, IA, USA

Month	Air temperature (°C)			Precipitation (mm)		
	30-year mean ^y	2022	2023	30-year mean ^y	2022	2023
June	21.8	21.4	21.5	120.8	135.6	81.5
July	23.3	23.2	21.7	95.9	87.4	87.9
August	22.2	22.1	22.3	115.3	61.7	102.9
September	18.5	18.1	19.1	77.6	45.2	84.8
October	11.0	9.7	11.7	62.8	21.8	70.6
Growing season mean	19.3	18.9	19.3	103.0	70.4	85.5
Total (June–October)				472.4	351.8	427.8

^xCover crop treatments were seeded on 17 June 2022 and 16 June 2023 and terminated on 28 July 2022 and 2023. Cabbage was transplanted on 9 August 2022 and 15 August 2023 and harvest completed on 17 October 2022 and 18 October 2023.

^yIowa Environmental Mesonet 1993–2023.

The study was arranged as a randomized complete block design with eight treatments and four replications. Each plot was 6 m × 7.6 m with a 1.5 m buffer around each plot, maintained through tillage. Cover crop species represented three functional groups: grass, legume, and nonlegume broadleaf. Treatments included monocultures of browntop millet (*Urochloa ramosa*; BTM; grass), buckwheat (*Fagopyrum esculentum*; BW; nonlegume broadleaf), cowpea (*Vigna unguiculata*, var. Iron and Clay; CP; legume), and sunnhemp (*Crotalaria juncea*; SH; legume); a three-way mixture with browntop millet, buckwheat, and cowpea (3-CP); a three-way mixture with browntop millet, buckwheat, and sunnhemp (3-SH); and a four-way mixture with all four evaluated species (4-W). A weedy fallow control with no cover crop was also established for each replication (Control). Cover crop seeds were untreated and buckwheat seed was certified organic. Browntop millet was sourced from Green Cover Seeds (Bladen, NE, USA) and buckwheat, sunnhemp, and cowpea were sourced from Albert Lea Seeds (Albert Lea, MN, USA). Sunnhemp and cowpea were inoculated with OMRI-approved Exceed Superior Legume Inoculant (Visjon Biologics; Henrietta, TX, USA) at a manufacturer-recommended rate of 70.9 g per 22.7 kg of seed at the time of seeding.

Cover crop establishment, biomass, and termination

To prepare the sites for cover crop establishment, the previous cover crop of oats or red clover was mowed, the plot was rototilled, and the soil was cultipacked. About two weeks following oat or red clover termination, on 17 June 2022, and 16 June 2023, cover crops were seeded manually using a Gandy drop spreader (Gandy Company; Owatonna, MN, USA). Each species was seeded separately in the mixtures. Seeds were incorporated with a drag harrow and immediately watered with overhead sprinkler irrigation. No additional irrigation was supplied for the duration of cover crop growth.

Seeding rates for each treatment are presented in Table 2. The 4-W seeding rates were determined with the substitutive approach (Smith et al., 2014), dividing the monoculture seeding rate of each species by the total number of species in the mixture, resulting in 25% of the monoculture seeding rate for each species. The 3-CP and 3-SH seeding rates used 60% of the monoculture seeding rate for the legume in the mixture (cowpea or sunnhemp) and 20% of the monoculture seeding rate for buckwheat and browntop millet. The ratio of the seeding rates for all species in the mixtures summed to one. Three-way mixture legume and grass/nonlegume broadleaf seeding proportions were developed to control interspecific

competition and targeted a total biomass C:N ratio ≤30:1 to limit N immobilization (Creamer and Baldwin, 2000). Dry weight biomass and C:N ratio values for the evaluated species, as reported by Bilenky et al. (2022), were used to estimate the C:N ratio of the combined species in the mixture.

Aboveground cover crop biomass samples were collected 40 days after seeding (DAS), on 27 July 2022 and 26 July 2023 from each treatment using the quadrat method. Two 50 cm × 50 cm quadrats were randomly placed in each plot and all aboveground biomass was cut at the soil level. Biomass was sorted by cover crop species, broadleaf weed, or grass weed. All sorted samples were placed in a forced-air oven at 67°C until a constant weight was reached. The dry weight was then recorded for each sample and the two quadrats were averaged by treatment/replication. Dry cover crop samples were ground to a fine particle size and a 10 g sample of each treatment in all replicates, separated by species in the mixtures, was sent to Ward Laboratories, Inc. (Lincoln, NE, USA) for tissue analysis. Tissue concentrations of cover crop mixtures were calculated as a weighted average based on the biomass of each species in the mixture for each treatment/replication.

Cover crops were terminated by flail mowing on 28 July 2022 and 2023 (41 DAS). About one week later, on 5 August 2022 and 4 August 2023, the field was rototilled to incorporate residue.

Table 2. Cover crop treatment seeding rate (kg·ha⁻¹)^x by species

Treatment	Browntop millet	Buckwheat	Cowpea	Sunn hemp
BTM ^y	34	–	–	–
BW	–	79	–	–
CP	–	–	84	–
SH	–	–	–	56
3-CP	7	16	50	–
3-SH	7	16	–	34
4-W	9	20	21	14

^xSeeding rates are based on bulk seed weights.

^yBTM = browntop millet monoculture; BW = buckwheat monoculture; CP = cowpea monoculture; SH = sunnhemp monoculture; 3-CP = cowpea, browntop millet, and buckwheat mixture; 3-SH = sunnhemp, browntop millet, and buckwheat mixture; 4-W = browntop millet, buckwheat, cowpea, and sunnhemp mixture. 3-CP is 60% monoculture rate for cowpea and 20% monoculture rate for browntop millet and buckwheat. 3-SH is 60% monoculture rate for sunnhemp and 20% monoculture rate for browntop millet and buckwheat. 4-W is 25% of monoculture rate for each species.

Cabbage production and weed biomass

Cabbage (*Brassica oleracea* var. Caraflex; High Mowing Organic Seeds; Wolcott, VT, USA) was seeded on 29 June 2022 and 28 June 2023 in 72-cell flats with Berger OM6 (Saint-Modeste, QC, Canada) OMRI-approved growing media at the Department of Horticulture greenhouses at Iowa State University. Cabbage seedlings were fertilized with 5-1-1 AquaPower® Liquid Fish Fertilizer (JH Biotech, Inc.; Ventura, CA, USA) at a concentration of 150 mg L⁻¹ on 22 and 29 July 2022 and 19 July, 27 July, and 8 August 2023.

Following cover crop termination, Sustane® 8-2-4 OMRI-approved fertilizer (Cannon Falls, MN, USA) was spread at a rate of 75 kg N ha⁻¹ across the entire research plot area. This rate represented 67% of the recommended total N rate (Phillips, 2024) without adjustments of expected plant-available N mineralization. Previous cover crop research has indicated a 67% reduction to allow for crop growth without masking treatment effects (Cherr et al., 2006b; Allar and Maltais-Landry, 2022). In 2022, a Gandy drop spreader was used to apply the fertilizer and tillage was used to incorporate. In 2023, The Andersons, Inc. rotary spreader (Model SR2000; Maumee, OH, USA) was used to apply the fertilizer, and it was incorporated during bed preparation using a plastic mulch layer.

Each cover crop treatment/replication included four 6 m long raised beds: two exterior guard beds and two interior beds for data collection. Raised, plastic mulch beds with drip irrigation were established using a plastic mulch layer. Cabbage was transplanted on 9 August 2022 and 15 August 2023 with a water wheel transplanter in staggered double rows on each bed. Rows were 30 cm apart with plants 30 cm apart within rows.

Cabbage was regularly scouted for pests and OMRI-approved *Bacillus thuringiensis*, subspecies *kurstaki* (Javelin WG; Certis LLC; Columbia, MD, USA) was sprayed to control lepidopterous larvae every six to ten days from 26 August to 2 October 2022 and 7 September to 4 October 2023.

Cabbage was harvested from the first replication on 14 October 2022 and from the rest of the field on 17 October 2022. In 2023, all cabbage was harvested on 18 October 2023. In both years, the first two heads in each data row were not used for data collection. The subsequent 12 heads were harvested and used for data collection, for a total of 24 heads per treatment/replication. Cabbage heads were then graded as marketable and nonmarketable due to insect damage or small size. In 2022, cabbage heads were categorized as small with a circumference <30 cm. In 2023, due to generally reduced cabbage head size, any head <25 cm circumference was categorized as small. Two marketable heads per treatment/replication were placed in a forced air oven at 67°C until a constant weight was reached, ground to fine particle size, and a 10 g sample from each replicate was sent to Ward Laboratories, Inc. for tissue analysis.

Soil sampling, microbial biomass, and labile carbon

Soil samples were collected three times each year: prior to cover crop seeding (before seeding), following cover crop termination (at termination), and after cabbage harvest (after harvest). Sampling occurred on 17 June, 28 July, and 21 October 2022 and 15 June, 28 July, and 20 October 2023. Samples were collected with a 5 cm diameter soil probe to a depth of 15 cm. Five individual cores were collected per plot and homogenized. Soil samples were sieved to 2 mm diameter and sent to AgSource Laboratories, Inc. (Ellsworth, IA, USA) for analysis of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), zinc

(Zn), pH, cation exchange capacity (CEC), organic matter (OM), nitrate-nitrogen (NO₃-N), and ammonium-nitrogen (NH₄-N).

After harvest soil samples were additionally analyzed for permanganate oxidizable carbon (POXC) and microbial biomass carbon (MBC) as indicators of habitat for microorganisms. Microbial biomass analysis was performed with the soil chloroform fumigation-extraction procedure, modified from Vance et al. (1987). One 5 g soil sample from each treatment/replication was fumigated in a vacuum chamber at 75 kPa pressure with 25–30 mL of chloroform for 24 hours, labeled as the ‘fumigated’ sample. An unfumigated 5 g sample from each treatment/replication and the fumigated sample were shaken at 200 rpm with 0.5 M potassium sulfate (K₂SO₄) for one hour and then left to settle for 30 minutes. After settling, extractions were poured through plastic funnels with Whatman #1 filter paper and immediately placed in a –20°C freezer. Samples were thawed and combusted on a Shimadzu TOC-L analyzer with TN functions (Shimadzu Corporation; Kyoto, Japan). MBC measurements are reported as the difference between the fumigated and unfumigated samples.

POXC was measured using methods adapted from Culman et al. (2021). Five grams of soil from each plot were reacted with a 0.2 M potassium permanganate (KMnO₄) and deionized water solution. The supernatant was then diluted and pipetted into a 96-well plate (Corning Life Sciences; Tewksbury, MA, USA), and the absorbance value was determined at 550 nm with a microplate spectrophotometer (iMark, Bio-Rad Laboratories; Hercules, CA, USA). Absorbance values were used to calculate mg POXC·kg⁻¹ soil.

Anion exchange membranes

Anion exchange membranes (AEMs) inserted in the soil were used to quantify the NO₃-N released from cover crop and soil mineralization during cabbage production. The AEMs were sourced from Suez WTS Solutions (Model AR204E; Westborough, MA, USA) and cut into 1.5 cm × 7 cm strips. One AEM strip was inserted vertically 2–3 cm below the soil surface in each data row in each treatment/replication using a putty knife, for a total of two AEMs per plot. AEMs remained in the soil for 9 or 10 days, were removed, placed in labeled plastic bags, and brought to the laboratory for extraction. New AEMs were immediately placed in the soil. A total of six AEM deployments occurred in 2022 and 2023, beginning on 10 August 2022 and 17 August 2023 and concluding on 5 October 2022 and 13 October 2023.

Following an adapted procedure from Subler et al. (1995), removed AEMs were extracted for NO₃-N in the laboratory. First, AEMs were rinsed with deionized water to remove soil particles and placed in labeled 50 mL centrifuge tubes with 50 mL of 2.0 M potassium chloride (KCl). Tubes were shaken for one hour and the extraction was poured through Whatman #1 filter paper into scintillation vials. To determine NO₃-N adsorbed to AEMs, 10 µL of the extraction from each scintillation vial was plated onto a 96-well microplate with 200 µL of vanadium chloride reagent, following methods adapted from Doane and Horwath (2003). After a 5-hour incubation period, absorbance values were determined at 540 nm with a microplate spectrophotometer. Each plate also contained a set of standards ranging from 0 to 10 mg·L⁻¹ of NO₃-N made from ammonium nitrate. The absorbance values of the standards were used to plot a standard curve, from which the cover crop treatment NO₃-N concentrations were determined.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis Software program (SAS ver. 9.4, SAS Institute, Inc.; Cary, NC, USA) and the GLIMMIX procedure. All data were analyzed as a randomized complete block design by year, date (when applicable), and treatment. Replication by treatment was the random effect. The assumptions of normality and homogeneity of variance were checked with the Shapiro–Wilk test and Levene tests. Significant interactions between years were found for many variables, therefore, results from each year are presented separately. Orthogonal contrasts were used with the GLM procedure to compare treatment effects between monocultures (BTM, BW, CP, and SH) and mixtures (3-CP, 3-SH, and 4-W), CP and mixtures containing cowpea (3-CP and 4-W), and SH and mixtures containing sunnhemp

(3-SH and 4-W). To determine a correlation between cover crop treatment biomass production and weed suppression, a linear regression was performed.

Results

Cover crop productivity and performance in mixtures

Cover crop biomass was significant by treatment, but not by year, although, significant interactions between treatment and year were found. Cover crop biomass ranged from 4.7 to 0.7 Mg ha⁻¹. The cowpea monoculture (CP) produced the lowest biomass in both years, although BW had a similar biomass in 2022 and SH was similar in 2023 (Table 3). In 2022, BTM biomass was the greatest, although similar to all but CP and BW. In 2023, BW produced the

Table 3. Cover crop and weed biomass prior to termination^y in 2022 and 2023

Treatment	Cover crop biomass (Mg·ha ^{−1})	Weed biomass (Mg·ha ^{−1})			
		Broadleaf	Grass	Total	% of Plot biomass ^w
2022					
BTM ^x	4.2 a ^y	0.7	0.3 b	1.0 d	19
BW	1.5 b	2.1	0.8 b	2.9 ab	66
CP	1.7 b	1.4	0.9 b	2.3 bc	58
SH	3.3 a	1.1	0.3 b	1.4 cd	30
3-CP	3.1 a	1.0	0.4 b	1.4 cd	31
3-SH	3.6 a	1.0	0.6 b	1.7 cd	32
4-W	3.2 a	1.5	0.4 b	1.9 bcd	37
Control	NA	1.8	2.0 a	3.8 a	NA
<i>P-value</i>	0.0015	0.1943	0.0007	0.0002	
Contrasts ^z		Significance			
<i>Monoculture vs. mixture</i>	**	NS	NS	NS	
<i>CP vs. 3-CP & 4-W</i>	*	NS	*	NS	
<i>SH vs. 3-SH & 4-W</i>	NS	NS	NS	NS	
2023					
BTM	3.7 ab	0.5	2.4 bc	2.9 c	44
BW	4.7 a	0.3	1.0 d	1.2 d	20
CP	0.7 c	0.4	3.4 ab	3.8 bc	84
SH	1.3 c	0.9	3.6 a	4.5 ab	78
3-CP	3.1 b	0.5	2.5 bc	3.1 c	50
3-SH	3.2 b	0.4	2.3 b	2.6 c	45
4-W	2.8 b	0.5	2.4 bc	2.9 c	51
Control	NA	1.4	2.0 a	5.3 a	NA
<i>P-value</i>	0.0001	0.3833	0.0003	<.0001	
Contrasts		Significance			
<i>Monoculture vs. mixture</i>	NS	NS	NS	NS	
<i>CP vs. 3-CP & 4-W</i>	**	NS	**	*	
<i>SH vs. 3-SH & 4-W</i>	**	NS	**	**	

^yTermination occurred 41 days after seeding (DAS).

^wPercent of plot biomass is the percentage of weed biomass + cover crop biomass that is made up of weed biomass.

^xBTM = browntop millet monoculture; BW = buckwheat monoculture; CP = cowpea monoculture; SH = sunnhemp monoculture; 3-CP = cowpea, browntop millet, and buckwheat mixture; 3-SH = sunnhemp, browntop millet, and buckwheat mixture; 4-W = browntop millet, buckwheat, cowpea, and sunnhemp mixture.

^yMeans followed by the same letter within the same column and year are not significantly different at $P \leq 0.05$.

^zOrthogonal contrast significance at $P \leq 0.05 = **$, $P \leq 0.10 = *$, and NS = nonsignificant.

Table 4. Percent of total biomass of cover crop mixtures by species and seeding rate of each species in mixture as a percent of full seeding rate in 2022 and 2023

Treatment	Species	% of Full seeding rate ²	2022	2023
			% of Total biomass	% of Total biomass
3-CP	Browntop millet	20	73	75
	Buckwheat	20	9	16
	Cowpea	60	17	9
3-SH	Browntop millet	20	41	50
	Buckwheat	20	4	17
	Sunnhemp	60	55	33
4-W	Browntop millet	25	51	60
	Buckwheat	25	9	26
	Cowpea	25	8	3
	Sunnhemp	25	31	11

²Full seeding rate of browntop millet = 39 kg ha⁻¹, buckwheat = 79 kg ha⁻¹, cowpea = 84 kg ha⁻¹, and sunnhemp = 56 kg ha⁻¹.

largest biomass, which was similar to BTM. In both years, the mixtures containing cowpea (3-CP and 4-W) produced a greater biomass than CP. Similarly, in 2023, the mixtures containing sunnhemp (3-SH and 4-W), produced a greater biomass than the sunnhemp monoculture (SH). Orthogonal contrasts of mixtures and monocultures indicated mixtures produced a larger biomass

than monocultures in 2022, although only significant at an alpha level of 0.1 ($P = 0.0640$).

The proportion of total biomass represented by each species in the mixtures did not correlate well with the seeding rate reductions (Table 4). For instance, browntop millet made up, on average, 58% of total biomass in mixtures despite a 20% full seeding rate in three-way mixtures and a 25% full seeding rate in the four-way mixture. In contrast, cowpea and sunnhemp made up, on average, 9% and 33% of total biomass in mixtures, respectively, but were seeded at 60% of the full seeding rate in the three-way mixtures.

Weed suppression

In both years, at the time of cover crop biomass sampling, all treatment plots had a similar broadleaf weed biomass as Control, indicating a lack of broadleaf weed suppression by the treatments (Table 3). Grass weed biomass was significantly lower in all cover crop plots compared to Control in 2022. Total weed biomass in 2022 was lowest in BTM, SH, 3-CP, 3-SH, and 4-W. In 2023, all treatments, excluding CP, had lower grass weed biomass compared to Control, with the lowest grass weed biomass in BW. Total weed biomass in 2023 was lowest in BTM, BW, 3-CP, 3-SH, and 4-W. A strong inverse relationship between cover crop biomass and total weed biomass was found ($R^2 = 0.57$) (Fig. 1).

Cover crop biomass C:N ratio and tissue nutrient concentrations

Cover crop tissue nutrient concentrations differed between treatments, as well as between monocultures and mixtures. The treatment with the highest C:N ratio in both years was BTM, similar only to 3-CP in 2023, which was made up of 75% browntop millet (Table 5). The lowest C:N ratios were found in the two legume monocultures, CP and SH, in both years. Orthogonal contrasts

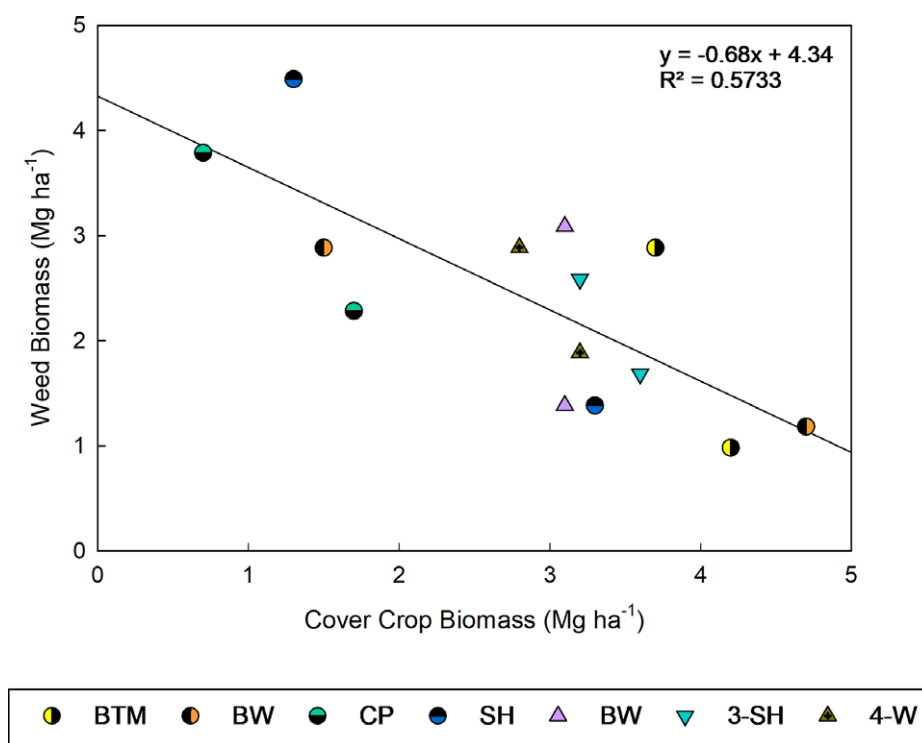


Figure 1. Relationship between cover crop and total weed biomass (Mg ha⁻¹) at time of cover crop termination. Both 2022 and 2023 data are included in the figure. Monocultures shown with circle (●) and mixtures shown with triangle (▲).

revealed significantly higher C:N ratios in mixtures containing cowpea (3-CP and 4-W) in both years, and those containing sunnhemp (3-SH and 4-W) in the first year, than in CP and SH monocultures. On average, mixtures had a significantly higher C:N ratio than monocultures in both years.

CP was among the treatments with the greatest tissue concentration of most macro- and micronutrients, including % N, % P, % Ca, Zn (ppm), Mn (ppm), Cu (ppm), and B (ppm) in both years, and % K, % S, and % Mg in 2022 (Table 5 and Supplementary Material). Treatments with similar nutrient concentrations to CP were often the treatments that also included cowpea, including

similar % P, % K, % Mg, and % Zn in 3-CP and 4-W to CP in 2022. BW had similar % P and % Mg to CP in 2022. SH was similar to CP in % Cu in both years and % N and % P in the second year.

Vegetable yield and quality

In 2022, cabbage grown following BTM had significantly more small (nonmarketable) cabbage heads compared with the other treatments, resulting in the lowest number of marketable cabbage heads (Fig. 2). CP, SH, 3-SH, and 4-W produced more marketable

Table 5. Cover crop tissue macronutrient concentration (%) and quantity ($\text{kg}\cdot\text{ha}^{-1}$) 40 days after seeding in 2022 and 2023

Treatment	C:N	N		% P		% K		% S		% Ca		% Mg	
		%	kg·ha ^{−1}	%	kg·ha ^{−1}	%	kg·ha ^{−1}	%	kg·ha ^{−1}	%	kg·ha ^{−1}	%	kg·ha ^{−1}
2022													
BTM ^x	48.9 a ^y	0.9 f	38.2 cd	0.34 b	14.5 a	2.4 bc	101.2 a	0.18 b	7.7 a	0.33 e	13.9	0.30 c	12.6
BW	36.3 b	1.3 ef	19.5 d	0.41 a	6.1 b	2.0 c	30.5 c	0.15 c	2.2 c	1.04 bc	15.6	0.42 ab	6.3
CP	13.3 e	3.3 a	56.1 abc	0.40 a	6.8 b	2.9 a	49.1 bc	0.28 a	4.8 bc	1.66 a	28.2	0.49 a	8.4
SH	17.0 de	2.7 b	89.1 a	0.31 b	10.3 ab	2.2 c	71.9 ab	0.20 b	6.5 ab	1.12 b	37.0	0.39 b	12.9
3-CP	26.2 c	1.7 de	52.7 bcd	0.44 a	13.7 a	2.8 ab	85.3 ab	0.20 b	6.2 ab	0.77 d	23.9	0.41 ab	12.8
3-SH	21.3 cd	2.1 c	75.6 ab	0.34 b	12.4 a	2.3 bc	83.9 ab	0.19 b	7.0 a	0.83 d	29.9	0.39 b	14.1
4-W	22.8 cd	2.0 cd	64.0 abc	0.41 a	13.2 a	2.7 ab	85.1 ab	0.20 b	6.5 ab	0.85 cd	27.2	0.43 ab	13.6
<i>P</i> -value	<.0001	<.0001	0.0076	0.0001	0.0167	0.0044	0.0157	<.0001	0.0036	<.0001	0.0880	0.0115	0.1921
Contrasts ^z	Significance												
<i>Monocultures vs. mixtures</i>	**	NS	NS	**	**	*	*	NS	*	**	NS	NS	*
<i>CP vs. 3-CP & 4-W</i>	**	**	NS	NS	**	NS	**	**	*	**	NS	*	NS
<i>SH vs. 3-SH & 4-W</i>	NS	**	NS	**	NS	*	NS	NS	NS	**	NS	NS	NS
2023													
BTM	33.6 a	1.3 c	48.1 b	0.24 d	8.8 a	3.23	119.5 b	0.18	6.8 a	0.53 e	19.6 bc	0.55	20.4 ab
BW	25.9 b	1.8 b	84.6 a	0.27 cd	12.5 a	3.52	165.4 a	0.14	6.4 a	1.10 c	51.7 a	0.54	25.6 a
CP	15.5 c	2.8 a	19.6 c	0.35 a	2.4 b	2.92	20.4 c	0.20	1.4 c	1.77 a	12.4 c	0.62	4.3 d
SH	15.8 c	2.8 a	36.4 bc	0.32 ab	4.2 b	2.58	33.5 c	0.17	2.2 bc	1.39 b	18.1 bc	0.47	6.1 cd
3-CP	28.7 ab	1.5 bc	46.5 b	0.29 bc	9.1 a	3.35	103.9 b	0.17	5.4 a	0.70 de	21.7 bc	0.52	16.0 b
3-SH	24.5 b	1.9 b	60.8 b	0.30 c	9.5 a	3.02	96.6 b	0.18	5.7 a	0.87 d	27.8 b	0.50	16.1 b
4-W	26.1 b	1.7 bc	44.8 b	0.32 ab	8.9 a	3.45	96.6 b	0.16	4.5 ab	0.80 d	22.4 bc	0.48	13.5 bc
<i>P</i> -value	<.0001	<.0001	0.0005	<.0001	0.0014	0.0992	<.0001	0.1784	0.0025	<.0001	0.0002	0.2060	0.0005
Contrasts	Significance												
<i>Monocultures vs. mixtures</i>	**	**	NS	NS	*	NS	NS	NS	NS	**	NS	NS	NS
<i>CP vs. 3-CP & 4-W</i>	**	**	**	**	**	NS	**	**	**	**	*	**	**
<i>SH vs. 3-SH & 4-W</i>	**	**	NS	NS	NS	**	**	NS	**	**	NS	NS	**

^xBTM = browntop millet monoculture; BW = buckwheat monoculture; CP = cowpea monoculture; SH = sunnhemp monoculture; 3-CP = cowpea, browntop millet, and buckwheat mixture; 3-SH = sunnhemp, browntop millet, and buckwheat mixture; 4-W = browntop millet, buckwheat, cowpea, and sunnhemp mixture.

^yMeans followed by the same letter within the same column and year are not significantly different at $P \leq 0.05$.

^zOrthogonal contrast significance at $P \leq 0.05 = **$, $P \leq 0.10 = *$, and NS = nonsignificant.

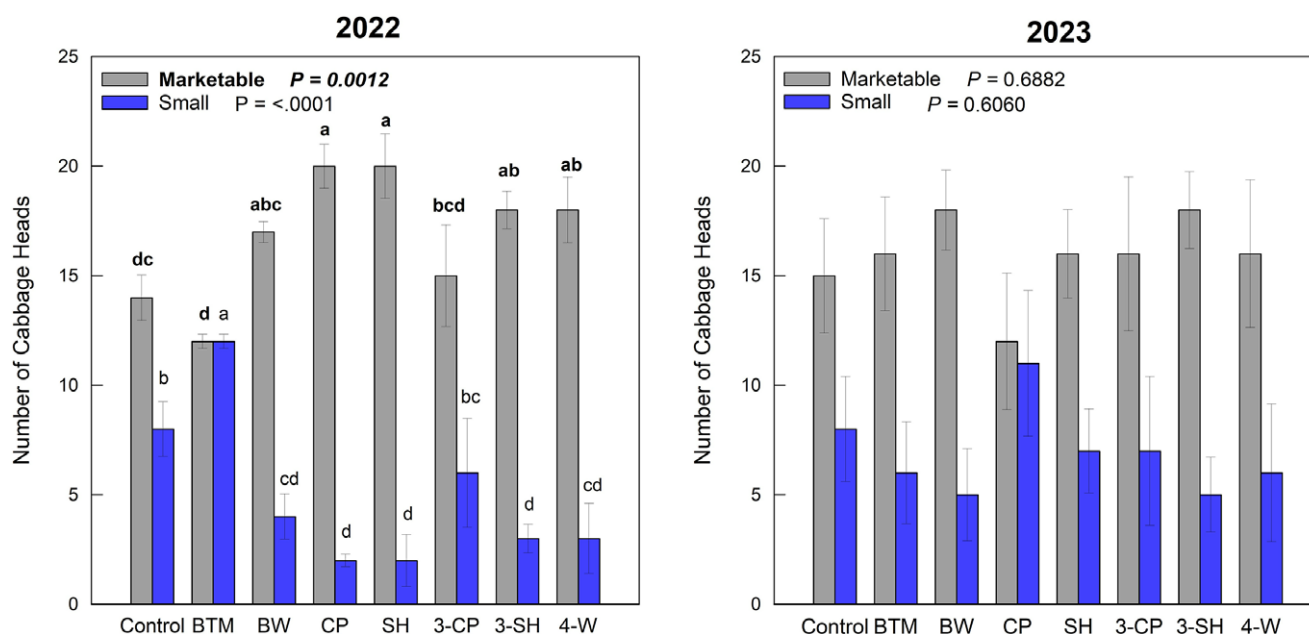


Figure 2. Marketable and nonmarketable cabbage head yield following cover crop treatment in 2022 and 2023 from 24 heads in 6 m row length. Marketable and nonmarketable cabbage head yields analyzed separately by treatment.

cabbage heads than BTM and Control. 3-CP produced a similar number of marketable cabbage heads as the other mixtures, as well as the lowest-yielding BTM and Control. BW was similar to both the top producers and Control. CP plots produced a greater number of marketable cabbage heads than mixtures containing cowpea (3-CP and 4-W) in 2022. In 2023, no statistically significant differences were found in cabbage yield between treatments.

Cover crop treatment had a slight effect on the cabbage head nutrient concentrations grown following cover crop incorporation. Cabbage grown following CP and BW had greater % P and % Ca than other cover crop treatments in 2022, although no differences in any cabbage head macronutrient concentrations were found between treatments in 2023 (Table 6). Orthogonal contrasts revealed cabbage had lower nutrient concentrations following monocultures than following mixtures for those nutrients with reported differences, including % P in both years, % Ca in 2022, and % K and % Mg in 2023. Additionally, cabbage grown following SH had a higher % N than those grown following mixtures containing sunnhemp in 2022.

Soil microbial biomass, labile carbon, and soil nutrients

Differences in MBC were significant by year ($P = 0.0184$) but not by treatment ($P = 0.3792$). MBC was, on average across all treatments, 15% higher in 2022 than in 2023. Orthogonal contrasts revealed that MBC was greater in plots with mixtures than with monocultures in 2022, by 17.9% (Fig. 3). In 2023, no statistical differences were found for MBC between mixtures and monocultures.

POXC was not different by year or by cover crop treatment. Mixtures had a slightly greater POXC than monocultures in 2022, by 7.5%, at an alpha level of 0.1 ($P = 0.0628$) (Fig. 4), although no differences were found in 2023.

Most soil nutrients were not impacted by cover crop treatment in either year. At the time of cover crop termination in 2022, inorganic N ($\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$) was higher in SH than in the other treatment plots, although this trend was not seen in the second year (Supplementary Material). In 2023, no differences were found for

any soil nutrients between treatments or between monocultures and mixtures.

Anion exchange membranes

Nitrate-N mineralization, as measured by AEMs, differed by date of sampling and by year, but not by treatment (Fig. 5). Total cumulative $\text{NO}_3\text{-N}$ mineralized was on average 83% higher in 2023 than in 2022. Although no statistical differences were found, the treatment with the greatest cumulative $\text{NO}_3\text{-N}$ mineralization in 2022, SH, had 56% greater mineralization than BTM, the treatment with the lowest mineralization.

Discussion

Productivity was not reliably increased in mixtures

Productivity among monocultures was congruent with our current understanding of productivity from cover crop functional groups (Baraibar et al., 2018; McKenzie-Gopsill et al., 2022), as leguminous monocultures produced the lowest biomass while grass and non-legume broadleaf monocultures produced the greatest.

When investigating cover crop mixtures, researchers have questioned the applicability of the diversity-productivity hypothesis (Florence and McGuire, 2020), which purports that productivity should be higher in mixtures. Our results indicated that although mixtures were similarly productive to the best monoculture (BTM) in the first year, they did not exceed this productivity. Therefore, our study also shows a lack of support for the diversity-productivity hypothesis in cover crop mixtures.

Furthermore, cover crop biomass productivity did not increase as the number of species in the mixtures increased, as 4-W biomass was similar to 3-CP and 3-SH. Finney et al. (2016) found evidence for a positive relationship between the number of species and productivity in a study of cover crop mixes with up to eight species, including both winter hardy and winter killed functional groups. Functional diversity is seen to drive increased productivity more

Table 6. Cabbage tissue macronutrient concentrations following cover crop treatment in 2022 and 2023

Treatment	% N	% P	% K	% S	% Ca	% Mg
2022						
BTM ^x	2.2	0.44 c ^y	3.3	0.57	0.51 c	0.20
BW	2.4	0.52 ab	3.7	0.63	0.72 a	0.24
CP	2.7	0.56 a	3.9	0.60	0.63 ab	0.25
SH	3.0	0.48 bc	3.6	0.57	0.58 bc	0.23
3-CP	2.6	0.44 c	3.4	0.56	0.53 bc	0.22
3-SH	2.6	0.47 bc	3.4	0.58	0.53 bc	0.22
4-W	2.2	0.46 c	3.4	0.55	0.54 bc	0.20
Control	2.9	0.53 ab	3.8	0.62	0.58 bc	0.24
<i>P</i> -value	0.1559	0.0038	0.3246	0.6052	0.0290	0.1909
Contrasts ^z	Significance					
Monoculture vs. mixture	NS	**	NS	NS	**	NS
CP vs. 3-CP & 4-W	NS	**	*	NS	*	**
SH vs. 3-SH & 4-W	**	NS	NS	NS	NS	NS
2023						
BTM	3.7	0.49	3.5	0.88	0.65	0.23
BW	3.9	0.53	3.6	0.87	0.67	0.24
CP	3.6	0.46	3.3	0.72	0.61	0.22
SH	3.7	0.51	3.4	0.74	0.59	0.22
3-CP	3.6	0.45	3.3	0.76	0.60	0.22
3-SH	3.6	0.45	3.3	0.71	0.66	0.23
4-W	3.7	0.49	3.4	0.70	0.59	0.22
Control	3.6	0.48	3.3	0.76	0.59	0.22
<i>P</i> -value	0.2666	0.1112	0.0848	0.1501	0.1094	0.0516
Contrasts	Significance					
Monoculture vs. mixture	NS	**	**	*	NS	**
CP vs. 3-CP & 4-W	NS	NS	NS	NS	NS	NS
SH vs. 3-SH & 4-W	NS	NS	NS	NS	NS	NS

^xBTM = browntop millet; BW = buckwheat; CP = cowpea; SH = sunnhemp; 3-CP = cowpea, browntop millet, and buckwheat mixture; 3-SH = sunnhemp, browntop millet, and buckwheat mixture; 4-W = browntop millet, buckwheat, cowpea, and sunnhemp mixture.

^yMeans followed by the same letter within the same column and year are not significantly different at $P \leq 0.05$.

^zOrthogonal contrast significance at $P \leq 0.05 = **$, $P \leq 0.10 = *$, and NS = nonsignificant.

than species diversity (Tilman et al., 1997; Finney et al., 2016). In our study, 4-W included an additional legume, but not an additional functional group, compared with the three-way mixtures. This potentially led to overlapping resource niches and greater competition between the legume species in 4-W, inhibiting biomass production.

Weed suppression dependent on cover crop biomass and performance of individual species

Results from our study support the established logic that while cover crops in general can provide some weed suppression (MacLaren et al., 2019; Bilenky et al., 2022), grasses and nonlegume broadleaves provide superior suppression than legumes in monoculture (Baraibar et al., 2018; Creamer and Baldwin, 2020), and productivity is a major determiner of how well the cover crop can

provide this function (Finney et al., 2016; MacLaren et al., 2019; Bilenky et al., 2022). We identified a negative relationship between cover crop biomass and total weed biomass ($R^2 = 0.57$), with the greatest weed suppression coming from the most productive treatments, BTM in 2022 and BW in 2023. The ability of SH to suppress weeds in our study was related to its productivity. In the first year, SH suppressed weeds similar to BTM (the top performer), but, as SH productivity dropped from 3.3 Mg ha⁻¹ to 1.3 Mg ha⁻¹ in the second year, SH plots had similar total weed biomass as Control. Bilenky et al. (2022) found sunnhemp productivity averaged 3.5 Mg ha⁻¹ and reduced weed biomass by over 100% compared with cowpea and mungbean.

One of the goals of a cover crop mixture is to account for the expected poor weed suppression of legumes by growing them with other species that can better perform this function (McKenzie-Gopsill et al., 2022). In our study, we found weed suppression from

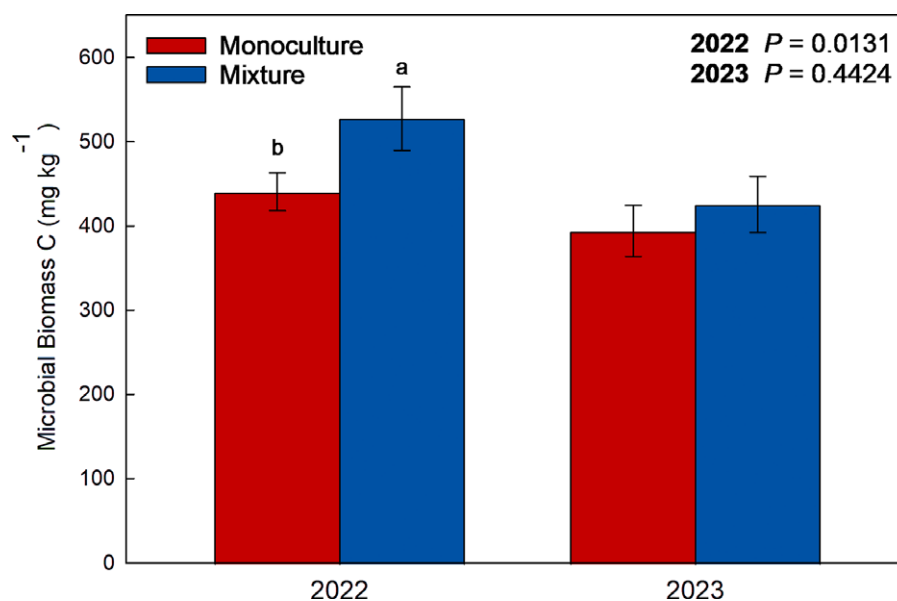


Figure 3. Microbial biomass carbon after cabbage harvest in 2022 and 2023 in monoculture and mixture treatments. Monoculture treatments included BTM, BW, CP, and SH. Mixture treatments included 3-CP, 3-SH, and 4-W.

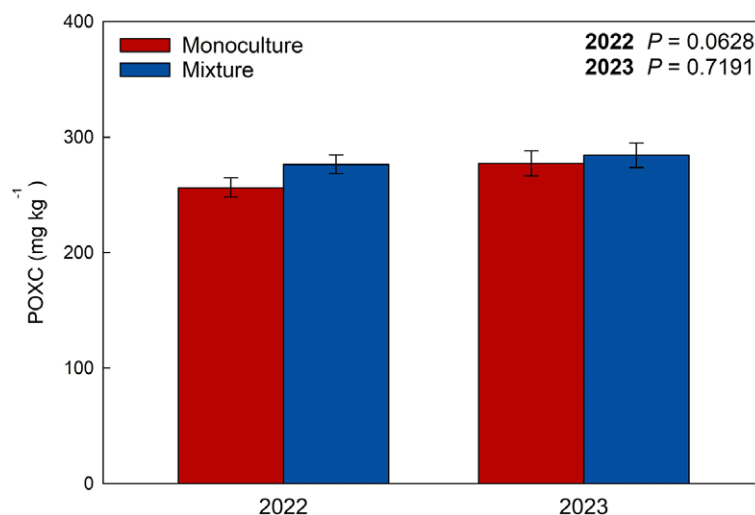


Figure 4. Soil permanganate oxidizable carbon (POXC) after cabbage harvest in 2022 and 2023 in monoculture and mixture treatments. Monoculture treatments included BTM, BW, CP, and SH. Mixture treatments included 3-CP, 3-SH, and 4-W.

mixtures to be similar to, but not necessarily better than, the best monocultures. Although orthogonal contrasts revealed total weed biomass was similar between monocultures and mixtures in both years, the proportion of plot biomass made up of weeds in legume monocultures was reduced by, on average, 11% in 2022 and 32% in 2023 in mixtures (Table 3). Many studies have found a lack of evidence for increased weed suppression from increased diversity in mixtures (Baraibar et al., 2018; Florence and McGuire, 2020), instead finding weed suppression driven by highly competitive species in the mixture (Holmes et al., 2017; Baraibar et al., 2018; MacLaren et al., 2019; McKenzie-Gopsill et al., 2022). Our results indicate the high productivity of browntop millet, making up on average 74% of 3-CP biomass and 56% of 4-W biomass, likely accounted for the similar weed suppression in 3-CP and 4-W compared with the top performers.

The exact mechanism of weed suppression is unclear. Allelopathic compounds produced by cowpea, sunn hemp, and buckwheat are known to inhibit weeds (Adler and Chase, 2007; Sturm et al., 2018) and may have played a role. Additionally, weed suppression may have been influenced by increased resource capture of available soil N, soil moisture, and light (MacLaren et al., 2019), and the growth rate or canopy cover (McKenzie-Gopsill et al., 2022) of the additional individual species in the mixture.

While substitutive seeding rates are recommended by researchers to compare findings across studies and decipher differences between mixtures and monocultures (Florence and McGuire, 2020), they may not serve to meet grower demands (Bybee-Finley et al., 2022). In our study, seeding browntop millet at 20% of its full rate in the three-way mixtures and 25% in 4-W allowed for similar weed suppression as in a monoculture. Baraibar et al. (2018) also found cereal rye and oats

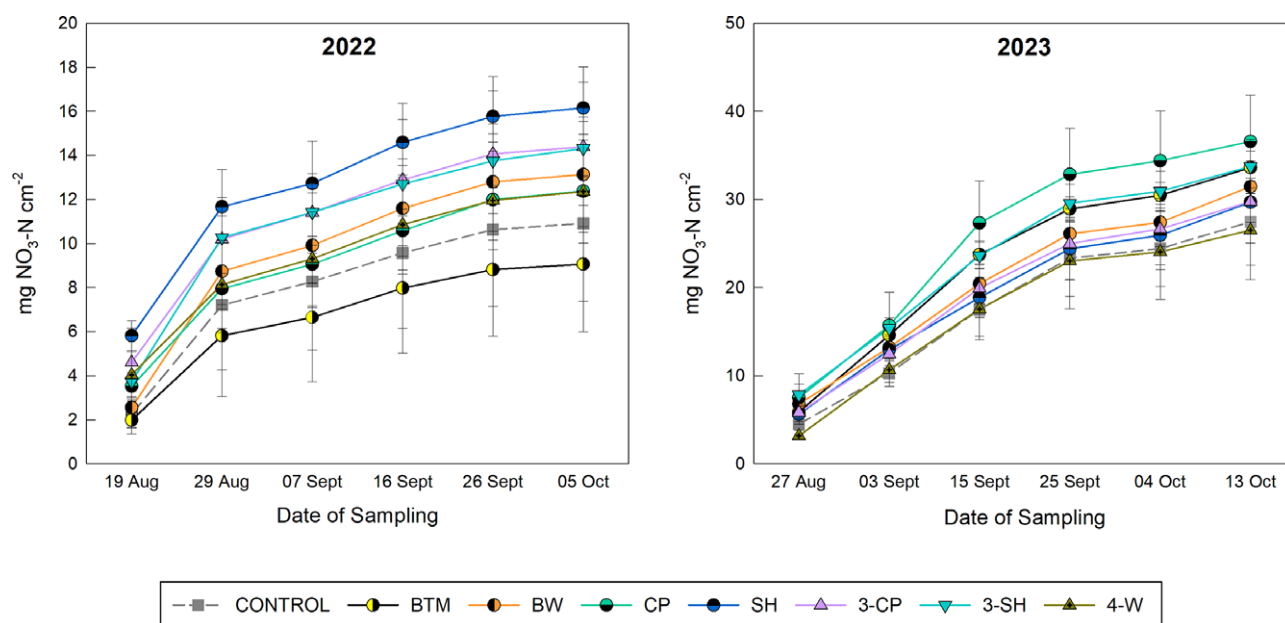


Figure 5. Cumulative soil $\text{NO}_3\text{-N}$ measured by anion exchange membranes (AEMs) during cabbage growth following cover crop treatment termination in 2022 and 2023. Monocultures shown with circle (●) and mixtures shown with triangle (▲).

suppressed weeds to a similar degree as in a monoculture when seeded at 20% of their monoculture rates in mixtures. A 20% of full rate seeding rate of competitive grass species can, therefore, serve as a preliminary threshold for those developing mixtures for weed suppression. Future research should investigate seeding rate thresholds for cover crop mixtures, providing guidance to growers looking to develop purposeful mixtures which maintain the functions of multiple species.

Performance of browntop millet, buckwheat, cowpea, and sunnhemp in mixtures

Buckwheat biomass production was very similar to the seeding rate in all mixtures, seeming to compete with browntop millet, but not suppress legumes. Others have found buckwheat to perform similarly, increasing evenness in mixtures, especially those containing sorghum sudangrass (McKenzie-Gopsill et al., 2022). This provides support for the inclusion of buckwheat in summer cover crop mixtures to maintain the production of other species in the mix and temper aggressive grass species.

Our study showed browntop millet exerted strong dominance in mixtures, making up the majority of biomass in 3-CP and 4-W, as well as a proportion of biomass far exceeding its 20% of full seeding rate in 3-SH. Many researchers have also found highly productive grass species to exert strong interspecific competition in mixtures, leading to legume suppression. For example, McKenzie-Gopsill et al. (2022) found that when sorghum sudangrass, a similarly highly productive warm-season grass to browntop millet, was included in a mixture, the evenness of productivity of the various species in the mixture decreased. While interspecific competition is a challenge, the high productivity of browntop millet can increase overall biomass production and provide important functions in a mixture, such as weed suppression.

Cowpea was suppressed to a much greater degree than sunnhemp in mixtures. Contrastingly, other researchers have found cowpea to be highly competitive (Creamer and Baldwin, 2020), including making up 93% of total biomass in a biculture with

cowpea and foxtail millet when cowpea was seeded at 73 kg ha^{-1} (O'Connell et al., 2015). Allar and Maltais-Landy (2022) found cowpea, seeded at 95 kg ha^{-1} , represented on average 28% of total biomass in a 5-way mixture including sunnhemp, sorghum sudangrass, buckwheat, and sunflowers. In our study, cowpea was seeded at 50 kg ha^{-1} in 3-CP but made up only 13% of biomass. Seeding rate can play a large role in the productivity of each species in a mixture and adjusting rate is often the main way to manage interspecific competition (Bybee-Finley et al., 2022). The seeding rate used in our study was not sufficient to allow cowpea to balance the aggressiveness of browntop millet. If used in a mixture with browntop millet or other grass species, cowpea seeding rates should be maintained near the full seeding rate, or above 50 kg ha^{-1} .

Sunnhemp has an upright growth habit, in contrast with the bush quality of cowpea. Morphology is an important aspect of complementarity, leading to increased resource capture and suitability in a mixture (Chapagain et al., 2020; Finney et al., 2016). Cherr et al. (2006b) found that sunnhemp intercepted more photosynthetically active radiation (PAR) than the other legume cover crop species, leading to greater biomass production. Allar and Maltais-Landy (2022) used sunnhemp in summer cover crop mixtures in Florida, finding it dominated a biculture with sorghum sudangrass. Our results indicate that sunnhemp was competitive with browntop millet, making up 55% of 3-SH biomass, while browntop millet accounted for 41% in 2022. Additionally, sunnhemp has a low C:N ratio and high biomass production, supporting N contributions and weed suppression in cover crop mixtures. Sunnhemp can be characterized as a highly productive, competitive legume which performs well in mixtures, but attention to substitutive or reduced seeding rates is advised.

Cover crop performance in mixtures is also dependent on environmental factors. Higher levels of soil inorganic N have been found to favor the productivity of grasses and nonlegume broadleaves in a mixture, while legumes dominate when there are low levels of soil inorganic N (Baraibar et al., 2020; Allar and Maltais-Landy, 2022). Legacy N mineralization from terminated red clover increased cumulative $\text{NO}_3\text{-N}$ mineralization by 84% in the second

year. This may have contributed to the decrease in the proportion of cowpea and sunnhemp in the second year compared with the first, by 7% and 21%, respectively, and increases in proportions of browntop millet and buckwheat. Similarly, in a 5-way mixture of summer cover crop species, Allar and Maltais-Landry (2022) found that sunnhemp accounted for 53% of total biomass and sorghum sudangrass account for 8% in one year but, when soil inorganic N was 50% higher in the second year, sunnhemp made up only 11% of total biomass and sorghum sudangrass accounted for 67%.

Cover crop C:N ratios and tissue nutrient concentrations

The C:N ratios of the cover crop monoculture treatments met expectations and were consistent with previous work (Bilenky et al., 2022), showing the grass monoculture, BTM, to have the highest C:N ratio, and the legume monocultures, CP and SH, to have a low C:N ratio, <20:1. The seeding rate proportions used in the three-way mixtures, 60% of the legume full rate and 20% of the full rate of both browntop millet and buckwheat, achieved the target of a C:N ratio $\leq 30:1$. Additionally, orthogonal contrasts revealed the C:N ratio of mixtures to be greater than monocultures. This can be partly explained by the high proportion (58% on average) of browntop millet, the species with the largest C:N ratio out of those evaluated, in the mixtures.

All cover crop treatments were terminated at 41 DAS to avoid buckwheat, the fastest maturing species evaluated, from setting seed. This prevented the other species from reaching the optimal growth stage for cover crop termination, potentially limiting ecosystem service provisioning by reducing productivity and impacting tissue quality (Clark, 2012; Balkcom et al., 2016). Often leguminous cover crops are terminated at flowering, when N fixation peaks (Clark, 2012), occurring at approximately 60 DAS for cowpea and sunnhemp. Browntop millet, like other grass cover crop species, should be terminated prior to setting seed, occurring 60 DAS depending on environmental conditions (Sheahan, 2014). With a more extended growth period, these three species would likely have produced greater amounts of biomass and had larger C:N ratios. For example, Wauters et al. (2021) found sunnhemp biomass production increased by, on average, 3.0 Mg ha⁻¹ and the C:N ratio increased by 41% when terminated 50 DAS instead of 30 DAS. Increased productivity offers an opportunity to increase legume N contribution and weed suppression but must be balanced with biomass C:N ratio to prevent N immobilization following termination. Variability in maturation rate shows the challenge in developing a mixture which can provide reliable productivity among species (Baraibar et al., 2020) and ensure provisioning of targeted ecosystem services.

Certain cover crops can scavenge nutrients better than cash crops, and when the residue is incorporated, supply a labile form of the nutrient for subsequent crop uptake (Fageria et al., 2005). In our study, cowpea produced high-quality tissue with superior micro- and macronutrient concentrations, including the highest % N, % K, % S, % Ca, Mg (ppm), Zn (ppm), Mn (ppm), and B (ppm) among monocultures and increasing mixture concentrations in 3-CP and 4-W. Limited research has documented the impact of nutrients from incorporated cover crop residue on vegetable crop uptake. Nutrient release from organic forms in cover crop tissue is a complex, biologically driven process dependent on soil pH, moisture, temperature, soil type (Prescott, 2005), and the proportion of other nutrients in the tissue and soil environment (Vardaka et al., 1997). Although tissue degradation was not a primary focus of this research, we identified increased % P and % Ca in cabbage following

CP and BW in 2022, correlating with increased cover crop tissue concentrations. Buckwheat is known to scavenge for P, releasing organic acids in the soil to increase availability for uptake (Clark, 2012). Furthermore, buckwheat and cowpea support symbiotic relationships with arbuscular mycorrhizal fungi (AMF) which enhance P uptake (Boglaienko et al., 2014; Johnson et al., 2016), although sunnhemp, and many other non-brassica cover crop species, also strongly support AMF colonization (Soti et al., 2023). Further research should explore the function of cover crops to supply labile forms of nutrients for crop uptake following degradation, and consider the inclusion of cowpea in cover crop mixtures to support nutrient contributions to cash crops.

It is important to note that weeds accounted for a large proportion of biomass in all cover crop treatment plots and were not analyzed for tissue nutrient content. Weed residue quality, such as C:N ratio and lignin content, impacts nutrient contributions to cash crops and can play a large role in nutrient mineralization in the soil (Promsakha Na Sakonnakhon et al., 2006). Therefore, without accounting for the weed tissue nutrient content, interpretations of a correlation between cover crop tissue and cabbage head nutrient content are incomplete.

Decreased N contribution and cabbage yield with high browntop millet biomass

There is evidence of N immobilization from BTM in 2022. BTM biomass produced 38 kg N ha⁻¹ but had a high C:N ratio of 49:1. Cumulative NO₃-N mineralization was numerically lowest in BTM plots and more than 50% of cabbage heads in BTM plots were classified as small (Fig. 2). On the other hand, in 2022, BW biomass had a C:N ratio of 36:1 and similar cabbage yield to legume monocultures. This supports other findings suggesting N mineralization occurs from cover crops with a C:N ratio $\leq 40:1$ (O'Connell et al., 2015).

Two of the evaluated mixtures, 3-SH and 4-W, had a similar cabbage yield to the legume monocultures (CP and SH) in 2022, but 3-CP experienced a lower cabbage yield than CP and SH. Strong interspecific competition from browntop millet in 3-CP resulted in cowpea making up only 17% of 3-CP biomass in 2022, limiting the amount of N produced by the mixture to 53 kg N ha⁻¹, in contrast to 76 kg N ha⁻¹ in 3-SH and 64 kg N ha⁻¹ in 4-W. Additionally, 3-CP biomass had a slightly higher C:N ratio (26.2), compared with 21.3 in 3-SH and 22.8 in 4-W. Although no differences in cumulative NO₃-N were found between the treatments, the combination of a smaller supply and slightly increased C:N ratio may have slowed N mineralization for cabbage uptake and lowered marketable yield in 3-CP. Additionally, the substantial amount of weed biomass in incorporated residue across treatments may have significantly altered plot biomass C:N ratios, influencing cumulative NO₃-N mineralization and potential cover crop treatment effects.

Limited impact on microbial biomass and labile carbon from mixtures

Significantly higher MBC following cover crop mixtures than monocultures in 2022 gives partial evidence of an increase in microbial populations, and infers mixtures may offer a more beneficial habitat for soil microorganisms. Similarly, using permanganate oxidizable C (POXC) as a measure of labile carbon and an indicator of microbial food supply, we revealed a marginal increase following mixtures compared to monocultures in the first year, 7.5%. This may be associated with an increase in types of C sources

from the biomass of the additional species in the mixtures and may have contributed to the higher MBC in mixtures in the same year. Although, no differences in POXC or MBC between mixtures and monocultures were found in 2023. Measures of soil labile C and microbial communities in field conditions are influenced by a complex of edaphic, management, and climatic factors (Finney et al., 2016; De Souza et al., 2023) and may not reveal changes after only one year (Bielenberg et al., 2023). Additionally, greater cover crop biomass is generally associated with greater soil organic C, including labile forms, such as POXC (Ghimire et al., 2019), but we found no statistical differences between treatments, and no correlation between total biomass, kg C·ha⁻¹, or C:N ratio with POXC. Similar to the other evaluated ecosystem services, the large proportion of weed biomass within cover crop plots may have largely influenced POXC and the lack of correlations with other metrics.

The timing of MBC and POXC sampling may have also limited our ability to adequately measure the cover crop treatment effects on the microbial community. For example, some researchers have found increases in microbial communities and diversity in plots with cover crop mixtures during cover crop growth (Finney et al., 2016), or following termination (Chavarría et al., 2016), but we sampled at the end of the experiment, following the cabbage harvest. Other research has found cover crops mixtures did not influence microbial communities (De Souza et al., 2023) nor promote soil biology better than monocultures (Florence and McGuire, 2020). Additional methods of measuring microbial communities, such as 16S rRNA sequencing, may better reveal the impact cover crop mixtures have on the soil microbiome.

Conclusion

Although cover crop mixtures may not exceed the performance of monocultures in the provisioning of individual ecosystem services, the maintenance of multiple functions from the varying species in a mixture may allow for multifunctionality. Our study evaluated cover crop mixture and monoculture provisioning of four ecosystem services: weed suppression, N contribution, enhanced crop yield, and habitat for soil microbes in an organic vegetable production system. Results indicate that mixtures were able to bring high weed suppression, although the inclusion of browntop millet and overall productivity were more important factors than number of species in the mixture. Cumulative NO₃-N supplied by the treatments was similar, although high weed populations in cover crop plots may have masked cover crop treatment differences. Mixtures provided similar cabbage yields as legume and buckwheat monocultures, but only when interspecific competition of a highly productive, high C:N ratio grass species (browntop millet) did not suppress other species in the mixture, as occurred in 3-CP. Microbial biomass C was increased following cover crop mixtures in one year and further investigation of this service is warranted. Therefore, we can conclude that cover crop mixtures achieved partial multifunctionality, especially if measured as the maintenance of distinct species functions and prevention of tradeoffs, rather than an ability to exceed monoculture functions. In all, cover crop mixtures may increase microbial biomass, provide better weed suppression than legume monocultures, and maintain similar N contributions and cabbage yields when an appropriately balanced mixture is achieved. Additional species compositions of cover crop mixtures should be explored under varying growing conditions, addressing questions of threshold seeding rates and the impact of environmental conditions on cover crop performance. Substantial weed growth in cover crop plots and termination

timing of cover crops played a role in our results and should be taken into account in future studies. As more growers employ cover crop mixtures to meet ecosystem service and crop rotation goals, research which addresses practical questions will best serve these communities and advance our understanding of cover crop mixture limitations and potential.

Supplementary material. To view supplementary material for this article, please visit <http://doi.org/10.1017/S1742170525000031>.

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Competing interest. The authors declare none.

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