University of Michigan

Master's Thesis

Effects of staggered planting on cover crop mixture evenness and multifunctionality

by
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1.0 INTRODUCTION

Cover crops, or non-harvested crops planted in windows between primary crops in rotations, can increase on-farm diversity, ecosystem functioning, and soil health, especially when planted in mixtures containing diverse plant functional groups with complementary traits (Blesh, 2018; Finney & Kaye, 2017; Isbell et al., 2017; Schipanski et al., 2014; Snapp et al., 2005; Storkey et al., 2015; Weidlich et al., 2017). Plants of the same functional group share functional traits, or characteristics that determine how they affect the surrounding environment (Díaz & Cabido, 2001). For example, brassicas have large tap roots that can reduce soil compaction and allelochemicals that reduce pest pressure; grasses have fibrous roots that are adept at retaining nutrients; and legumes can fix nitrogen (N) gas from the atmosphere through biological N fixation (BNF) (CTIC, SARE & ASTA, 2020; Jacobs, 2012; Snapp et al., 2005) and have low carbon-to-nitrogen ratio (C:N) litter, both of which increase N availability in the soil (Blesh, 2018; Schipanski et al., 2012). The varying impacts of plant species on ecosystem processes both across and within functional groups can be predicted using their functional traits, such as specific leaf area (SLA), height, leaf %N, and C:N (Garnier & Navas, 2012; Wood et al, 2015).

Cover crop mixtures that include species with diverse sets of functional traits can increase the provisioning of multiple ecosystem functions (i.e. multifunctionality) (Blesh, 2018; Davis et al. 2012; Finney et al. 2017; Hector & Bagchi, 2007). For example, diverse agroecosystems tend to be more resilient to extreme weather events and pest outbreaks, especially when functions such as improved N supply, water and nutrient retention, and weed management allow for reduced use of external inputs (Finney & Kaye, 2017; Kremen & Miles, 2012; Snapp et al. 2005). Yet, limited diversity-associated increases in multifunctionality may be achieved if one functional group outcompetes another, because abundance is a key predictor of contributions of species to the functioning of ecosystems (Grime, 2002). Ensuring that cover crop species representing distinct functional groups have adequate representation within a mixture poses a challenge given that growth rates and competitive potential vary across species such that some functional groups tend to consistently competitively exclude others.

While competitive exclusion presents a challenge to building and maintaining diverse cover crop species mixtures, priority effects present a possible solution. Priority effects refer to the variation in effects that a species has on its environment resulting from its order of establishment within a community. The order of establishment of species within a community can influence the overall species richness, composition, and biomass of the community, which in turn can affect ecosystem functioning (Fukami, 2015). Priority effects are caused by two mechanisms: niche preemption and niche modification. Niche preemption occurs when two species require the same resources and an early-arriving species reduces the resources available to a later-arriving species, thus influencing subsequent community composition and function (Fukami, 2015). Niche modification occurs when an early-arriving species enhances or degrades the resources required by later-arriving species, thereby affecting their establishment, which also influences subsequent community composition and function (Fukami, 2015).

While niche preemption may be expected to consistently reduce diversity in cover crop mixtures, niche modification could either increase or decrease the diversity of cover crop communities. For example, beneficial niche modification may occur when an early-establishing legume supplies N to soil, which could benefit later-establishing species and increase the productivity of the community, especially within the first growing season as found by Weidlich et al. (2017). In contrast, early-establishing grasses may reduce the growth of later-establishing species by taking up space and resources with dense roots. In agroecosystems, priority effects can potentially be managed to support evenness of cover crop mixtures by staggering the planting of each species to control order of establishment.

Biotic interactions, and their impacts on co-existence and subsequently the diversity of a community, are influenced by abiotic factors. Soil fertility is particularly important in influencing species diversity and evenness in plant mixtures, as high-fertility soils tend to result in lower species richness due to strong competitors taking advantage of the abundance of nutrients (i.e., selection effect) (Buckland & Grime, 2000; Ejrnæs et al., 2006; Kardol et al., 2013; Weidlich et al., 2017). These impacts of fertility may be sensitive to the order of arrival of the different species into the community. In a microcosm experiment, increasing the time between each species' establishment resulted in stronger priority effects that led to greater differences in community composition, and this effect was more pronounced in more fertile soil (Kardol et al., 2013). Conversely, in grasslands, there was no effect of soil fertility on the impact of order of arrival of distinct functional groups on the resulting diversity of the system (Weidlich et al., 2017). To our knowledge, no studies have tested whether priority effects impact evenness in cover crop mixtures and subsequent multifunctionality in agroecosystems, and how these effects are influenced by soil fertility levels.

To understand how mixture evenness and ecosystem functions provided by cover crop mixtures are impacted by priority effects and soil fertility, we established five planting treatments in a field experiment using one brassica, one grass, and two legume species, which were planted in low- and high-soil fertility treatments in a fully factorial design. We measured the following ecosystem functions to determine multifunctionality: above- and belowground N retention, carbon (C) and N mineralization, BNF, and weed suppression. We also measured four plant functional traits: maximum plant height, specific leaf area, leaf %N, and shoot C:N.

We hypothesized that the legume-first, low soil fertility treatment would lead to the highest mixture evenness, given that prior research has found high fertility treatments to become dominated by the strongest competitor. Although legumes may have a competitive advantage in low-fertility soils due to their ability to host N-fixing bacteria, we did not expect this advantage to supersede the relatively stronger competitive abilities of grasses and brassicas when co-planted. The earlier planting date for legumes may improve their establishment by reducing competition, and may also benefit subsequent species by supplying N through BNF. Second, we hypothesized more even mixtures to have higher levels of multifunctionality given that there would be adequate representation of all three functional groups. And, third, we expected to find significant differences in plant functional trait expression for each species between the staggered planting and soil fertility treatments. For instance, we expected to find higher leaf %N and lower C:N in the legume-first treatments due to legumes' ability to fix N, perhaps facilitating other species' abilities to acquire N.

2.0 METHODS

2.1 Experimental Design

The cover crop mixture in the experiment included four species from three functional groups – brassicas, grasses, and legumes. Oilseed radish (*Raphaneus sativus [L.] var. oleiferus*) represented the brassica, and the grass was represented by oats (*Avena sativa*). We selected two legumes, field pea (*Pisum sativum*) and balansa clover (*Trifolium michelianum*). These four species have similar phenologies and are recommended species to plant in temperate cropping systems (Johnny's Selected Seeds, 2022; Midwest Cover Crops Council, 2011).

The experiment was conducted at the University of Michigan Campus Farm in Ann Arbor, Michigan, USA (42.29759N, 83.66649W). We established plots in a 0.05 ha section of a field with low fertility status based on an initial soil characterization (Section 2.2), which provided a low baseline fertility level, permitting us to create a contrasting higher fertility treatment by amending soil with compost. The field had been in agricultural production for five years, with addition of pelletized, composted chicken manure the previous year, and contained watermelons, sweet corn, and broccoli. Marginal amounts of crop residue were present in blocks 1, 2, 3, and 5, with watermelon vines in blocks 1-3 and broccoli stalks in block 5 (Figure 1). Block 4 contained more substantial amounts of crop residue, with approximately five rows of dried corn stalks. There is a slight slope across the field, averaging a grade of 5.4%. We therefore placed our experimental blocks across the topographic gradient.

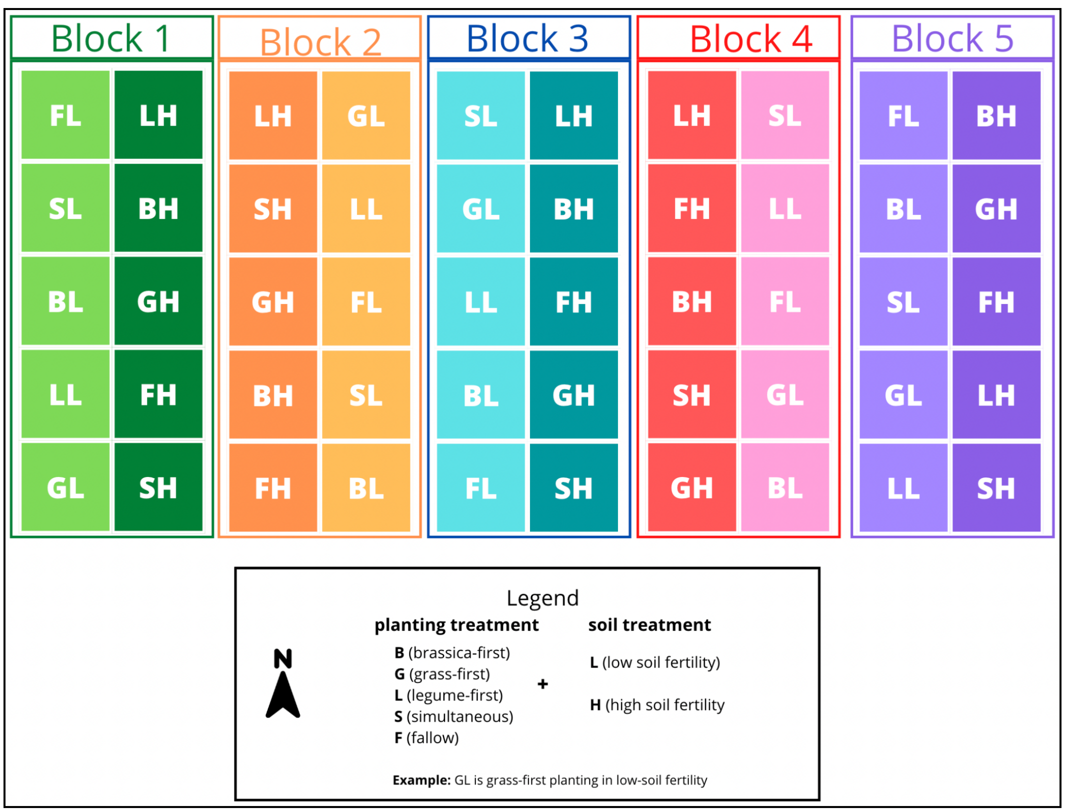

Figure 1: Experimental design showing the individual plot layout for five planting treatments across two levels of soil fertility, with five replicate blocks

The experiment was planted in a fully factorial, randomized, split-block design with five replicates, with each block split to contain one strip each of the high and low soil fertility treatment. Each treatment plot was 9 m^2 (Figure 1). Treatments included planting brassicas first (B), grasses first (G), and both legumes first (L); in each treatment the the two remaining functional groups were planted 18 days after the first group, to test for priority effects based on order of planting and establishment. Treatment S had all functional groups planted simultaneously and treatment F was a weedy fallow control.

2.2 Baseline Soil Sampling and Analysis

On April 12, 2022, we collected six, 5 cm diameter soil cores to 20 cm depth from each block. Cores were homogenized and a subsample of soil was sieved to 2 mm for analysis of inorganic N (NH_4^+ and NO_3^-) and potentially mineralizable N (PMN) using 2 M KCl extraction and a seven-day anaerobic incubation (Drinkwater et al. 1997). Extracts were frozen and later analyzed colorimetrically for inorganic N on a discrete analyzer (*AQ2; Seal Analytical, Mequon, WI).* Bulk density was measured by collecting three random cores of 4.8 cm diameter to 5 cm depth from each block, drying each core at 60 ºC for forty-eight hours, and weighing each core. A subsample of soil was also sent to A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN) for a standard soil test including macro- and micro-nutrients, soil texture, and pH. Baseline soil samples indicated consistent pH, organic matter, texture, and nutrient levels across all five blocks. The soil was slightly alkaline with a mean pH of 7.38 and 1.92 % organic matter. The bulk density was an average of 1.49 g/cm^3. The soil texture was classified as a sandy loam and had an average cation exchange capacity of 6.52. The soil contained an average of 17.8 ppm P, 70.7 ppm K, 131 ppm Mg, and 1,050 ppm Ca.

2.3 Site Preparation

Overwintering crop residue was incorporated with shallow tillage (5 cm depth) immediately following baseline soil sampling on April 12. On April 29, the high-fertility treatment plots were amended with RevitaPro 3-4-3, an organic granular fertilizer (composted chicken manure) at a rate of 890 kg/ha (Ohio Earth Food). Both the low and high fertility plots were then tilled again to 5 cm depth to incorporate the compost into the high-fertility plot and to create the same level of soil disturbance in the low-fertility plot.

2.4 Planting

We calculated seeding rates for each species in the mixture by dividing monoculture rates by four (Table 1). Immediately following compost application and tillage, seeds were broadcast for the first cover crop planting event on April 29. The plots were monitored until the plants reached sufficient emergence (the first true-leaves have appeared on the dicots and the oats, and monocots were at least 5 cm tall). Eighteen days later, on May 17, we broadcast-seeded the remaining functional groups into treatments B, G, and L.

Table 1: Seeding rates

Species	Seeding Rate (kg/ha)
Balansa Clover (*T. michelianum*)	2.4 kg/ha
Field Pea *(P. sativum*)	12.2 kg/ha
Oats (*A. sativa*)	16.3 kg/ha
Oilseed Radish (*R. sativus* [L.] var. *oleiferus*)	4.1 kg/ha

2.5 Plant Sampling & Analysis

On June 5 and 6, aboveground biomass was collected from all plots, including fallow plots, by placing a 0.25 m^2 quadrat over a representative area of cover crops within each plot and clipping aboveground biomass at soil surface level and sorting plants by species, with weeds grouped into one category. The biomass was then dried for 48 hours at 60 ºC before being weighed and ground to 2 mm in a Wiley Mill for analysis of C and N using dry combustion with a Leco TruMac CN Analyser. C and N concentrations were multiplied to biomass dry weight to determine aboveground C and N inputs, then divided (C/N) to determine shoot C:N.

To estimate belowground biomass and root:shoot ratio in each treatment, we collected one root core per cover crop species in each plot for a total of four cores per plot. The core was 8 cm diameter and we sampled directly over each target species to a depth of 20 cm. The cores were combined into a composite sample. In the event a particular species could not be found, a duplicate core of another species was taken to maintain even volume, with peas and clover standing in for the other since they are both legumes, and oats and radishes standing in for the other since they are both non-legumes. Soil was washed from roots by placing the composite sample in water, agitating the soil to free all roots, then sieving the root slurry through 2 mm, then 0.5 mm sieves to extract the roots from the water. Roots collected in the 2 mm sieve were picked through for ten minutes to remove any non-root debris, while a subset of roots collected in the 0.5 mm sieve from each treatment were picked through for one hour to remove non-root debris. The difference in pre- and post- cleaning weights for the 0.5 mm roots were used to calculate a correction factor to apply to remaining fine root samples for each treatment.

Following washing, roots were dried at 60 ºC for a minimum of 48 hrs before being weighed and ground into a fine powder. Each sample was analyzed for C and N concentration on the Leco TruMac CN Analyser; a subsample of roots was also ashed to correct for any soil material that might have contaminated the sample. C and N concentrations were multiplied by root dry weight to determine belowground C and N inputs, and then divided (C/N) to determine belowground C:N. The weight of total belowground biomass (roots) was divided by total aboveground biomass excluding weeds (shoots) to determine community-level root:shoot. The treatments in which a replacement species was sampled also had root C:N calculated at the community level, but the cores from the replacement species were not used to calculate root:shoot given the mass difference between radish and oat roots.

We sampled species within each treatment for functional traits (height, leaf %N, shoot C:N, root:shoot ratio, and specific leaf area (SLA)) when the majority of plants were reaching reproductive maturity. The heights and leaves for SLA and leaf %N were collected for the first-planting oilseed radishes on June 13, forty-five days after the first planting, given that they had reached reproductive maturity earlier than the other species. The locations of the oilseed radishes sampled on June 13 were marked and avoided during later aboveground biomass collection.

Sixty-seven days following the first planting (forty-nine days following the second planting), on July 5 and 6, we sampled the remaining species for maximum plant height and SLA. Maximum plant height was measured for three representative individuals of each species in each plot. We measured SLA as the ratio of leaf surface area to dry weight with leaves collected from three representative plants of each species. We calculated leaf area using the software ImageJ (Schneider et al., 2012), and then dried the leaves at 40 ºC for a minimum of 48 hours, at which point they were weighed and ground into fine powder before being analyzed for leaf %N using dry combustion with the LECO TruMac CN Analyser (Leco Corporation, St. Joseph, MI). Fallow plots and weeds were not sampled for plant functional traits or roots. Following field sampling, cover crops were mowed and tilled into the soil, making sure to maintain plot boundaries.

2.6 Ecosystem Functions

To estimate total aboveground N, we multiplied aboveground biomass for each species by their respective N concentrations and summed the N contributions from each species. Similarly, for total belowground N, we multiplied root belowground biomass for each size class (0.5-2 mm and >2 mm) by their respective N concentrations and summed their N contributions. In order to quantify N inputs from legume N fixation, we used literature values to estimate the % of aboveground N derived from fixation for each legume species. Specifically, we multiplied the estimate of percent of N derived from air (Ndfa) by total legume N (in kg ha^{-1}) to estimate BNF. Ovalle et al (2006) found balansa clover to derive 92.6% of its N from air when in granitic, sandy soils (similar to our site) and Schipanski & Drinkwater (2012) found that N derived from air for fields peas was between 56-86%; we used the average of those percentages (71%).

Weed suppression was calculated as:

$$\%\text{Weed Suppression} = \frac{(\text{Fallow Weed Biomass - Treatment Weed Biomass})}{(\text{Fallow Weed Biomass})} \times 100$$

We used an aerobic incubation to measure C and N mineralization rates in each treatment following cover crop termination. Two weeks following cover crop incorporation, we collected soil from each plot. Within each plot, three 5 cm diameter soil cores were collected to 20 cm depth and composited before a subsample was sieved to 4 mm. A subsample of soil was immediately analyzed for extractable inorganic N (as described in Section 2.2) to set a baseline, while another subsample was run through a fourteen-day aerobic incubation, modifying methods outlined in Drinkwater et al (1996). Briefly, 30g of field-moist soil from each plot was placed in a quart-sized jar. Bulk density was estimated for each sample, which was then used to determine the amount of water to add to each sample to bring it to 60% water-filled pore space (WFPS) following a 24-hour degassing period. The jars were then tightly capped with lids with rubber septa, and set in a dark area at 20ºC for 14 days. After 14 days, two air samples were extracted from each jar with a syringe and injected into a LiCor-820 to be analyzed for CO_2 content, which was compared to baseline CO_2 content collected at the beginning of the aerobic incubation. The soil in the jars was then sieved to 2 mm and analyzed for inorganic N in triplicate using 2 M KCl extraction. Pre-incubation baseline CO_2 and N measurements were subtracted from post-incubation measurements, then divided by fourteen days to calculate C and N mineralization rate per day.

2.7 Mixture Evenness & Multifunctionality

Mixture evenness was calculated using the Shannon Diversity Index and then dividing those values by H'_{max}, which is ln(4) since there are four species in the mixture, excluding weeds.

Shannon's Diversity Index: $H' = - \Sigma p_i * \ln(p_i)$

Evenness = H' / H'_{max}

where p_i is the proportion of aboveground biomass of species i within the mixture.

To calculate multifunctionality, we used the threshold approach outlined in Byrnes et al. (2013). First, we defined the maximum potential level of each function as the mean of the top 10% of values. This was to reduce the likelihood of the maximum potential level being set by an outlier. We then calculated the percentage of the maximum that each treatment achieved. Multifunctionality for each treatment was then calculated as the average % function across all functions.

2.8 Statistical Analyses

Statistical analyses were conducted in R (The R Foundation for Statistical Consulting, Vienna, Austria, v3.5.1). We used two-way ANOVA models using the lmer function with planting and soil treatments as fixed effects and block as a random effect. If planting or soil treatments indicated significance at $\alpha \leq 0.05$, we then performed a post-hoc Tukey HSD test using the glht function to determine which treatments had significant differences. If the planting and soil interaction was significant, we used emmeans pairwise comparisons.

3. RESULTS

Overall, we found no significant effect of soil fertility on any of the measured response variables, except for one trait; maximum plant height for radish and clover (Section 3.4).

3.1 Above- and belowground biomass

Total aboveground biomass was twice as great in both the simultaneous and grass-first treatments compared to the brassica-first treatment ($p = 0.01$ and $p = 0.04$, respectively) and 4.8 times larger than the legume-first treatment ($p < 0.001$ and $p < 0.01$, respectively; Figure 2). Overall, biomass for each species was greatest in the treatments in which they were planted first. Oats dominated the grass-first and simultaneous treatments, while radish and the two legume species comprised the majority of aboveground biomass in the respective treatments in which they were planted first. Total belowground biomass was 1.5 times higher in the simultaneous planting treatment compared to the legume-first treatment ($p < 0.01$; Figure 2).

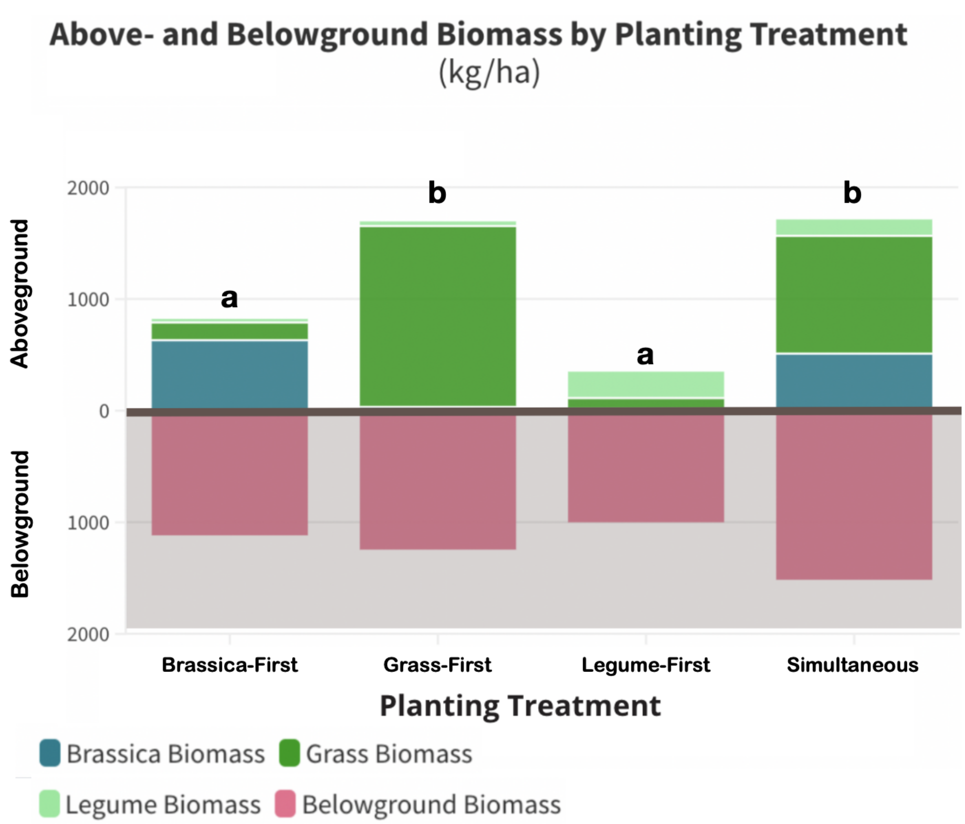

Figure 2: Above- and belowground biomass by planting treatment (kg ha^{-1}). Aboveground biomass is shown at the species-level,while belowground biomass is shown at the treatment-level.

3.2 Mixture evenness

Mixture evenness was significantly lower in the grass-first planting treatment compared to the brassica-first ($p = 0.04$), legume-first ($p = 0.02$), and simultaneous ($p = <0.001$) planting treatments. There were no significant differences in evenness between the brassica-first, legume-first, or simultaneous treatments (Table 2).

Table 2: Mixture evenness values by planting treatment (Values closer to 1 are more even)

Treatment	Index Value	Tukey Letters
B	0.46	a
G	0.22	b
L	0.49	a
S	0.56	a

3.3 Ecosystem functions & multifunctionality

Differences in ecosystem functions across treatments suggest there are some tradeoffs associated with planting treatment, but not soil fertility treatment. N supply from BNF, for example, was nearly seven- and five-fold higher in the legume-first planting treatment compared to the brassica-first ($p < 0.01$) and grass-first planting treatments ($p = 0.01$), respectively. Total aboveground N retention was significantly greater in the simultaneous treatment compared to the brassica-first and legume-first planting treatments ($p = 0.02$). Similarly, total belowground N retention was also significantly greater in the simultaneous treatment compared to the brassica-first planting treatment ($p < 0.01$), but unlike total aboveground N, belowground N in the simultaneous treatment was greater than the grass-first planting treatment ($p = 0.02$) rather than the legume-first treatment. Weed suppression was also 56% greater in the simultaneous, and 50% greater in the grass-first planting treatments compared to the legume-first treatment ($p = 0.001$). The only area where we did not observe trade-offs in ecosystem functioning was for C and N mineralization rates, which were similar across all treatments. Mean values and standard errors for each measured function are in the appendix (Table A1).

Table 3: Ecosystem functions across treatments. Values represent the percent of the maximum function level achieved by each planting treatment, with multifunctionality calculated as the average of the six functions for each treatment.

		Treatment			
		B	G	L	S
Function	Aboveground BNF	7.39	10.44	51.29	29.51
	C Mineralization	66.96	70.23	71.66	71.32
	N Mineralization	53.18	51.18	54.14	53.15
	Total Aboveground N	28.79	47.75	27.88	57.21
	Total Belowground N	53.82	55.87	59.26	77.59
	Weed Suppression	32.72	54.55	4.41	60.44
	Multifunctionality	34.69	41.42	38.38	49.89

Overall, multifunctionality was highest in the simultaneous planting treatment, but only statistically significant compared to the brassica-first planting treatment ($p = 0.01$). Figure 3 demonstrates the ecosystem benefits and trade-offs associated with each planting treatment.

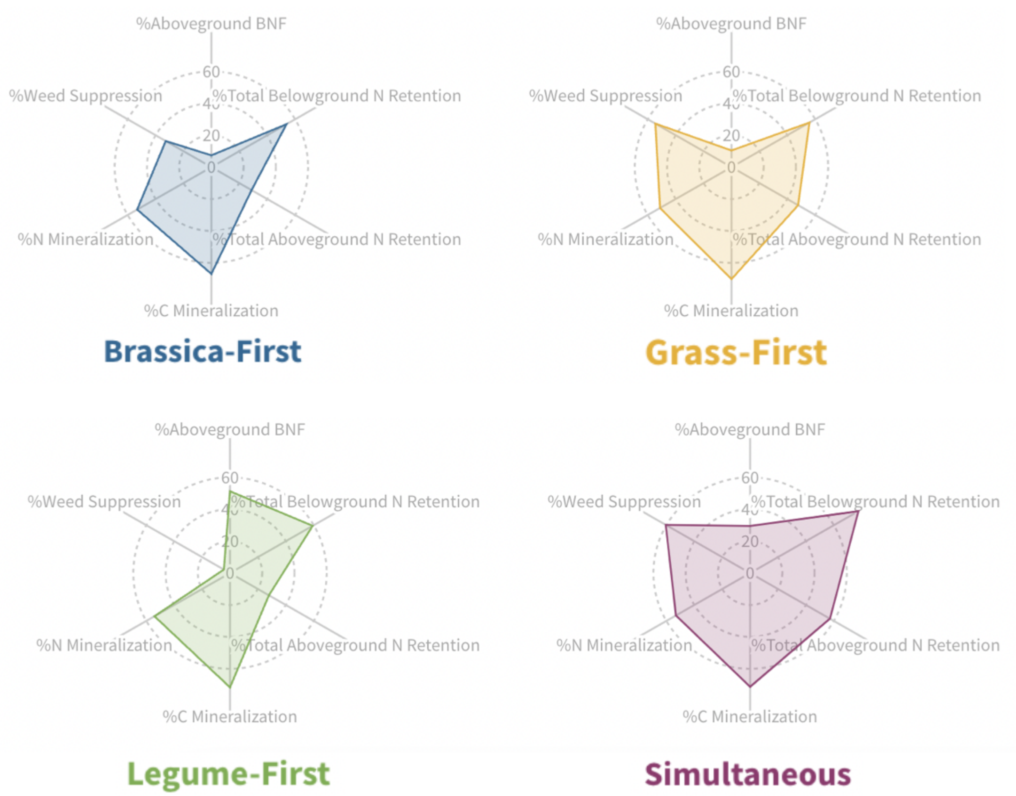

Figure 3: Radar charts for each planting treatment showing the percentage of the maximum level of six ecosystem functions achieved.

3.4 Plant functional traits

At the community level, root:shoot was greater in the legume-first treatment than the grass-first planting treatment (p = 0.02). Total aboveground and belowground C:N were significantly lower in the legume-first planting treatment compared to all other planting treatments (p = < 0.01) (Table 4). The grass-first planting treatment had a significantly higher aboveground C:N compared to the brassica-first (p = < 0.001) and simultaneous (p < 0.01) treatments.

Table 4: Community Root:Shoot, Aboveground C:N, and Belowground C:N

	Community Root:Shoot			Aboveground C:N			Belowground C:N		
Treatment	Mean	SE	Tukey Letters	Mean	SE	Tukey Letters	Mean	SE	Tukey Letters
B	1.44	0.20	ab	27.27	0.93	a	32.76	1.61	a
G	0.95	0.22	a	33.90	0.58	b	29.671	1.21	a
L	3.05	0.80	b	18.61	1.19	c	23.632	1.19	b
S	1.18	0.28	ab	29.17	1.22	a	29.92	1.35	a

At the species level, oat traits varied the most across treatments. Oat SLA, height, leaf %N, and shoot C:N were significantly different when comparing planting treatments B to G, B to S, G to L, and L to S, with high degrees of significance (Table 4).

Clover was taller in treatment B than in treatment S, but only in high soil fertility (p = 0.03). There was insufficient clover biomass to measure leaf %N, and there were no significant differences in clover SLA across treatments.

Field peas did not display any significant differences in C:N or leaf %N across treatments. However, field pea SLA was significantly greater in the grass-first planting treatment when compared to all other treatments. Field pea height was significantly taller in the simultaneous planting treatment compared to the grass-first planting treatment.

Although there were no significant differences in radish SLA across treatments, radish leaf %N was significantly greater in the legume-first planting treatment when compared to the simultaneous and brassica-first planting treatments. Radish leaf %N was also significantly greater in the grass-first planting treatment than the brassica-first planting treatment (p = 0.04).

Across planting treatments, radish height was greatest in the brassica-first and simultaneous treatments compared to the grass- and legume-first treatments. However, there was also a significant interaction between planting and soil treatments for radish height. Specifically, radishes in treatments B and S were significantly taller in high soil fertility than low (p = <0.01), but this trend did not hold true for treatments G and L.

Table 5: Functional trait means and standard errors for each species by planting treatment Different lowercase letters indicate significance at $p < 0.05$

		Treatment	C:N Mean (SE)	C:N Tukey Letters	Height Mean (SE)	Height Tukey Letters	Leaf %N Mean (SE)	Leaf %N Tukey Letters	SLA Mean (SE)	SLA Tukey Letters
Species	Clover	B	*Insufficient Biomass*	a	5.27 (0.81)	a	*Insufficient biomass to analyze clover leaf %N.*		525.89 (70.16)	a
		G	*Insufficient Biomass*	a	7.04 (0.81)	a			549.14 (93.81)	a
		L	18.29 (0.88)	a	7.48 (0.92)	a			511.22 (79.28)	a
		S	17.29 (3.23)	a	7.98 (1.04)	a			359.54 (27.78)	a
	Oat	B	26.89 (1.03)	a	49.32 (2.67)	a	2.82 (0.16)	a	239.56 (7.25)	a
		G	35.52 (0.51)	b	80.07 (1.64)	b	2.20 (0.08)	b	202.83 (5.58)	b
		L	26.26 (1.17)	a	54.20 (2.07)	a	2.94 (0.11)	a	246.54 (15.33)	a
		S	33.51 (1.00)	b	79.80 (1.02)	b	2.26 (0.10)	b	199.16 (4.08)	b
	Pea	B	17.12 (0.96)	a	28.87 (1.47)	ab	3.82 (0.18)	a	289.02 (13.96)	a
		G	15.66 (0.87)	a	27.77 (2.24)	a	3.87 (0.19)	a	383.42 (38.81)	b
		L	16.93 (0.40)	a	35.47 (2.79)	ab	3.62 (0.12)	a	248.62 (8.73)	a
		S	17.16 (0.78)	a	38.07 (3.52)	b	3.63 (0.25)	a	283.37 (17.75)	a
	Radish	B	28.46 (1.03)	a	65.10 (2.7)	a	2.42 (0.09)	a	227.39 (14.14)	a
		G	16.52 (1.24)	b	13.27 (1.01)	b	2.93 (0.15)	bc	310.11 (19.45)	a
		L	17.77 (3.01)*	b	11.68 (1.22)	b	3.07 (0.16)	c	313.48 (23.99)	a
		S	27.32 (1.40)	a	70.32 (3.47)	a	2.57 (0.13)	ab	271.76 (12.07)	a

**Data for radishes in treatment L includes low soil fertility plots only*

4.0 DISCUSSION

Our study tested the impacts of staggered planting and soil fertility on cover crop mixture evenness and multifunctionality, using priority effects as an ecological framework. By assessing plant functional trait variation across treatments, we also aimed to gain insights into the interactions and mechanisms driving cover crop outcomes. Although previous ecological research has tested the effects of staggered planting and soil fertility on plant community composition and function (Kardol et al., 2013; Weidlich et al., 2017), the priority effects lens has yet to be applied in an agroecological context. Hayden et al. (2015) began to bridge this gap with staggered planting of a rye-vetch cover crop mixture, but did not consider soil fertility and evaluated a relatively small suite of functions. The present work integrates all of these concepts to assess a potential strategy for improving cover crop performance and agroecosystem function.

4.1 Mixture Evenness

Adequate representation (e.g., biomass) of a functional group is necessary for that group to meaningfully perform its function in an ecosystem (e.g., Grime, 2002). In this study, we measured mixture evenness as an indicator of how evenly distributed the three functional groups were, and in turn, able to perform their respective functions. In general, there was relatively low mixture evenness across all the staggered planting treatments, while the simultaneous treatment achieved slightly higher evenness. Each functional group ended up dominating the treatment in which it was planted first, potentially reflecting either niche preemption, or simply that the later-planted species had less time to accumulate biomass. Since oats dominated both the grass-first and simultaneous treatments, this suggests grasses will outperform other functional groups regardless of planting order, which is unsurprising given the vigorous competitive ability of grasses (Helenius & Jokinen, 1994; Wang et al., 2022).

Moreover, our hypothesis that the legume-first, low-soil fertility treatment would be the most even due to beneficial niche modification by the legumes was not supported. This may be because the legumes had relatively poor growth, even when planted first. Given that the amount of N supplied by legumes is largely driven by legume biomass (Crews et al. 2016), there was likely insufficient N from BNF to result in beneficial niche modification, and, in turn, promote growth of the other species beyond that of the simultaneous treatment. Alternative seeding methods or rates could help improve legume performance in future studies, although even with greater biomass the mechanism of niche modification may not be important within mixtures of annual cover crops, particularly with a short growth period like in this study. For instance, the two-week head start provided by the staggered planting was likely too short to allow for sufficient legume growth and N fixation prior to establishment of the remaining species. Kardol et al. (2013) found that priority effects are more pronounced the longer the amount of time between species establishment, so it is possible that a longer lead time for the legumes may have resulted in stronger niche modification for later-planted species. Kardol et al. (2013) also found high soil fertility to have a strong influence on the magnitude of priority effects and resulting community composition, which we did not find here. Instead, our results fall more in line with Weidlich et al. (2017), who also did not find significant interactions between order of arrival and soil fertility. If the low fertility soil in our study was not sufficiently N-limited to provide legumes with a competitive advantage (which may be the case in many agroecosystems), this could have minimized the effect of soil fertility on cover crop outcomes.

The fact that the simultaneous treatment had the greatest evenness agrees with the findings of Kardol et al. (2013), who found that species planted simultaneously in fertile soil were able to better establish than later-planted species in a staggered planting regime, which led to a more balanced community composition. In low soil fertility, they found no difference in species establishment between simultaneous and staggered planting treatments. Although we did not observe a significant soil fertility effect, this could be in part due to strong weed pressure across all plots, which would have contributed to niche preemption in staggered planting treatments regardless of soil fertility status. This could also explain why the simultaneous treatment was the most even, since the establishment of all four cover crop species at once helped reduce weed pressure, as well as why the legume-first treatment performed so poorly given how much weed competition the legumes faced.

4.2 Multifunctionality

The simultaneous treatment had both the greatest evenness and multifunctionality, which aligns with our expectation that adequate representation of each functional group is critical for achieving a diverse set of ecosystem functions (Grime 2002). However, the evenness and multifunctionality trends of the staggered planting treatments did not support our hypothesis. For example, the grass-first treatment was the least even, but scored nearly as high as the simultaneous treatment for multifunctionality. This reflects the fact that grasses generally excel at several of the functions measured here, including weed suppression and total N retention given that grasses are highly productive and excellent nutrient scavengers (Dabney et al., 2007; Dozier et al, 2017). On the other hand, even though the legume-first treatment was not significantly less even than the simultaneous treatment, it had lower multifunctionality due to poor weed control and lower total N retention. The legume-first treatment did excel at BNF, as expected, with five times as much BNF as the grass- and brassica-first treatments, and almost twice as much as the simultaneous treatment. These results highlight clear tradeoffs in functions across treatments driven by differences between legumes and non-legumes. The only functions without major trade-offs were soil C and N mineralization, which was surprising given the differences in aboveground and belowground C:N inputs (Table 4). However, the high abundance of weeds in the experiment may have masked differences between cover crop treatments; fields with lower weed pressure may thus be more likely to reap the benefits of legume-first planting. Alternatively, it is also possible that background soil organic matter pools were more important drivers of decomposition than the effect of fresh litter inputs from the short-term experiment.

4.3 Plant functional trait variation

We found wide variation in plant functional traits both at the community- and species-level. At the community level, root:shoot ratio was greatest in the legume-first treatment, and lowest in the grass-first planting treatment, while the brassica-first and simultaneous treatment had intermediate ratios.The high root:shoot ratio in the legume-first treatment could reflect the strong weed pressure in this treatment; because the legumes were not effective at suppressing weeds at the beginning of the cover crop growing season, the later-planted species faced strong competition for soil resources and thus may have invested more energy belowground. In the case of the grass-first treatment, weeds were more effectively suppressed, potentially allowing for less investment belowground, which, coupled with the fact that oats are highly productive, contributed to a low root:shoot ratio. Community level above- and belowground C:N ratios were lowest in the legume-first treatment, indicating high-quality litter additions to the soil, which could help compensate for the limited amount of biomass entering the soil from this mixture and support subsequent cash crop growth (Fragaria et al., 2004; Hoorman, 2009).

At the species level, most trait variation for the non-legumes was driven by differences in plant growth stage at the time of sampling resulting from the staggered planting. For example, oat shoot C:N and height were significantly higher, while SLA and leaf %N were lower, when oats were planted at the first planting date (i.e., grass-first and simultaneous treatments). These trends reflect that the oats from the first planting date were closer to the reproductive growth stage when traits were sampled (Zhang et al., 2013; Tsai & Chang, 2022). This was also the case for radish C:N, height, and SLA. However, we also observed a significant interaction between planting and soil treatment for radish height, wherein radishes were taller in higher fertility than in lower fertility soils for the brassica-first and simultaneous treatments. This could indicate that radishes were better able to compete for sunlight with the other cover crop species, especially oats, in the simultaneous treatment, and with weeds in the brassica-first treatment, when nutrient availability was high at the outset of the growing season. Radish leaf %N in the brassica-first treatment, where the radish was included in the first planting, was significantly lower than the radishes in the legume-first treatment, suggesting that radish may have benefited from greater N availability specifically when planted following legumes.

Except for plant height, there were no significant differences in clover traits, which could be due in part to its poor growth overall. Clover was significantly taller in the simultaneous treatment compared to the brassica-first treatment, but only in the high soil fertility treatment. This could reflect that clover faced strong competition for light in the high fertility simultaneous treatment, likely from oats, but not when following radish.

Although there were no significant differences in pea shoot C:N or leaf %N across treatments, SLA and height did show considerable variation. Pea SLA was greatest in the grass-first treatment, suggesting competition for resources - either light or water - as peas tried to establish themselves when planted after the oats. Pea height was significantly taller in the simultaneous planting treatment compared to the grass-first planting treatment as well, but likely not due to being planted earlier, as the legume-first peas were not significantly taller than the grass-first peas. This suggests functional complementarity, as the peas may be using the oats as structural support when they are co-planted. However, pea biomass (and N fixation input) was higher when planted first, rather than simultaneously with other species, indicating that even though peas grew taller in the simultaneous treatment, they were more productive and contributed more to ecosystem functioning when given a head start.

4.4 Limitations and future directions

Given the poor performance of legumes, they likely would have performed better with a higher seeding rate and drilling as the seeding method rather than being broadcast seeded. Not only do clovers and peas perform better when drilled over being broadcast, but our seeding rates were on the low end of recommended ranges for broadcast planting, which may have contributed to low biomass production (Satell et al., 1998; St Aime et al., 2021). In addition, better weed control would likely have improved the quality of our results. Twenty-four of the forty plots had greater aboveground weed biomass compared to the biomass of all four target species combined. Given that weed suppression was one of the target functions of our study, we did not actively manage weeds. However, if weed suppression was not included in the study, it would allow for weed management which could enhance the clarity of other functions, perhaps C and N mineralization most of all.

We chose not to irrigate given that farmers are unlikely to water their cover crops, yet it is possible that irrigation would have allowed our cover crops to produce stronger results. There was a small rainshower on the day following the second planting (1.04 cm rainfall), but the following eight days were hot and relatively dry, with total precipitation throughout that time only amounting to 0.76 cm (National Weather Service, 2022). Although irrigating the cover crops may diminish the real-world applicability of the study, offering optimal conditions for cover crop growth in a controlled experiment may strengthen overall responses and interactions of target functions and traits, making for more substantial contributions to ecological understanding.

4.5 Implications for management

Our findings continue to build on management suggestions for maximizing cover crop functions in agroecosystems. The trade-offs in overall N retention, BNF, and weed suppression across planting treatments indicate that farmers should select a planting treatment based on their specific management goals. If a farmer’s goals include weed suppression and retention and recycling of existing N in their soil, they should consider the simultaneous or grass-first treatments. Given that farmers could plant all functional groups of the cover crop at once using the simultaneous planting treatment, the simultaneous treatment would be the easiest option. Furthermore, using cover crops to suppress weeds in lieu of herbicides can improve the environmental quality of their land, decrease the development of herbicide-resistant weeds, and reduce the health risk associated with proximity to herbicides, especially when planted early in the season (Dorn et al., 2015; Hashimi et al., 2020; Mennan et al., 2020; Osipitan et al., 2018; CTIC, SARE & ASTA, 2016).

However, farmers who primarily seek to increase N supply in their soil, for example, should consider the legume-first treatment since it had the highest N input from BNF. Utilizing BNF as a source of N instead of N fertilizers can benefit the environment as well as future cash crops (CTIC, SARE & ASTA, 2016). N fertilizer production is energy intensive, requiring up to 10.8 GJ of energy to support a hectare of maize (Alluvione et al., 2011). In addition to the emissions associated with fertilizer production, the application of N fertilizer is responsible for the emissions of another potent greenhouse gas: N_2O (Jensen, Hauggaard-Nielsen, 2003; Shcherbak et al, 2014). In contrast to synthetic N fertilizers, atmospheric N fixed by legumes is a renewable resource powered by the sun. N from BNF is also less prone to losses from leaching and runoff compared to soluble N fertilizers (Jensen & Hauggaard-Nielsen, 2003). Reductions in N leaching are essential for reducing eutrophication within aquatic ecosystems, which may further reduce greenhouse gas emissions (Jensen & Hauggaard-Neilsen, 2003; Li et al., 2021). Therefore, planting legumes first in a cover crop mixture can potentially benefit the field - through increasing N supply - as well as the broader environment.

V. CONCLUSION

Findings from our field experiment indicate that there are limited tradeoffs in evenness and multifunctionality when manipulating priority effects through the staggered planting of cover crop mixtures. The one exception was that N supply from BNF was improved by planting legumes ahead of grasses and brassicas. Even though the grass-first treatment had low mixture evenness, it provided high levels of multiple ecosystem functions. Overall, simultaneous planting of cover crop mixtures may be the best option for farmers because of the ease of planting and similar levels of multifunctionality compared to the staggered planting treatments tested here. iRegardless of the cover crop planting strategy farmers select, harnessing the functions provided by diverse groups of cover crops in place of herbicides and fertilizers can contribute to more environmentally friendly agricultural practices.

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CITATIONS

1. Alluvione, F., Moretti, B., Sacco, D., & Grignani, C. (2011). EUE (energy use efficiency) of cropping systems for a sustainable agriculture. Energy, 36(7), 4468-4481. https://www.sciencedirect.com/science/article/pii/S036054421100243X?casa_token=huCM9lBHQK8AAAAA:XCMc-18N4ykiFi7fQDBU9EthybWiIyZp7OjHg6WXNydS9AmL87TMuSlVFz4GTZt-w409iKCVAQ

2. Blesh, J. (2018). Functional traits in cover crop mixtures: Biological nitrogen fixation and multifunctionality. Journal of Applied Ecology, 55(1), 38-48. https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2664.13011

3. Buckland, S. M., & Grime, J. P. (2000). The effects of trophic structure and soil fertility on the assembly of plant communities: a microcosm experiment. Oikos, 91(2), 336-352. https://onlinelibrary.wiley.com/doi/abs/10.1034/j.1600-0706.2000.910214.x?casa_token=HEUSGWA-bucAAAAA%3AzurboEotHQYvMYg28HzBZrMGGxuvuMx7-YRWwjDem44REZIXdmhfsVQMoUwrq7ryWutzkAwQxW1Kg7uX

4. Byrnes, J. E., Gamfeldt, L., Isbell, F., Lefcheck, J. S., Griffin, J. N., Hector, A., & Emmett Duffy, J. (2013). Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. Methods in Ecology and Evolution, 5(2), 111-124. https://doi.org/10.1111/2041-210X.12143

5. Crews, T. E., Blesh, J., Culman, S. W., Hayes, R. C., Jensen, E. S., Mack, M. C., ... & Schipanski, M. E. (2016). Going where no grains have gone before: From early to mid-succession. Agriculture, Ecosystems & Environment, 223, 223-238. https://www.sciencedirect.com/science/article/abs/pii/S0167880916301402?casa_token=3EHBHwYsUHAAAAAA:7KtD4rIWWePN6FDgJ3H4d89TveK6_wu4RRHezSJfcEGsfDEA4iyEqNM8_0b6OuUvIJtiU1Zvbg

6. CTIC, SARE & ASTA. 2016. 2015-2016 annual report: Cover crop survey. North Central Sustainable Agriculture Research and Education Program and Conservation Technology Innovation Center, St Paul. MN. https://www.sare.org/wp-content/uploads/2015-2016-Cover-Crop-Survey-Report.pdf

7. CTIC, SARE & ASTA (2020). 2019-2020 Annual Report: Cover Crop Survey. North Central Sustainable Agriculture Research and Education Program and Conservation Technology Innovation Center. https://www.sare.org/wp-content/uploads/2019-2020-National-Cover-Crop-Survey.pdf

8. Dabney, S. M., Delgado, J. A., & Reeves, D. W. (2001). Using winter cover crops to improve soil and water quality. Communications in Soil Science and Plant Analysis, 32(7-8), 1221-1250. https://www.tandfonline.com/doi/abs/10.1081/CSS-100104110

9. Davis, A. S., Hill, J. D., Chase, C. A., Johanns, A. M., & Liebman, M. (2012). Increasing cropping system diversity balances productivity, profitability and environmental health. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0047149

10. Díaz, S., & Cabido, M. (2001). Vive la différence: plant functional diversity matters to ecosystem processes. Trends in ecology & evolution, 16(11), 646-655. https://www.sciencedirect.com/science/article/abs/pii/S0169534701022832?casa_token=6LinxdGre-UAAAAA:P3xRrniwP8IiDHo2XDbG4cKe7AKoluObNe9d_hdZP8e6ltAp8tsOBb7Vpt3n85Y0LQvWZ3UJYg

11. Dorn, B., Jossi, W., & van der Heijden, M. G. (2015). Weed suppression by cover crops: comparative on‐farm experiments under integrated and organic conservation tillage. Weed Research, 55(6), 586-597. https://onlinelibrary.wiley.com/doi/full/10.1111/wre.12175?casa_token=_BpXQtyNcikAAAAA%3ALPoPdmlfCN1c97lCZT9K9jb2Gnz7NvShyZHZ__nonoUMTXlkA4diXHngDCfC_LBacO2SpS5kunoc8E5J

12. Dozier, I. A., Behnke, G. D., Davis, A. S., Nafziger, E. D., & Villamil, M. B. (2017). Tillage and cover cropping effects on soil properties and crop production in Illinois. Agronomy Journal, 109(4), 1261-1270. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/agronj2016.10.0613

13. Drinkwater, L. E., Cambardella, C. A., Reeder, J. D., & Rice, C. W. (1997). Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen. Methods for assessing soil quality, 49, 217-229. https://acsess.onlinelibrary.wiley.com/doi/abs/10.2136/sssaspecpub49.c13

14. Ejrnæs, R., Bruun, H. H., & Graae, B. J. (2006). Community assembly in experimental grasslands: suitable environment or timely arrival?. Ecology, 87(5), 1225-1233. https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1890/0012-9658%282006%2987%5B1225%3ACAIEGS%5D2.0.CO%3B2?casa_token=Ph5uq_2y5x0AAAAA%3AEy0YL9HU-PHJvk_FCuSjqFzEGr7dfoLKN_is9fd5lRM7GgVs_I6oRvLCZL4VnJbP6hB_DKlnEzUe0aU8

15. Finney, D. M., & Kaye, J. P. (2017). Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. Journal of Applied Ecology, 54(2), 509-517. https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2664.12765

16. Finney, D. M., Murrell, E. G., White, C. M., Baraibar, B., Barbercheck, M. E., Bradley, B. A., Cornelisse, S., Hunter, M. C., Kaye, J. P., Mortensen, D. A., Mullen, C. A., & Schipanski, M. E. (2017). Ecosystem Services and disservices are bundled in simple and diverse cover cropping systems. Agricultural & Environmental Letters, 2(1), 170033. https://doi.org/10.2134/ael2017.09.0033

17. Fukami, T. (2015). Historical contingency in community assembly: Integrating niches, species pools, and priority effects. Annual Review of Ecology, Evolution, and Systematics, 46(1), 1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

18. Garnier, E., & Navas, M. L. (2012). A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. Agronomy for Sustainable Development, 32, 365-399. https://link.springer.com/article/10.1007/s13593-011-0036-y

19. Grime, J. P. (2002). Benefits of plant diversity to ecosystems: Immediate, Filter and founder effects. Journal of Ecology, 86(6), 902–910. https://doi.org/10.1046/j.1365-2745.1998.00306.x https://besjournals.onlinelibrary.wiley.com/doi/full/10.1046/j.1365-2745.1998.00306.x

20. Hashimi, M. H., Hashimi, R., & Ryan, Q. (2020). Toxic effects of pesticides on humans, plants, animals, pollinators and beneficial organisms. APRJ, 5(4), 37-47. https://www.researchgate.net/profile/Rahmatullah-Hashimi/publication/343470477_Toxic_Effects_of_Pesticides_on_Humans_Plants_Animals_Pollinators_and_Beneficial_Organisms/links/5f2c121c299bf13404a68a22/Toxic-Effects-of-Pesticides-on-Humans-Plants-Animals-Pollinators-and-Beneficial-Organisms.pdf

21. Hayden, Z. D., Ngouajio, M., & Brainard, D. C. (2015). Planting date and staggered seeding of rye–vetch mixtures: Biomass, nitrogen, and legume winter survival. Agronomy Journal, 107(1), 33-40. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/agronj14.0237?casa_token=TBMUC7Kro0AAAAAA%3AS809qCS-EDL_bkLqbMPLz8nf_DzC-knYC_kz5qNE1JB8iLdX03dM9BjfpekqPSePVIQs9Qq7tkgY0-Zb

22. Hector, A., Bagchi, R. Biodiversity and ecosystem multifunctionality. Nature 448, 188–190 (2007). https://doi.org/10.1038/nature05947

23. Helenius, J., & Jokinen, K. (1994). Yield advantage and competition in intercropped oats (Avena sativa L.) and faba bean (Vicia faba L.): Application of the hyperbolic yield-density model. Field Crops Research, 37(2), 85-94.

24. Hoorman, J. J. (2009). Using cover crops to improve soil and water quality. Lima, Ohio: Agriculture and Natural Resources, The Ohio State University Extension. http://www.blanchardriver.org/wp-content/uploads/2019/06/Using-Cover-crops-SAG-08-09.pdf

25. Isbell, F., Adler, P. R., Eisenhauer, N., Fornara, D., Kimmel, K., Kremen, C., & Scherer‐Lorenzen, M. (2017). Benefits of increasing plant diversity in sustainable agroecosystems. Journal of Ecology, 105(4), 871-879. https://besjournals.onlinelibrary.wiley.com/doi/pdfdirect/10.1111/1365-2745.12789

26. Jacobs, A. 2012. Plant fact sheet for oilseed radish (Raphanus sativus var. oleiformis). USDA-Natural Resources Conservation Service, Booneville Plant Materials Center, Booneville, AR. https://adminplants.sc.egov.usda.gov/factsheet/pdf/fs_rasa2.pdf

27. Jensen, E. S., & Hauggaard-Nielsen, H. (2003). How can increased use of biological N 2 fixation in agriculture benefit the environment?. Plant and soil, 252, 177-186. https://link.springer.com/article/10.1023/A:1024189029226

28. Johnny's Selected Seeds. (2022). Crimson Clover - Cover Crop Seed. https://www.johnnyseeds.com/farm-seed/legumes/clovers/crimson-clover-cover-crop-seed-982.html

29. Johnny's Selected Seeds. (2022). 4010 Field Pea Organic Cover Crop Seed. https://www.johnnyseeds.com/farm-seed/legumes/field-peas/4010-field-pea-organic-cover-crop-seed-92G.html?cgid=field-peas#start=1

30. Johnny's Selected Seeds. (2022). Oats (Common) Organic Cover Crop Seed. https://www.johnnyseeds.com/farm-seed/grains/oats/oats-common-organic-cover-crop-seed-2509G.html?cgid=oats#start=1

31. Johnny's Selected Seeds. (2022). Oilseed Radish Cover Crop Seed. https://www.johnnyseeds.com/farm-seed/brassicas/oilseed-radish-cover-crop-seed-2906.html?cgid=brassicas

32. Kardol, P., Souza, L., & Classen, A. T. (2013). Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. Oikos, 122(1), 84-94. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-0706.2012.20546.x?casa_token=sQL_YHZoeOEAAAAA%3AQFmOsaH4TZP8C2uopMjef9YF1-BljzMEuUUf478myrm5n57umU2zmCDONZLOn91cAD6hKKF8GxlNseUn

33. Kremen, C., & Miles, A. (2012). Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. Ecology and society, 17(4). https://www.jstor.org/stable/26269237?seq=1#metadata_info_tab_contents

34. Li, Y., Shang, J., Zhang, C., Zhang, W., Niu, L., Wang, L., & Zhang, H. (2021). The role of freshwater eutrophication in greenhouse gas emissions: A review. Science of The Total Environment, 768, 144582. https://www.sciencedirect.com/science/article/pii/S0048969720381134?casa_token=fNLa0aZhSZ8AAAAA:P-zXOTiBTL9jvbgUr-qKNqU1mC0wJX7YEVjhh4_cdUyqsZkPYgvSvLYlBx6uFynemuKtVJp-XA

35. Mennan, H., Jabran, K., Zandstra, B. H., & Pala, F. (2020). Non-chemical weed management in vegetables by using cover crops: A review. Agronomy, 10(2), 257. https://www.mdpi.com/2073-4395/10/2/257

36. National Weather Service. (2022). United States. [Web Archive] Retrieved from the National Oceanic and Atmospheric Administration, https://www.weather.gov/wrh/Climate?wfo=dtx

37. Ohio Earth Food. ReVita Compost Plus 3-4-3 50# bag. https://ohioearthfood.com/products/re-vita-compost-plus-3-3-3

38. Osipitan, O. A., Dille, J. A., Assefa, Y., & Knezevic, S. Z. (2018). Cover crop for early season weed suppression in crops: Systematic review and meta‐analysis. Agronomy Journal, 110(6), 2211-2221. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/agronj2017.12.0752

39. Ovalle, C., Urquiaga, S., Pozo, A. D., Zagal, E., & Arredondo, S. (2006). Nitrogen fixation in six forage legumes in Mediterranean central Chile. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 56(4), 277-283. https://www.tandfonline.com/doi/full/10.1080/09064710500310246

40. Sattell, R., Dick, R., Hemphill, D. D., & McGrath, D. M. (1998). Field pea (Pisum sativum L. or Pisum sativum L. ssp. arvense (L.) Poir. https://ir.library.oregonstate.edu/concern/open_educational_resources/6682x4227

41. Schipanski, M. E., Barbercheck, M., Douglas, M. R., Finney, D. M., Haider, K., Kaye, J. P., & White, C. (2014). A framework for evaluating ecosystem services provided by cover crops in agroecosystems. Agricultural Systems, 125, 12-22. https://www.sciencedirect.com/science/article/abs/pii/S0308521X13001492?casa_token=tsIRKSxkNYQAAAAA:07JONg3YqExN_s-HwstOTvsrPm5VM2F-1F2Gy-kFsJPvcdbuIo2bFURR6WSfzx7mFI4Uld-qiQ

42. Schipanski, M. E., & Drinkwater, L. E. (2012). Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. Plant and Soil, 357, 147-159. https://link.springer.com/article/10.1007/s11104-012-1137-3

43. Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671-675. doi:10.1038/nmeth.2089

44. Shcherbak, I., Millar, N., & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. Proceedings of the National Academy of Sciences, 111(25), 9199-9204. https://www.pnas.org/doi/abs/10.1073/pnas.1322434111

45. Snapp, S. S., Swinton, S. M., Labarta, R., Mutch, D., Black, J. R., Leep, R., ... & O'neil, K. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. Agronomy journal, 97(1), 322-332. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/agronj2005.0322a?casa_token=jM3ArvsF_PQAAAAA%3A3Xp_SWvP6xw2ZvrOrSGy73eXFY0mly3atCqEsv9IUCAz6n1DUKj1VAY2FmnchFmGkc17so1X_TOZlfmv

46. St Aime, R., Noh, E., Bridges Jr, W. C., & Narayanan, S. (2021). A Comparison of Drill and Broadcast Planting Methods for Biomass Production of Two Legume Cover Crops. Agronomy, 12(1), 79. https://www.mdpi.com/20A Comparison of Drill and Broadcast Planting Methods for Biomass Production of Two Legume Cover Crops73-4395/12/1/79

47. Storkey, J., Döring, T., Baddeley, J., Collins, R., Roderick, S., Stobart, R., & Watson, C. (2015). A framework for designing multi-functional cover crops. Aspects of Applied Biology, 129, 7-12. https://www.researchgate.net/profile/Jonathan-Storkey/publication/280304708_A_framework_for_designing_multi-functional_cover_crops/links/55b5f37708aed621de02fd98/A-framework-for-designing-multi-functional-cover-crops.pdf

48. Tsai, S. S., & Chang, Y. C. A. (2022). Plant Maturity Affects Flowering Ability and Flower Quality in Phalaenopsis, Focusing on Their Relationship to Carbon-to-nitrogen

Ratio. HortScience, 57(2), 191-196.
https://journals.ashs.org/hortsci/view/journals/hortsci/57/2/article-p191.xml

49. Wang, B., Deng, J., Wang, T., Ni, W., Feng, Q., & Lan, J. (2022). Effect of Seeding Options on Interspecific Competition in Oat (Avena sativa L.)–Common Vetch (Vicia sativa L.) Forage Crops. Agronomy, 12(12), 3119.
https://www.mdpi.com/2073-4395/12/12/3119

50. Weidlich, E. W., Von Gillhaussen, P., Delory, B. M., Blossfeld, S., Poorter, H., & Temperton, V. M. (2017). The importance of being first: exploring priority and diversity effects in a grassland field experiment. Frontiers in Plant Science, 7, 2008.
https://www.frontiersin.org/articles/10.3389/fpls.2016.02008/full

51. Wood, S. A., Karp, D. S., DeClerck, F., Kremen, C., Naeem, S., & Palm, C. A. (2015). Functional traits in agriculture: agrobiodiversity and ecosystem services. Trends in ecology & evolution, 30(9), 531-539.
https://www.sciencedirect.com/science/article/abs/pii/S0169534715001652?casa_token=kGfCwmAwqFgAAAAA:U8ZW-CxPR-qs9odgIDsgw4YEYZdpn4LOHWKVun-TPH6bBi3MqJDdMB7ew5KLDefsbvj9e5g1nQ

52. Zhang, H., Wu, H., Yu, Q., Wang, Z., Wei, C., Long, M., & Han, X. (2013). Sampling date, leaf age and root size: implications for the study of plant C: N: P stoichiometry. PLoS One, 8(4), e60360.
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0060360

Appendix

Appendix Table A1: True Values of Function Means and Standard Error

Appendix Table A1: True Values of Function Means and Standard Error				
	B	G	L	S
Aboveground BNF (kg/ha)	1.24 (0.28)	1.75 (0.31)	8.60 (2.37)	4.95 (1.17)
Belowground N Inputs (g/m^2)	7.53 (0.74)	7.82 (0.74)	8.30 (0.35)	10.81 (0.91)
Aboveground N Inputs (kg/ha)	14.20 (1.30)	23.55 (3.92)	13.75 (3.29)	28.22 (4.98)
C Mineralization (μg CO_2-C/g/day)	4.73 (0.36)	4.96 (0.37)	5.06 (0.35)	5.04 (0.43)
N Mineralization (mg N/kg soil / day)	0.26 (0.06)	0.25 (0.03)	0.26 (0.05)	0.26 (0.02)
Weed Suppression (%)	25.33 (7.64)	42.14 (8.92)	3.413 (8.25)	46.79 (11.01)