Final Report

Title: Legume Cover Crop Impacts on Soil Nitrogen Cycling and Soil Fertility on Organic Vegetable Farms

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Locations: Central and Southeastern Michigan

Research activities:

This participatory, on-farm research project addressed the following objectives:

1) Identify legume cover crops (grown in monocultures and mixtures) that build SOM and increase nutrient cycling capacity on organic vegetable farms.
2) Identify rotations and management practices that maximize BNF and reduce N surpluses.
3) Develop workshop activities and outreach materials to share information with small-scale, organic vegetable farmers of varying levels of experience.

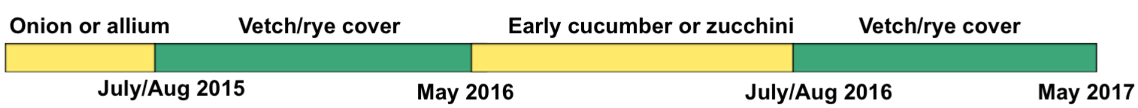
We established two different experiments on 10 vegetable farms in Michigan. One of the experiments (hereafter "rye/vetch") lasted three years, and the second experiment (hereafter "common garden") spanned one year. Both studies identified legume-based cover crop mixtures that build soil organic matter and enhance nutrient cycling capacity on organic vegetable farms in the Midwest.

First, we analyzed baseline soil samples collected from both experimental fields prior to initiating the experiments for a suite of soil health indicators, including macro- and micro-nutrients, pH, bulk density, soil organic matter, two fractions of particulate organic matter, potentially mineralizable nitrogen (using an anaerobic incubation method), and potentially mineralizable carbon (PMC; i.e., the short-term flux of carbon dioxide released following rewetting of dried soil). We also conducted detailed management history interviews with all participating farmers. The farms represented a gradient of different soil types and management histories, including number of years in organic vegetable production (1 to 13 years).

Next, we established the on-farm research, which was planted in two separate fields on all 10 farms. The rye/vetch experiment determined variability in legume N fixation inputs across the farms from a hairy vetch cover crop grown in monoculture, and in a mixture with cereal rye, over two full years. The second goal of this experiment was to measure changes in soil fertility and nutrient cycling with two years of winter cover compared to a no cover crop control plot. All treatments were replicated four times in a randomized complete block design. All farmers used the same tillage practice, seeding rates, seeding method (broadcast with light culti-packing), and timing. They also used the same cover crop incorporation method, cash crops in rotation, and all had a small area of the field in a no cover crop control, so that we could determine changes in soil quality over the three-year period (from the baseline sample) due to the presence of the cover crop mixture.

Figure 1. Example layout of the two different research fields (outlined in yellow) on one of the participating vegetable farms in Southern Michigan. The field labeled "rye/vetch" was in the three-year experiment, and the field labeled "common garden" was in the one-year mixture screening experiment.

"Rye/Vetch" Study: This experiment was planted in a field with the same crop rotation on all farms for the duration of the study. The rotation included a winter cover crop bi-culture of hairy vetch/cereal rye over two winters (2015 and 2016):



During the cover crop seasons, we set up replicated mini-plots to assess differences in N fixation by the vetch grown in mixture and monoculture. We hand-weeded out the vetch to get rye monoculture plots, and hand-weeded out rye to establish vetch monoculture plots (Figure 2).

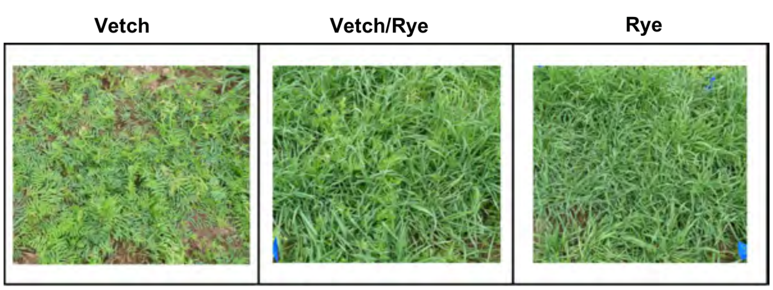


Figure 2. Example of the 1m x 1m sub-plots replicated four times per field to quantify N fixation inputs from hairy vetch grown in mixture and monoculture. The rye monoculture plot serves as the reference plant for the isotope method to estimate % of vetch N from the atmosphere.

In May of 2016 and 2017 we sampled the replicated cover crop treatments on all farms and measured: N fixation using the Natural Abundance (stable isotope) method, using the UC Davis stable isotope facility; total aboveground biomass and % N; and, vetch root nodule mass. We also sampled soil for inorganic N, potentially mineralizable N, and potentially mineralizable C. We then worked with farmers to plant the subsequent cash crop (Cucurbit family: either early cucumber or zucchini) and to weigh all crops harvested from the cover and no-cover treatments to determine yield differences due to the cover crop.

"Common Garden" Study: This field was planted in a one-year screening study where we evaluated different winter cover crop mixtures of interest to the group of farmer-participants. The experiment was planted in mid-August, 2015 on the same set of 10 farms. We planted different cover crop mixtures including legumes, grasses, and brassicas appropriate for the cool season niche, some of which overwinter. The idea was to mix species with complementary plant traits; e.g., reaching peak biomass in the fall or spring, N-fixing and non-N-fixing, and differences in shoot carbon-to-nitrogen ratio.

Cover crop treatment list	Seeding rate (lbs/ac)
Crimson clover/red clover/spring wheat (CC+RC+SW)	17/6/45
Austrian winter pea/oat/daikon radish (WP+OA+DR)	50/40/3
Lentil/yellow mustard/oat (LN+YM+OA)	30/8/40
Red clover/spring wheat (RC+SW)	8/50
Crimson clover/spring wheat (CC+SW)	22/50
Cereal rye/chickling vetch (CR+CV)	50/105
Austrian winter pea (WP)	100
Cereal rye (CR)	155
Spring wheat (SW)	155
No cover crop control	0

In one field per farm, we planted four replicate blocks of all 11 treatments, each of which was planted in an 8ft x 8 ft plot (i.e., 48 plots per field). For each plot on each farm, we measured aboveground biomass production, C and N content in biomass, N fixation (for a subset of treatments), and weed suppression (compared to the no-cover control) in the fall before cover crop dormancy and in the spring immediately before cover crop incorporation.

Results:

I. *Rye/Vetch Study*

In terms of biomass (Figure 3), the rye/vetch mixture performed similarly in both years, however there was much lower vetch biomass in both mixture and monoculture in the second year of the study. The second year of the study had more total growing degree days, so this result is a little puzzling, though it is possible that either: (i) vetch established poorly in the fall due to unfavorable conditions, or (ii) rye may have outcompeted the vetch during the cool spring weather. Total mixture and weed biomass were similar in both study years. Because of our study design – selected to compare vetch N fixation in mixture and monoculture at the same seeding density – the vetch monoculture biomass is not that representative of the vetch monoculture biomass a farmer could achieve with a higher seeding rate. However, the results for determining legume N fixation in mixture versus monoculture are more robust with this direct comparison at the same seeding density.

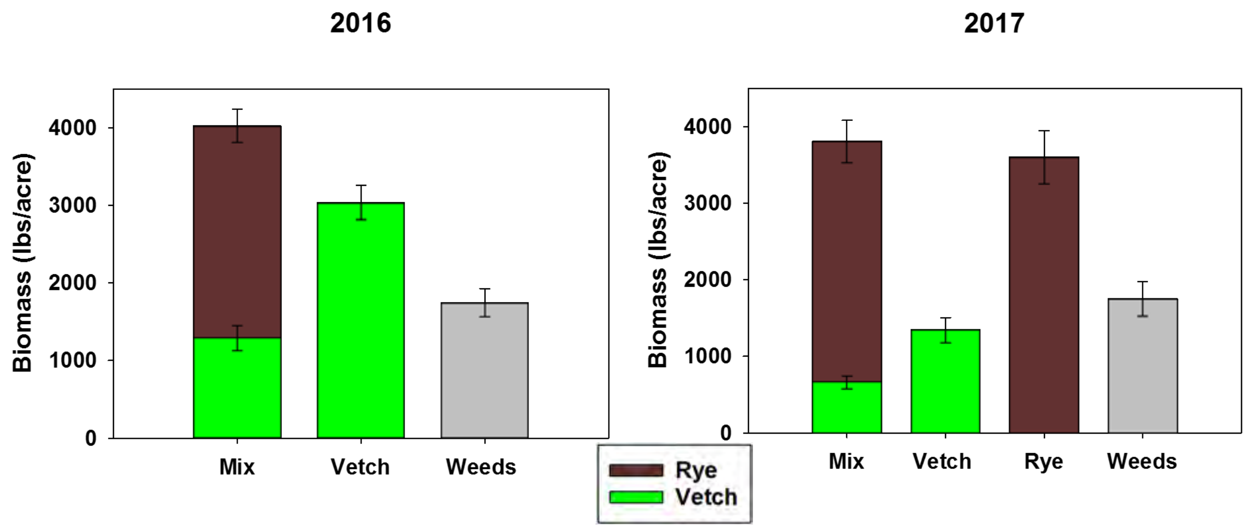


Figure 3. Total aboveground biomass for vetch/rye mixture ("Mix"), and monocultures (and weeds) in the two study seasons. Rye monoculture biomass was not measured in year 1.

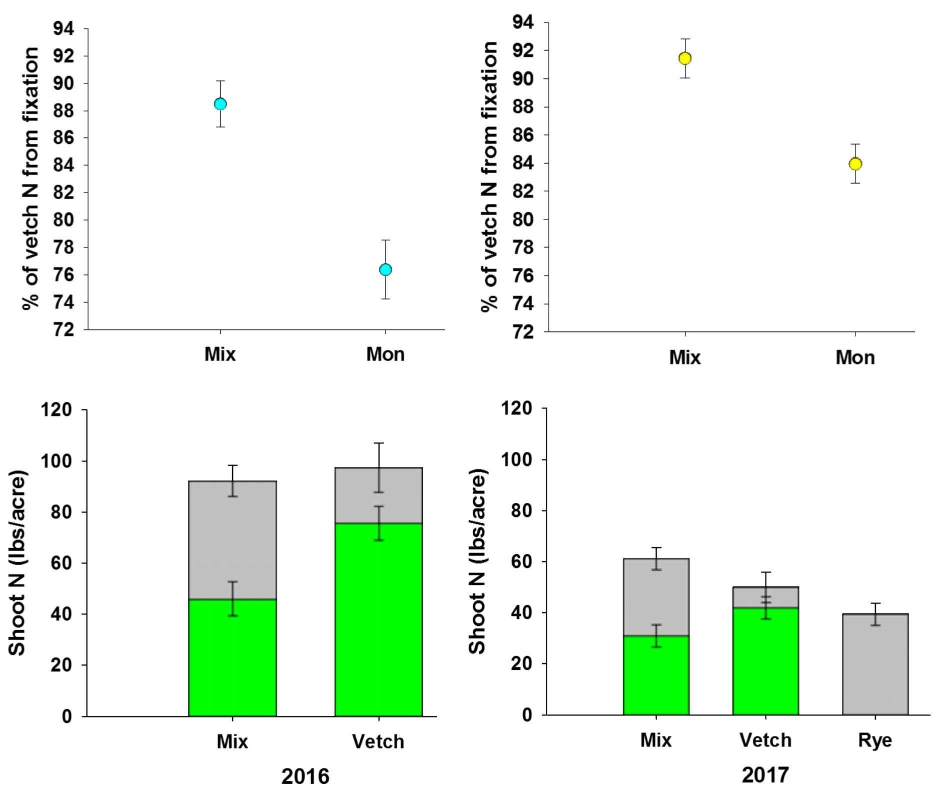


Figure 4. Percent of vetch N derived from fixation, and total N in aboveground biomass from vetch fixation and from the soil in 2016 and 2017.

Figure 4 shows the final results over two full cover crop seasons (2016 and 2017) for the proportion of vetch biomass N from the atmosphere (top panel) and for shoot N from vetch fixation (in green) and from the soil (in gray). In both years the vetch grown in mixture obtained a significantly greater proportion of its N from fixation due to competition for soil N from the rye. In 2016 this amount was 88.5% in mixture compared to 76.3% in monoculture, and in 2017 – when vetch biomass was much lower – the difference was 91.4% in mixture versus 84% in monoculture. Ultimately, the amount of legume biomass determines the total N supply to farm fields. However, particularly in 2016, the rye/vetch mixture provided a better balance of ecosystem services overall, because there was substantial N supply from the vetch alongside greater N scavenging by the rye (and likely weed suppression as well).

In 2017, when we measured both vetch and rye monoculture biomass in the replicate mini-plots, we were able to calculate the land equivalency ratio (LER) for the mixture compared to the monocultures. The distribution of LERs across all farms in the study is shown in Figure 5. On most farms, the mixture "overyielded" compared to the monocultures. A value of 1.5, for instance, would mean that a farmer would have to plant 15 acres of the monoculture to achieve the same biomass production as 10 acres of the mixture. Here, the LER was very high on some farms in part due to poor performance of the vetch monoculture. However, this is a general finding of cover crop mixtures, which has been reported in multiple studies.

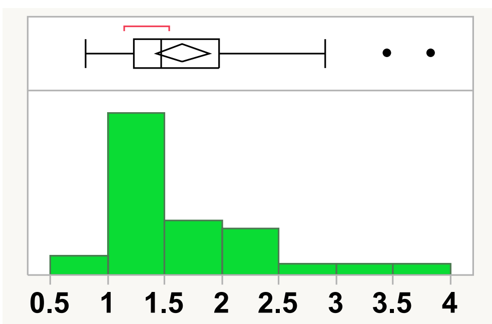


Figure 5. Distribution of land equivalency ratio (LER) values across all farms in the study in 2017. Most LERs were greater than 1, indicating "overyielding" by the cover crop mixture compared to the monocultures.

Between the 2016 and 2017 cover crop windows all farmers planted a Cucurbit crop in the experimental field. 9 of the 10 farms realized a yield increase in the cover cropped section of the field compared to the no cover control (Figure 6a; the farm with no change had very high baseline soil fertility). Because the total N supply from vetch was lower than that achieved in some field station experiments (e.g. 100-200 lbs of N/acre), we asked whether the N input from vetch was sufficient to balance the N removed in the harvested cash crop. The N supply from vetch was sufficient (or much greater than sufficient) on 7 of the 8 farms for which we calculated this N budget (Figure 6b). Many vegetable crops do not remove large amounts of N; hence, balancing N inputs with harvested N exports could increase sustainability of nutrient management on organic farms and potentially reduce costs on manure and compost inputs.

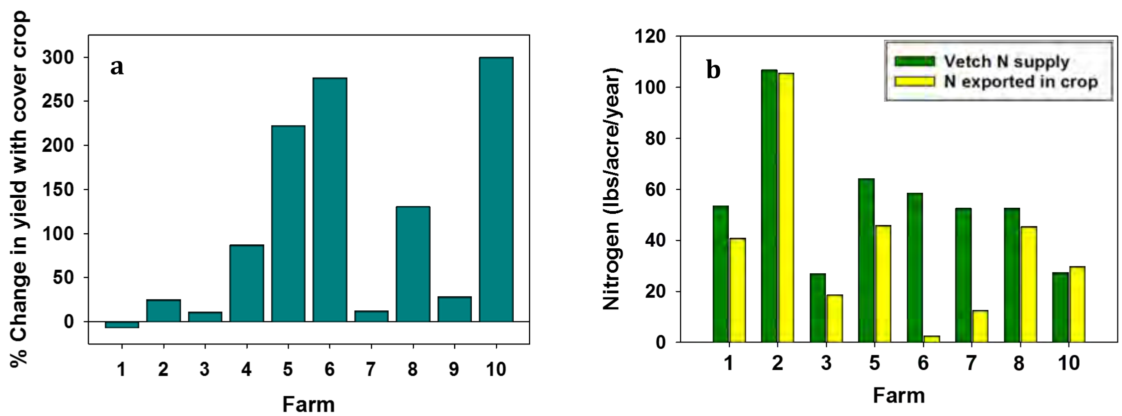


Figure 6. a) Percent change in Cucurbit yield following the vetch/rye cover crop, and b) Vetch N supply versus N exported from the field in the harvested portion of the Cucurbit crop.

Finally, we measured changes in multiple measures of soil health on all farms following two years with the vetch/rye cover crop compared to the no cover crop control. The final soil sampling occurred approximately three years after the baseline sampling. Following the study, there was no change in total organic matter (%OM) on the farms (on average), which we expected because total organic matter changes slowly following changes in management, and, depending on the soil type, total %OM can better reflect soil type differences on farms rather than management practices. However, there were substantial changes in multiple metrics of soil health that are more responsive to management changes. We measured two different fractions of particulate organic matter (POM), which turnover on timescales of a year to a decade. We also measured two different process rates that are indicative of soil biological activity.

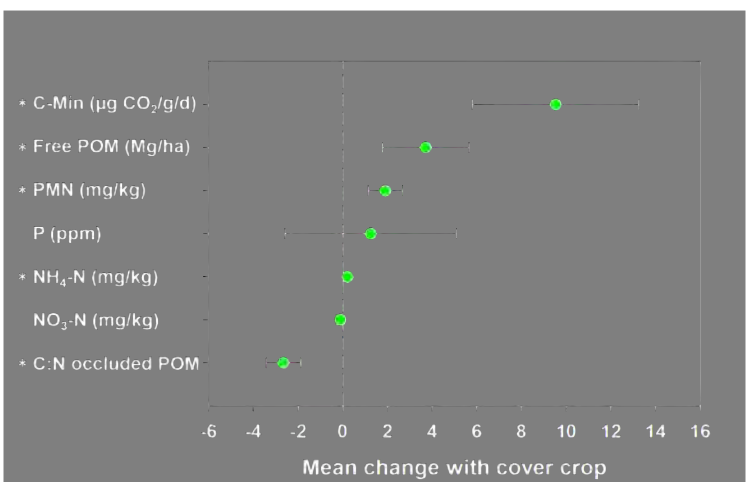


Figure 7. Mean change in soil health metrics across farms calculated as the difference between treatment (cover crop) and control (no cover crop), shown with 95% confidence intervals. Potentially mineralizable carbon (C-Min), potentially mineralizable nitrogen (PMN), the free particulate organic matter (POM) pool and the nitrogen concentration of the occluded POM pool all significantly increased (the latter is shown as reduced C:N). There was a lot of variation in Bray-1 phosphorus across farms, and it did not significantly increase. Soil inorganic N pools were small, though ammonium (NH_4^+) slightly increased over the study period.

In summary, vetch N fixation rates varied across farms with different levels of soil fertility due to management histories. The % of plant N from fixation was consistently higher in mixture compared to monoculture, suggesting the potential for realizing multiple ecosystem benefits at once with mixtures. Overall, vetch performance varied in the two study years, suggesting that for farmers who can afford the seed and establishment costs, higher vetch seeding rates within mixtures would be beneficial for more consistent mixture evenness. On average, farms realized a yield increase in their vegetable crops following the cover crop mixture, and N removed in the cash crop was balance by the N supply from the vetch cover crop. Finally, multiple biological and chemical indicators of soil health increased on participating farms following two full seasons of the cover crop mixture. This highlights the need for improved soil tests that give farmers this real-time, more holistic information about changes in soil characteristics due to management.

II. Common Garden Study

In Figure 8, the average results across all farms for biomass of the 11 different treatments are shown (10 cover crop treatments plus a weedy fallow control) along with the lbs N/acre retained in aboveground biomass. In the fall, mean biomass of all cover crops on individual farms ranged from 598 to 5145 lbs/acre (data not shown) (mean of 1249 lbs/acre) with the greatest treatment mean across all farms in the yellow mustard mix (Figure 8a). Spring cover crop biomass ranged from 967 to 11015 lbs/acre across individual farms (mean of 2498) with the greatest mean biomass in treatments with cereal rye. Mean aboveground biomass across farms was greater at the spring sampling time (Figure 8b) than the fall sampling time for all treatments except for lentil + yellow mustard + oat and spring wheat, which only included non-winter hardy species. In the fall, the winter pea + oat + daikon radish mixture tended to have higher biomass than the winter pea monoculture, although the difference was not statistically significant. The mean red clover biomass in the red clover + crimson clover + spring wheat mix was low in the fall, but increased at the spring sampling, even though crimson clover biomass was greater in both fall and spring.

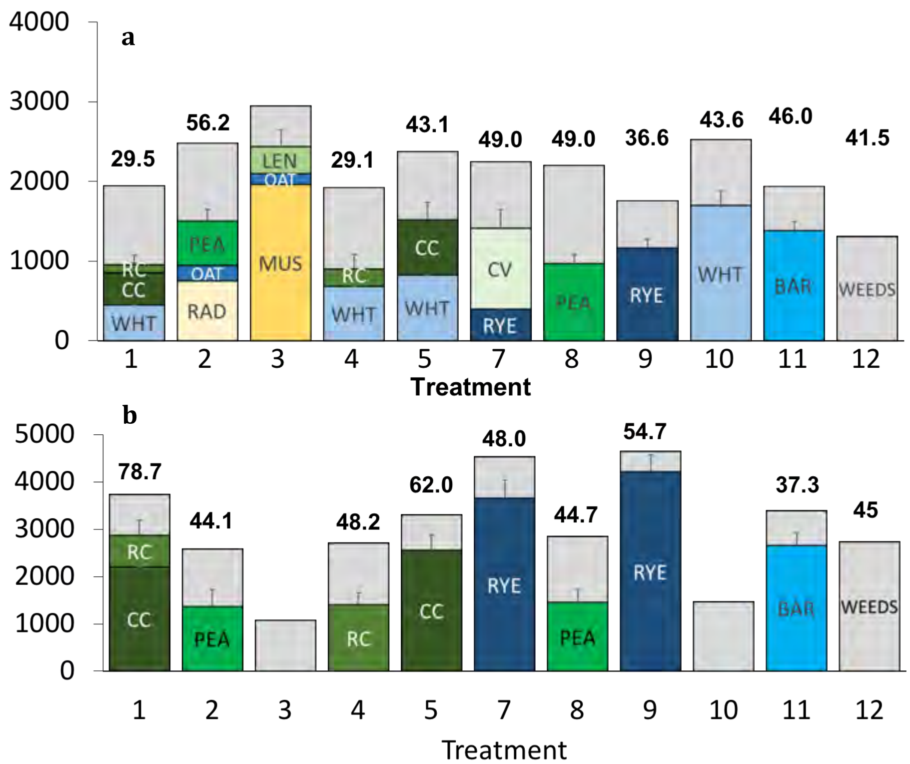


Figure 8. a) Mean fall biomass (lbs/acre; with standard errors) across all farms for each cover crop treatment, and b) mean spring biomass (lbs/acre; with standard errors) across all farms for each cover crop treatment. Numbers above the bars indicate the mean lbs N/acre retained in the aboveground biomass of each treatment across farms.

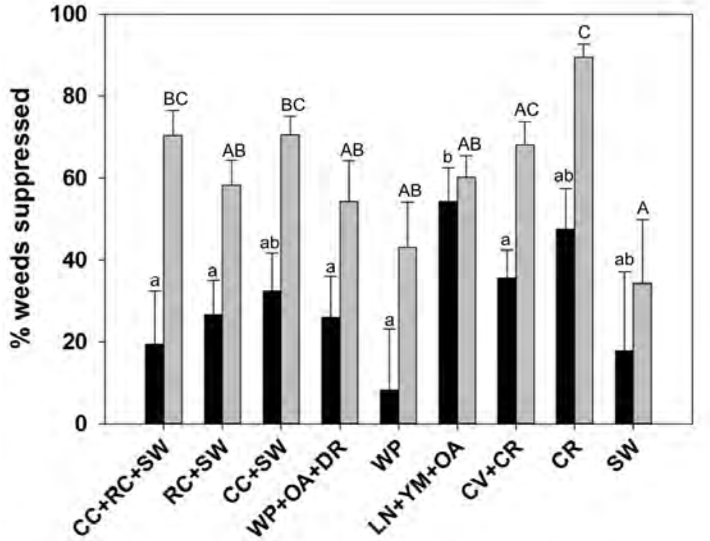
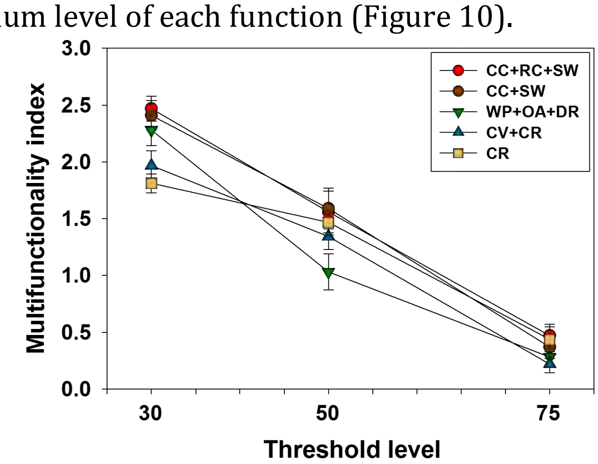


Figure 9. Mean weed suppression (% of weeds suppressed compared to the no cover crop control) with standard error for all treatments in the fall (black) and spring (gray). See table on page 3 for a key to species abbreviations. Statistics are for cover crop biomass at the treatment level, with lowercase letters for fall, and uppercase for spring. Mean values labeled with the same letter were not significantly different at P<0.05%.

Fall weed suppression (Figure 9) was low, ranging from 8.3% (WP) – 54.2% (LN+YM+OA). While the yellow mustard (YM) mixture had the best fall weed suppression, it was not significantly different from the cereal rye (CR) monoculture (47.5%), the crimson clover/spring wheat mixture (CC+SW mixture (32.3%), or from the spring wheat (SW) monoculture (17.6%; due to large variation in performance of SW across farms). In the spring, weed suppression was much higher, ranging from 34.3 (SW) – 89.4% (CR). The CR monoculture had the best spring weed suppression but was not significantly different from several mixture treatments: CC+RC+SW (70.5%), CC+SW (70.6%), or CV+CR (67.9%). Treatments that fully winterkilled sustained approximately 50% weed suppression in the spring compared to the no cover control; however, they did not suppress weeds as well as winter rye. The WP monoculture had the lowest weed suppression in the fall, but by spring it was not significantly different from most other treatments.

Even though cereal rye monoculture was one of the top performers (as expected) in terms of biomass production, N retention, and weed suppression, it did not provide the same level of *multifunctionality* (simultaneously providing multiple ecosystem services) as some of the legume-based mixtures did (CC+RC+SW, CC+SW, and WP+OA+DR), particularly at lower thresholds (30% and 50%) of the maximum level of each function (Figure 10).

Figure 10. Assessment of multi-functionality (provisioning multiple ecosystem services at once) for five of the cover crop treatments (mean index value with standard error) across farms. The highest possible index value was 3 (for the three ecosystem functions), and the index was assessed as the number of functions passing a 30%, 50%, or 75% threshold of the maximum level of each function measured in the study.



In addition, we found large variability in biological N fixation inputs from the legumes across the farms (Figure 11).

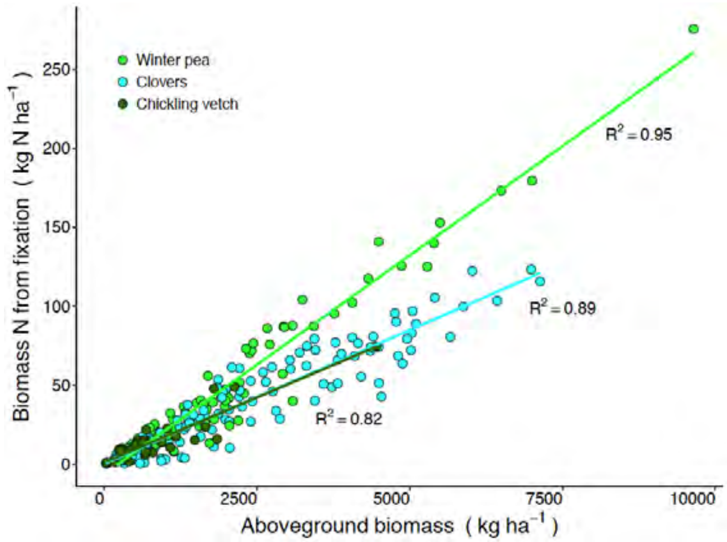


Figure 11. N supply from biological N fixation (y-axis) by winter pea, crimson and red clovers (combined), and chickling vetch across working organic vegetable farms in Michigan is explained by legume aboveground biomass production (x-axis).

What explained this variation in legume biomass within mixtures across farms? Results from linear regression models with soil properties (e.g., soil phosphorus, particulate organic matter (POM) fractions) indicated that legume biomass was higher in soils with lower N content in soil organic matter pools, particularly in the two POM fractions. Models for predicting winter pea and crimson clover biomass were especially robust (R^2=0.76 and R^2=0.54-0.67, respectively), and showed that legume biomass was negatively correlated with two measures of N availability from decomposition: the N concentration of occluded POM and the total quantity of free POM. Occluded POM, in particular, tends to be a reliable indicator of longer-term changes in soil fertility due to management—making it possible to differentiate whether SOM stocks reflect background soil type versus management practices. Legume biomass increased with plant-available phosphorus concentration in soil. Biomass of the grass species was also positively correlated with soil phosphorus, as well as with a lower carbon-to-nitrogen ratio of the free POM (i.e., greater N concentration of free POM).

Taken together, results from this screening study suggest that cover crop mixtures that combine complementary plant traits may be appealing to farmers because they provide several ecosystem services at once. However, mixtures were not able to sustain high levels of all of these services, indicating that there are trade-offs. From the perspective of ecological nutrient management, mixtures that include legumes are beneficial for retaining and recycling nutrients, suppressing weeds, and also supplying a new source of N to fields, which the non-legume cover crops cannot provide. In diversified vegetable farms, in particular, the N from legume N fixation in mixtures – while lower than N input from legume monocultures – may be sufficient to meet yield goals and crop N needs. Farmers in this study also explained that the cost of legume seed is generally not a large deterrent to them, since they have relatively small farms and high value crops.

Depending on specific management goals, however, monocultures may be more desirable. Rye is the most reliable cover crop choice for winter hardiness and weed suppression. And a legume monoculture may be desirable for large N supply. In both studies the performance of mixtures tended to vary more than the performance of monocultures (across farms in the common garden study, and across both farms and years in the rye/vetch study). This highlights the need for more research on mixtures to improve their management in variable environmental conditions.

Finally, our results show that variation in soil properties due to management history and soil type can explain variation in biomass of different cover crop species within mixtures. An important next step will be to build on this work to improve decision tools for farmers regarding cover crop mixtures. For instance, better understanding this variation would inform adjustments to seeding proportion and rate recommendations for mixtures in different soil conditions. Overall, increasing plant diversity in crop rotations with cover crop mixtures holds promise for moving all farms – both conventional and organic – along a continuum from reliance on purchased inputs to managing ecological processes for particular functions and goals, which can increase farm sustainability.

Outputs:

<u>Farmer Engagement</u>
Farmer engagement took several forms in this research. First, farmers were closely involved in study design and management decisions for the experimental fields. Much of this discussion occurred at annual project meetings with all participants. These were held on 3/9/2016, 3/15/2017, and 3/21/2018. Meetings were used to make plans for different phases of the experiments, and to share and discuss study findings throughout the project. At the 2018 meeting we discussed final results and focused on farmers' perceptions of study findings, solicited their input on tailoring final research reports to meet their needs, and we generated a list of future research ideas and questions that would be useful to address.

Second, early in the project we hosted two field days on participating farms. The first was on 9/3/2015, and the second was 9/8/2016. The field days were organized with collaborators at Michigan State University. During the events, attendees visited both experimental fields and we gave a presentation on our research goals and findings, and on principles of ecological nutrient management. We then facilitated a broader discussion about cover crops and soil fertility management on organic vegetable farms.

Figure 12. Sharing and discussing final project findings with the core farmer participant group at a project meeting in March of 2018, Ann Arbor, MI.

Third, to share findings with the broader farming community, PI Blesh attended the MOSES conference in LaCrosse, WI in both 2016 and 2017 with her lab manager, Beth VanDusen. In 2016, Blesh co-led a workshop at MOSES with Dr. Julie Grossman (University of Minnesota), which included early results from this study. We also presented a version of the results geared towards farmer outreach in a poster at the 2017 MOSES conference.

Publications

One paper from the common garden study has already been published, and is attached to this report:

Blesh, J. 2017. Functional traits in cover crop mixtures: biological nitrogen fixation and multifunctionality. Journal of Applied Ecology: doi:10.1111/1365-2664.13011.

This article was invited as part of a Special Feature in this journal on functional traits in agroecology.

A second publication from the Common Garden study is in preparation:
Blesh, J., VanDusen, B., and D. Brainard. In Prep. Ecosystem functions from cover crop mixtures on organic vegetable farms.

An additional three to four publications will result from the rye/vetch study. Because that project spanned multiple growing seasons, we are still in the final stages of data analysis and will begin writing those papers soon. Furthermore, two Master's thesis projects also resulted from this grant, leveraging the experimental plots already in place. One student, Tianyu Ying, measured decomposition dynamics in the vetch/rye plots on two of the participating farms. He used litter bags and anion resin beads to compare litter decomposition and nitrogen release rates in the cover cropped area to the no cover control over a summer following cover crop termination. The second student, Santiago Bukovsky-Reyes, analyzed root biomass and root traits such as root length and root diameter in the vetch/rye study. The goal of his study was to understand how belowground interactions between species in mixtures influence the aboveground processes we measured, like legume nitrogen fixation rates, and to determine how soil properties across the management gradient affect root traits. We expect to submit these remaining articles for publication by the end of summer 2018.

Presentations
The following is a list of presentations for both academic and farmer audiences in which findings from this research were shared.

Blesh, J. 2015 and 2016. Field Day: Organic Vegetable Twilight Meeting; Zilke Vegetable Farm, Milan, MI, September.

Grossman, J. and J. Blesh. 2016. Beyond rye: summer and winter cover crops. Co-led Workshop at the Midwest Organic and Sustainable Education Service (MOSES) Conference, La Crosse, WI, February 26.

VanDusen, B. and J. Blesh. 2017. Assessing performance of cover crop mixtures in organic vegetable cropping systems. Poster presentation at the Midwest Organic and Sustainable Education Service (MOSES) Conference, La Crosse, WI, February 23-25.

Blesh, J. 2018. The role of ecological and interdisciplinary research in developing multifunctional agroecosystems. Invited seminar series speaker, Graduate Degree Program in Ecology (Series theme, “Multi-Functional Ecology: Perspectives across Scales and Systems”), The Pennsylvania State University, State College, PA, March 19.

Blesh, J. 2017. Functional traits in agroecosystems: managing plant diversity for multifunctionality. Green Life Science Symposium, University of Michigan, Ann Arbor, MI, October 21.

Blesh, J. 2016. Assessing on-farm performance of cover crop mixtures in vegetable cropping systems. ASA-CSSA-SSSA International Annual Meeting, Phoenix, AZ, Nov. 6-9.

Blesh, J. 2016. Ecosystem management for resilient and multifunctional food systems. Invited Guest Seminar Intersections Seminar Series, Department of Geography, University of Toronto, Toronto, Ontario, Canada, November 18.

Blesh, J. 2016. Cropping system diversity, resilience, and multifunctionality. Center for Molecular and Clinical Epidemiology of Infectious Diseases (MAC-EPID) Symposium: Agriculture: Health Benefits, Health Risks, and Environmental Impact, Pre-symposium workshop, University of Michigan, April 14.

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FUNCTIONAL TRAITS IN AGROECOLOGY

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Functional traits in cover crop mixtures: Biological nitrogen fixation and multifunctionality

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Ceres Trust Organic Research Initiative

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Abstract

1. Cover crop mixtures with complementary plant functional traits including biological nitrogen fixation (BNF) may supply nitrogen (N) to farm fields while simultaneously providing other ecosystem functions such as N retention and weed suppression (i.e., multifunctionality). Understanding variation in these relationships across farms can help advance trait-based research in agroecology and ecological approaches to nutrient management.
2. This on-farm experiment explored the contributions of two- and three-species cover crop mixtures, which combined legumes, brassicas and cool season grasses, to ecosystem functions across a gradient of soil fertility levels driven by farm management history.
3. I evaluated the predictions that functional trait diversity of the cover crops would explain variation in multifunctionality, and that legume biomass and BNF within mixtures would be inversely correlated with indicators of soil N availability from organic matter across the farm gradient.
4. Ecosystem functions varied widely across farms. As expected, functional diversity was a significant predictor of multifunctionality, although the relationship was weak. Cover crop mixtures had significantly greater multifunctionality than a cereal rye monoculture, though not at the highest observed levels of each function, indicating trade-offs among functions. Linear regression models showed that legume biomass and BNF were negatively correlated with soil properties indicative of N availability from soil organic matter, whereas non-legume and weed biomass were positively correlated with other measures of soil fertility.
5. *Synthesis and applications*. Cover crop mixtures can increase functional diversity within crop rotations. Designing mixtures with complementary plant traits may be particularly effective for increasing multifunctionality and agroecosystem sustainability. On-farm research to understand variation in biological nitrogen fixation, which is both a plant trait and a key ecosystem function, across heterogeneous soil conditions, can inform management of soil fertility based on ecological principles.

KEYWORDS
agroecology, biological nitrogen fixation, cover crops, ecological nutrient management, functional diversity, functional trait, multifunctionality, on-farm research, plant traits, soil fertility

1 | INTRODUCTION

In agroecosystems, small increases in biodiversity can lead to large benefits for ecosystem function (Drinkwater, Wagoner, & Sarrantonio, 1998; Jackson, Pascual, & Hodgkin, 2007; Tiemann, Grandy, Atkinson, Marin-Spiotta, & McDaniel, 2015). The specific impacts of this "intended" biodiversity on agroecosystem processes can be evaluated based on species richness, other taxonomic diversity metrics, or more recently, plant functional traits (Garnier & Navas, 2012; Martin & Isaac, 2015; Petchey & Gaston, 2006; Wood et al., 2015). By managing functional trait diversity, farmers manipulate ecological interactions such as competition or facilitation to support ecosystem functions including nutrient supply, nutrient retention, weed and pest suppression, and organic matter accrual (Shennan, 2008).

Cover crop mixtures can increase the functional trait diversity of crop rotations during windows between cultivation of primary crops. Increasingly, a wide range of farmers express interest in planting multi-species cover crop mixtures to enhance ecosystem functions (CTIC, SARE & ASTA, 2016). To date, much of the empirical research on biodiversity and ecosystem function has focused on single functions, but there is growing interest in understanding the relationship between diversity and *multifunctionality*, which is defined as the simultaneous enhancement of multiple ecosystem functions (Byrnes et al., 2014). Recent research in natural ecosystems indicates that when considering multiple ecosystem functions together, increasing species richness may augment complementary functions (Mori et al., 2016; Zavaleta, Pasari, Hulvey, & Tilman, 2010).

Within agroecosystems, however, studies on cover crop mixtures have not found strong relationships between species diversity and multiple ecosystem functions even though the mixtures over-yielded compared to monocultures (Smith, Atwood, & Warren, 2014; Wortman, Francis, & Lindquist, 2012). Finney and Kaye (2017) found that increasing cover crop species richness of an agroecosystem only weakly correlated with multifunctionality. Instead, metrics of functional diversity based on plant functional traits of the cover crop mixtures—fall and spring growth rates and shoot C:N—better predicted multifunctionality in their field experiment. Similarly, Storkey et al. (2015) reported that cover crops of one- to four-species, which represented contrasts in functional traits such as biological N fixation (BNF) and phenology, enhanced the provisioning of ecosystem services compared to higher diversity mixtures. Taken together, these studies suggest that cover crop mixtures that combine complementary plant functional traits may optimize agroecosystem functions.

Symbiotic dinitrogen (N_2) fixation by legume species is a particularly valuable plant trait in agroecosystems. Crop rotations with BNF as the primary N source can have low or no N surpluses; that is, field-scale N inputs and harvested N exports are approximately in balance (Blesh & Drinkwater, 2013; Zhang et al., 2015). Legumes may down-regulate BNF and increase their dependence on soil N as soil organic N pools increase because of the energetic cost of supplying C to their symbiotic partners (Kiers, Rousseau, West, & Denison, 2003). Furthermore, overwintering legume cover crops reduce nitrate leaching because winter cover extends the timeframe of plant N uptake (Tonitto, David, & Drinkwater, 2006). Legumes can also contribute to long-term accumulation of soil organic matter (SOM) (e.g., Drinkwater et al., 1998).

Although BNF is often characterized as a binary functional category (Brooker et al., 2015)—presence or absence of legumes—the rate of N fixation (% N from fixation) varies within and across legume species. BNF can therefore be considered both a continuous plant trait *and* a critical ecosystem function that provides a new input of fixed N. The N fixation rate varies with competitive interactions in mixtures; for example, in legume-grass mixtures the legume's reliance on BNF increases due to competition for soil N by the grass species (e.g., Jensen, 1996). Legume N fixation is also likely to vary with soil fertility and management history. For instance, the outcome of competitive and facilitative interactions between legumes and non-legumes in mixtures may vary with soil fertility status and N supply from SOM (Schipanski & Drinkwater, 2011). However, as long as there are effective rhizobia in the soil, the N supply from BNF will largely be governed by total legume biomass production rather than by the % of legume N from fixation (Crews et al., 2016; Schipanski & Drinkwater, 2011).

This study integrates functional ecology and ecological nutrient management frameworks to assess how soil fertility status affects ecosystem functions from cover crop mixtures across working farms. The specific objectives are to: (1) test relationships between functional trait diversity of cover crop mixtures and multifunctionality; and (2) identify soil characteristics that explain variation in BNF in cover crop mixtures across farms. I evaluated nine cover crop treatments with 1, 2, or 3-species, along with a no cover crop control, on eight organic vegetable farms in southeastern Michigan. Treatments harnessed contrasts in several continuous and complementary plant traits: BNF, fall and spring growth rates, and shoot C:N ratio. I used three ecosystem functions to assess multifunctionality: N supply from BNF, weed suppression during the cover crop season, and N retention in above-ground biomass. I expected monocultures to maximize individual functions compared to mixtures, and functional diversity of the treatments to predict multifunctionality. I also predicted that legume biomass and BNF would be inversely correlated with measures of N availability from SOM, and that plant-available phosphorus (P) would correspond with greater legume and weed biomass.

2 | MATERIALS AND METHODS

2.1 | Experimental design

In the winter and spring of 2014, I recruited eight vegetable farmers in southeastern Michigan who manage their farms organically to investigate how cover crop mixtures combining diverse functional traits impact ecosystem functions. Farms had been in organic vegetable production from 1 to 13 years, and fields represented a gradient in soil fertility due to management history.

Six of the cover crop treatments were mixtures that included a legume and a grass species. The mixtures combined winter- and non-winter-hardy species, except for one treatment with three species that winter-kill (LN+YM+OA).[1] As a result, mixtures represented combinations of complementary plant functional traits: fall growth potential (kg/ha growing degree day [gdd]$^{-1}$), spring growth potential (kg ha^{-1} gdd^{-1}), C:N ratio of plant shoots, and BNF. The study also had three single-species treatments—including cereal rye (CR), which is the most common cover crop grown in the region and thus a useful benchmark for comparison—as well as a no cover crop control (Table S1, Appendix S1).

All experimental treatments were established on farms between 13–20 August 2015, in a randomized complete block design with four replicates. Each plot was 2.4 × 2.4 m (5.95 m^2). Legume seeds were inoculated with the appropriate inoculant (Nitragin® Gold or N-Dure®) at a rate of *c.* 4 g/kg seed.

2.2 | Soil sampling and analysis

Soil samples for baseline characterization of soil properties and metrics of soil nutrient cycling capacity were collected before establishment of the experiment from *c.* 20 soil cores (2 cm diameter by 20 cm depth), composited per experimental field to represent the initial conditions of each site. Since these were diversified vegetable farms, fields were relatively small (283–590 m^2, or 0.03–0.06 ha), flat, and homogeneous. I measured bulk density from the fresh weight of 8 cores per field using a field scale, and adjusted for soil moisture. Soil was processed immediately for soil moisture and extractable inorganic NO_3^- and NH_4^+). Triplicate soil subsamples were sieved for inorganic N determination and for a 7-day anaerobic N mineralization incubation (Drinkwater, Cambardella, Reeder, & Rice, 1996) followed by extraction with 2 M KCl. The amount of NH_4^+ and NO_3^- in each sample was analysed colorimetrically on a continuous flow analyser (AQ2; Seal Analytical, Mequon, WI). Remaining soil was air-dried before further analysis.

Soil organic matter has different fractions representing a continuum of accessibility to microbial decomposition, which therefore supply N over different time-scales. Soil particulate organic matter (POM) fractions, in particular, respond to changes in management on shorter time-scales (years to decade), and are indicators of soil nutrient supplying capacity relevant for guiding farm management decisions (Wander, 2004). Light fraction POM (also called free POM, or fPOM), and occluded POM (oPOM; i.e., physically protected POM), were separated on triplicate 40 g subsamples using a size and density fractionation method (Marriott & Wander, 2006, Appendix S2). Total soil C and N (to 20 cm) were measured by dry combustion on a Leco TruMac CN Analyser (Leco Corporation, St. Joseph, MI), and the C and N content of fPOM and oPOM were measured on a Costech ECS 4010 CHNS Analyser (Costech Analytical, Valencia, CA). A subset of *c.* 100 g of sieved dried soil was analysed for particle size (texture), pH, Bray-1 P, K, and other macro- and micro-nutrients at A & L Great Lakes Laboratories, Inc. (Fort Wayne, IN).

[1]List of treatments and abbreviations (see also, Table S1): (1) Crimson clover, Medium red clover, and spring wheat (CC+RC+SW); (2) Austrian winter pea, oat, and daikon radish (WP+OA+DR); (3) Lentil, yellow mustard, and oat (LN+YM+OA); (4) Medium red clover and spring wheat (RC+SW); (5) Crimson clover and spring wheat (CC+SW); (6) Chickling vetch and cereal rye (CV+CR); (7) Austrian winter pea (WP); (8) Cereal rye (CR); (9) Spring wheat (SW); and (10) weedy fallow control.

2.3 | Above-ground biomass sampling and C and N analysis

Above-ground biomass in all treatments was sampled in the fall between 5 and 22 October, 2015, and in the spring between 26 April and 18 May 2016 from one random 0.25 m^2 section of each replicate plot avoiding plot edges. Biomass was cut at the soil surface, separated by species (weeds were combined into one pool), dried at 60°C for 48 hr, weighed, and ground in a Wiley mill. Shoot biomass was analysed for total C and N by dry combustion on a Leco TruMac Analyser. Samples for isotope analysis were pulverized using a cyclone mill and analysed at the UC Davis Stable Isotope Facility (see Section 2.4).

2.4 | Legume N fixation

I estimated BNF using the natural abundance method (Shearer & Kohl, 1986). Briefly, legume and reference plant biomass (from CR and SW monocultures) were analysed for ^{15}N enrichment and total N content using a continuous flow Isotope Ratio Mass Spectrometer (Stable Isotope Facility, UC Davis).

The %N derived from fixation was calculated using the following mixing model:

$$\%\text{N from fixation} = 100 \times ((\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{legume}}) / (\delta^{15}\text{N}_{\text{ref}} - B))$$

where $\delta^{15}N_{ref}$ is the $\delta^{15}N$ signature of the reference plant, $\delta^{15}N_{legume}$ is the $\delta^{15}N$ signature of the legume, and *B* is defined as the $\delta^{15}N$ signature of a legume when dependent solely on atmospheric N_2. *B* values were determined by growing each legume species in the greenhouse in a N-free medium (Appendix S2).

2.5 | Calculation of functional diversity

To link ecosystem functions to functional diversity, I calculated Rao's Quadratic Entropy (Rao) (Rao, 1982; Schleuter, Daufresne, Massol, & Argillier, 2010) for each treatment using *FDiversity* software (Casanoves, Pla, Di Rienzo, & Dıaz, 2011) (Appendix S3). I used the total above-ground biomass at the fall sampling date to weight the index by abundance, since at that time all species, including the non-overwintering species, were represented in the plots (i.e., some of the mixture treatments became monocultures following winter-kill). The functional diversity index included four continuous plant functional traits: fall growth potential (kg ha^{-1} gdd^{-1}), spring growth potential (kg ha^{-1} gdd^{-1}), C:N ratio of plant shoots, and proportion of legume shoot N from fixation. To avoid scale effects, trait values were standardized to have zero mean and unit variance.

2.6 | Calculation of multifunctionality

Four ecosystem functions were measured: total above-ground biomass production, N retention in above-ground biomass, N supply from

BNF, and weed suppression (Appendix S3). Following Byrnes et al. (2014), I calculated a threshold-based index of multifunctionality, selecting three threshold levels potentially relevant to management (Table 1). Total above-ground biomass correlated with weed suppression, N retention, and BNF (see Section 3). I therefore did not include biomass in the calculation of multifunctionality, and the maximum score in the index was 3. I calculated multifunctionality at three different threshold levels (30%, 50%, and 75%) of the maximum observed level of each function, where the maximum value was the mean of the top 10 observations for each function across farms (Table 1). For example, a treatment would receive a multifunctionality score of 3 at the 30% threshold if BNF input was greater than 46 kg N/ha, and weed suppression was greater than 1,705 kg dm ha^{-1}, and soil N retained in biomass was greater than 59 kg/ha. I applied a square root transformation to the data for the three functions prior to calculating the maximum value since the distributions were skewed.

2.7 | Statistical analysis

All statistical analyses were computed in R (The R Foundation for Statistical Consulting, Vienna, Austria) using the *lme4* package for linear, mixed-effect models with treatment as a fixed effect and block nested in farm as a random effect. Comparison of least square means was performed using Tukey's honestly significant difference (HSD). Results are reported as statistically significant at $\alpha = 0.05$.

Since legume biomass governs the N supply from BNF (e.g., Crews et al., 2016), as well as the abundance of the N fixation trait within cover crop mixtures, I used linear regression to model above-ground biomass for each species in each treatment as a function of soil properties. I first selected a subset of soil predictors using information from the correlation matrix of all soil parameters (due to multicollinearity among soil variables and small sample size), and specific hypotheses about parameters that may drive variation across farms. Model selection was also informed by model comparisons to assess goodness-of-fit with the Akaike Information Criterion. In addition to legume biomass, I also modeled the BNF trait—both above-ground N from fixation and % of shoot N from fixation (and % from soil)—for the legume species. Along with the soil predictors, I also included legume and weed biomass in these regressions, and then dropped weed biomass for most of the models, which had better fits without this predictor.

TABLE 1 Multifunctionality assessment considering three ecosystem functions: N supply from BNF, weed suppression and N retention in above-ground plant biomass. Maximum levels for each function across all treatments (determined by taking the mean of the top 10 observations across sites), and three different threshold levels (i.e. 30%, 50% or 70% of the maximum level)

Ecosystem function	Maximum level	Threshold 30%	50%	75%
N supply from BNF (kg N/ha)	154	46	77	115
Weed suppression (kg dm ha^{-1})	5,683	1,705	2,842	4,262
N retained in biomass (kg N/ha)	196	59	98	147

3 | RESULTS

3.1 | Baseline soil properties

Soil analyses from farm fields indicated a gradient of soil fertility that reflected different farm management histories as well as underlying soil type (Table S2). All soils were Alfisols or Mollisols. Bray-1 P concentrations ranged from 4 to 88 ppm. Total organic C varied twofold from 27 to 52 Mg/ha. Potentially mineralizable N, fPOM pool size, and the quality of fPOM and oPOM pools (C:N) reflect soil N availability from more recent management practices and organic matter inputs (Wander, 2004); fPOM pool size varied from 8.6 to 27.1 Mg/ha and the N content of the oPOM pool ranged from 90.4 to 231.2 kg/ha (Table S2).

3.2 | Ecosystem functions during the cover crop season

Ecosystem functions for all treatments are shown in Figure 1. Of the monocultures, CR provided the greatest biomass production, weed suppression (relative to the no cover crop control), and N retention. Weed biomass was high, averaging 3,981.9 ± 352.9 kg/ha for the cover crop season. CR biomass was more than twofold greater than SW and WP monoculture biomass, and biomass in SW, WP, RC+SW and LN+YM+OA treatments was lower than weed biomass in the control. CV+CR mixture biomass was not significantly different from CR, and several mixtures were not significantly different from CR in terms of weed suppression (CV+CR, LN+YM+OA, CC+SW) and N retention (CV+CR, LN+YM+OA). Mean fixed N inputs were lowest in CV+CR (fall BNF only; 13.5 kg N/ha). For the treatments with overwintering legumes, mean BNF ranged from 33.4 (RC+SW) to 59.0 (CC+RC+SW) kg N/ha (Figure 1, top panel). Mean soil N retention in plant biomass varied from 30.8 to 101.9 kg N/ha (in WP and CR, respectively), and mean total above-ground N accumulation (soil plus fixed N) ranged from 54.0 to 118.9 kg N/ha (in SW and CC+CR+SW, respectively).

Ecosystem functions varied widely across farms, in part driven by differences in cover crop biomass (Figures 2 and 3). For soil N retained in cover crop biomass (Figure 2, top), treatments without legumes had a greater amount of soil-derived N per unit biomass than did the treatments with legume species, and for both plant types this relationship was relatively strong (non-legume $R^2 = 0.69$, and legume $R^2 = 0.42$). The relationship between biomass and weed suppression (Figure 2, bottom) was weaker, though significant ($R^2 = 0.31$ for non-legumes, and 0.17 for legumes; $p < .0001$), but the treatments with legume species had more variable weed suppression per unit cover crop biomass than did treatments with non-legumes only.

Across farms and legume species, the N supplied from BNF varied from 7 to 268 kg N/ha (Figure 3). There was a strong relationship between legume above-ground biomass and N from fixation (Figure 3; $R^2 = 0.95$, 0.91, and 0.82 for WP, CC and RC combined, and CV,

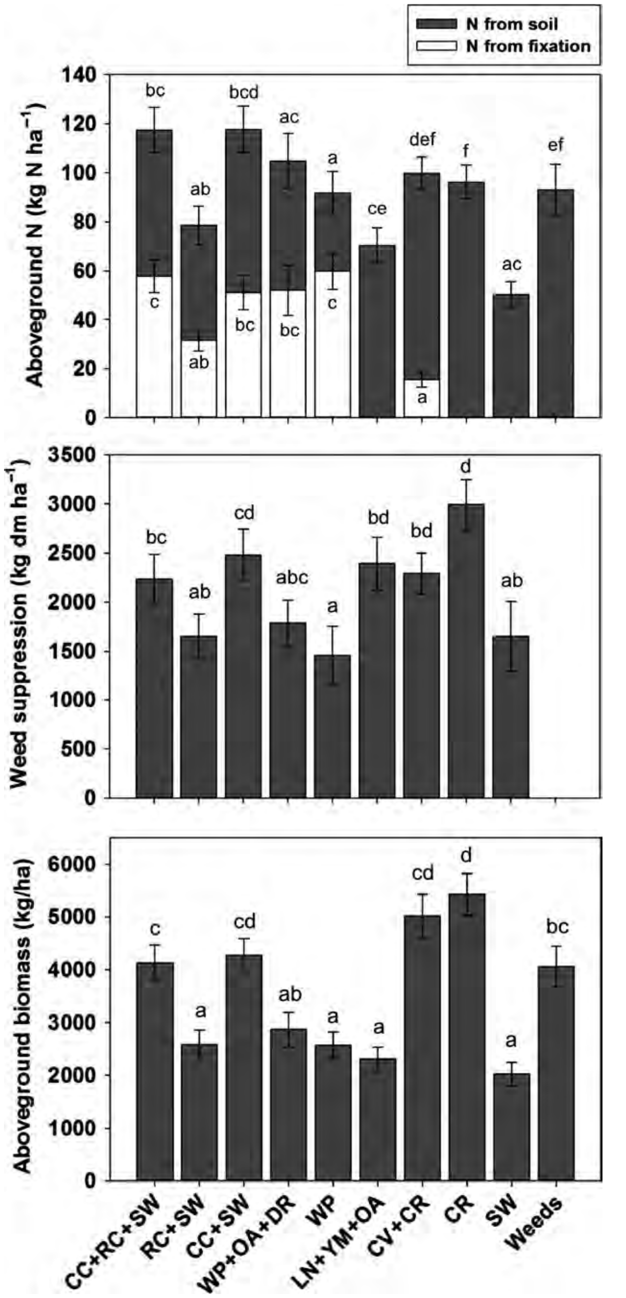

FIGURE 1 Treatment means and SEs for ecosystem functions across farms for fall and spring combined. Top panel: N retention (grey portion of bar) and biological N fixation (white portion of bar); Middle: weed suppression; and Bottom: Above-ground biomass, combining species, and fall and spring sampling times. “Weeds” is the no cover control. Mean values labelled with the same letter were not significantly different at $p < .05\%$ (Tukey’s HSD). CC = Crimson clover; RC = Red clover; SW = Spring wheat; WP = Austrian winter pea; OA = Oat; DR = Daikon radish; LN = Lentil; YM = Yellow mustard; CV = Chickling vetch; and CR = Cereal rye

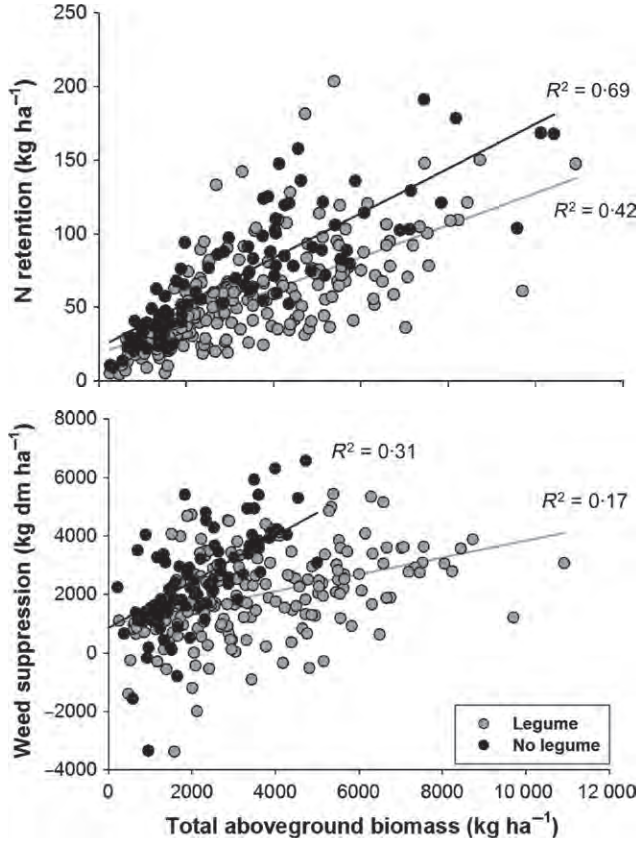

FIGURE 2 Regression relationships for total above-ground biomass (fall and spring sampling time points combined) and other ecosystem functions. Top: N retention (equal to total above-ground biomass N minus N from BNF), and Bottom: weed suppression (equal to weeds in control minus weeds in the treatment). Treatments are aggregated by those that include a legume species (grey symbols) and those that do not have a legume (black symbols)

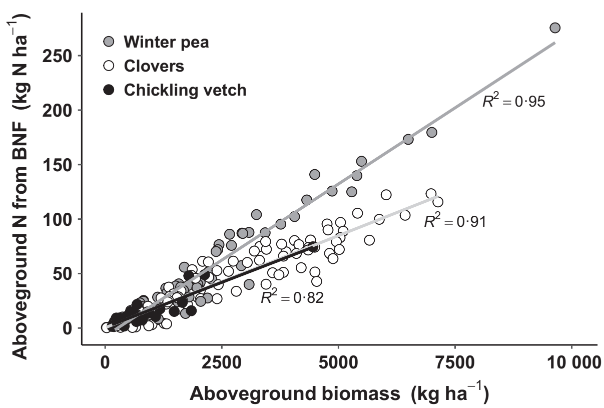

FIGURE 3 Relationships of legume above-ground biomass (combined fall and spring sampling points for winter pea and clovers) and the N supply function (total fixed N in above-ground biomass). Red clover and crimson clover are combined as ‘clovers’. Observations from all treatments were included in the analysis. Regression equations by species are: $y = 0.028x−7.46$ (winter pea; $N = 64$); $y = 0.017x + 0.70$ (chickling vetch; $N = 26$); $y = 0.018x−6.50$ (crimson clover; $N = 64$); and $y = 0.022x−0.84$ (red clover; $N = 64$)

respectively). The slope of this relationship was greatest for WP. For the mixtures, there was also a positive relationship between legume biomass as a proportion of total mixture biomass and N supply from BNF (data not shown).

3.3 | Multifunctionality

The CV+CR treatment had the greatest functional diversity score (Rao), followed by all other treatments with legumes, with the exception of LN+YM+OA in which the lentil (LN) performed poorly (Table 2). There was a significant relationship across treatments and farms between cover crop functional diversity and multifunctionality, but functional diversity only explained a small portion of the variation in multifunctionality (Figure 4a; $p = .0003$; $R^2 = 0.05$). At the 30% threshold, the CC+RC+SW mixture had the greatest mean multifunctionality index (Figure 4b and Table 2; 2.5). This score was not significantly different from WP+OA+DR (2.3), CC+SW (2.4), or the WP monoculture (2.2). The control (no cover) had the lowest level of multifunctionality at the 30% level. For all treatments, the mean number of ecosystem functions provided decreased as the threshold increased (Figure 4b). Comparing the mixtures to the CR monoculture, three mixtures had a score significantly greater than CR at the 30% threshold. At 50%, their scores started to overlap with CR, and at the 75% level, all of the multifunctionality scores were low, and were not different from CR (Table 2). There was a significant, but weak relationship between biomass and multifunctionality (Figure S1; $p < .0001$; $R^2 = 0.14$) for observations from all treatments and farms.

3.4 | Soil characteristics as predictors of BNF across farms

To understand drivers of variation in the N fixation trait across farms, regression using soil properties to model biomass across the 8 farm fields was separated into biomass for legume species (Table 3), non-legumes (grasses and brassicas, Table 4), and weeds (Table 5) for each treatment. Model selection identified soil properties to include in the regression models that: (1) were not strongly correlated with one another, and (2) tested hypotheses about SOM fractions that are responsive to management and have faster turnover times than the total SOM pool. As expected, biomass for some legume species was negatively correlated with soil properties indicative of soil N cycling capacity (Table 3): RC was positively related to the C:N of the fPOM (i.e., higher C:N reflects fPOM of lower N fertility), and CC and WP biomass were negatively correlated with the size of the fPOM pool (i.e., quantity of fPOM). Both CC and WP biomass were negatively related to the oPOM N pool. WP biomass in both monoculture and mixture was positively correlated with plant-available P, but this coefficient was not significant for other legume species. None of the models were significant for predicting RC biomass across the farms, and the model for CV had a low R^2 (0.31). Models for the other legume species were

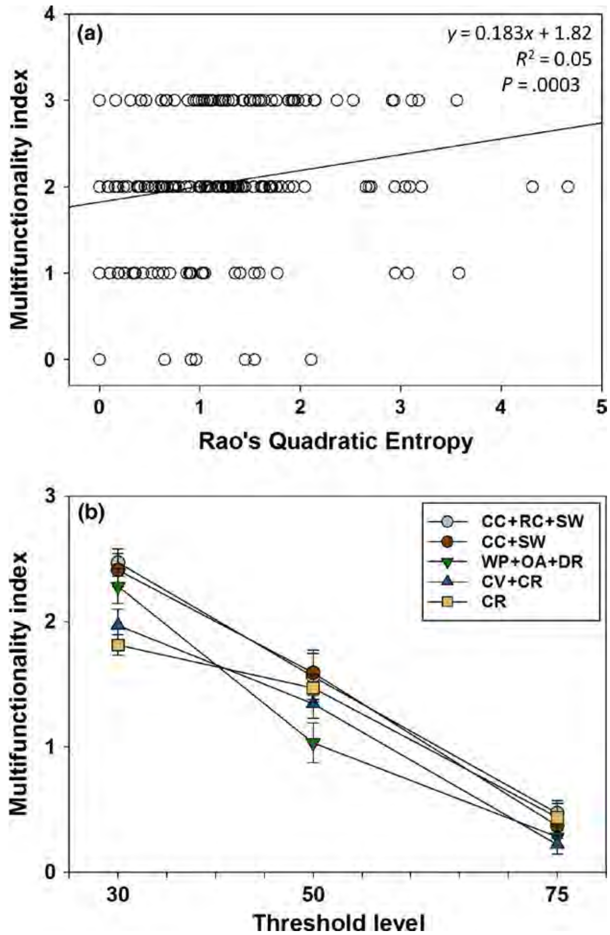

FIGURE 4 (a) Relationship between Rao's quadratic entropy (functional diversity) and multifunctionality for all cover crop treatments combined (for the 30% threshold level), and (b) mean multifunctionality index (with *SE*) at the 30%, 50% and 75% levels showing only the top five treatments at the 30% threshold level [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Mean (±*SE*) Rao's quadratic entropy (Rao) and multifunctionality index value by treatment. Values labelled with the same letter were not significantly different at $p < .05$% (Tukey's HSD). Treatments with the largest index for each multifunctionality threshold level are in bold font

Cover crop	Rao		MF 30%		MF 50%		MF 75%	
CC+RC+SW	1.2 ± 0.1	c	**2.5** ± 0.1	f	**1.6** ± 0.2	cd	0.5 ± 0.1	b
WP+OA+DR	1.4 ± 0.1	c	**2.3** ± 0.1	ef	1.0 ± 0.2	abc	0.3 ± 0.1	ab
LN+YM+OA	0.5 ± 0.1	b	1.7 ± 0.1	bc	0.9 ± 0.1	ab	0.3 ± 0.1	ab
RC+SW	1.1 ± 0.1	c	1.9 ± 0.2	cde	1.0 ± 0.2	abc	0	a
CC+SW	1.5 ± 0.1	c	**2.4** ± 0.1	f	**1.6** ± 0.2	d	0.4 ± 0.1	ab
CV+CR	2.1 ± 0.2	d	2.0 ± 0.1	cde	**1.3** ± 0.1	bd	0.2 ± 0.1	ab
WP	0	a	**2.2** ± 0.1	df	0.9 ± 0.2	ab	0.3 ± 0.1	ab
CR	0	a	1.8 ± 0.1	cd	**1.5** ± 0.1	cd	0.4 ± 0.1	b
SW	0	a	1.4 ± 0.1	ab	0.7 ± 0.1	a	0.1 ± 0.1	ab
Control (weeds)	—		1.0 ± 0.1	a	0.8 ± 0.1	a	0.3 ± 0.1	ab

TABLE 3 Regression coefficients and *SEs* (in parentheses) for regression analysis of legume biomass in each cover crop treatment using baseline soil properties as predictors. Coefficients in bold font are significant, and the estimated model fit is indicated by the R^2 and adjusted R^2

Legumes								
Treatment:	CC+RC+SW	CC+SW	OA+WP+DR	WP	Treatment:	CC+RC+SW	RC+SW	CV+CR
Species:	CC	CC	WP	WP	Species:	RC	RC	CV
Intercept	7,089 (1,783)	9,391 (1,787)	−1,593 (1,706)	2,853 (1,026)	Intercept	−405 (708)	−4,029 (1,023)	−25.9 (928)
Silt+Clay (%)	**83.7**** (27.9)	**90.7**** (28.0)	**93.4***** (24.0)	**60.9***** (16.1)	Clay (%)	9.5 (17.3)	16.4 (25.1)	−2 (22.8)
oPOM N	**−4,808***** (1,117)	**−5,978***** (1,119)	**−2,670***** (960.0)	**−2,008**** (642.6)	C:N fPOM	26.5 (34.1)	**255.9***** **(49)**	−1.9 (44.6)
fPOM pool	−98.8 (51.4)	**−138.8*** (51.5)	**−165.2**** (44.2)	**−111.9***** (29.6)	fPOM pool	24.1 (16)	12.6 (23)	**78.4***** **(21)**
Bray-1 P	12.5 (10.7)	18.4 (11.8)	**73.9***** (9.2)	**47.5***** (6.2)	Bray-1 P	0.13 (4.4)	3.5 (6.3)	−2.4 (5.7)
R^2	0.60	0.71	0.79	0.79	R^2	0.13	0.57	0.40
Adjusted R^2	0.54	0.67	0.76	0.76	Adjusted R^2	0	0.51	0.31
N	32	32	32	32	N	32	32	31

CC, Crimson clover; WP, Winter pea; RC, Red clover; CV, Chickling vetch.
Significance: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

strong (Table 3, R^2 = 0.60–0.79), particularly considering the relatively small number of sites and high variation typical of environmental data.

The models with the greatest fit for the non-legumes (Table 4) were for SW biomass in CC+SW (R^2 = 0.52), SW biomass in RC+SW (R^2 = 0.63) and CR biomass in CV+CR (R^2 = 0.58). Non-legume biomass was positively correlated with higher soil fertility; i.e., larger fPOM pool size, higher concentration of plant-available P, and higher % clay (for CR), which is often correlated with total SOM. Models for weed biomass within the cover crop treatments (Table 5) were weaker (R^2 = 0.20–0.37), with the highest R^2 for the model of weed biomass in the no cover crop control (R^2 = 0.46). Weed biomass was also positively correlated with indicators of soil fertility including % clay, a narrower C:N (i.e., a negative relationship with the C:N of the fPOM), soil P concentration (for weeds in LN+YM+OA and in the control), and with the size of the fPOM pool (for weeds in control).

Legume biomass was the strongest predictor in the models of BNF (i.e., shoot N from fixation in kg N/ha; p < .0001 for all species; Figure 3). Total shoot N fixed by WP in WP+OA+DR and RC in RC+SW was negatively correlated with weed biomass (p = .007 and .046, respectively); these two mixtures also had the lowest weed suppression (Figure 1, middle). BNF (kg N/ha) by CC in CC+RC+SW and CC+SW was negatively correlated with % clay. RC BNF was positively correlated with soil P for both RC treatments (p = .03 and .04), and CC and RC % N from soil was inversely related to soil P concentration (p = .008 for CC in CC+RC+SW; p = .0001 for all others). Models of % legume N from soil were also positively correlated with total legume biomass. In contrast with legume biomass and above-ground N from fixation, soil properties did not predict the % of above-ground N from fixation for any species, although CC % N from fixation in the spring (in both treatments) was positively related to increasing C:N of the fPOM pool (i.e., lower N availability). Models for legume biomass as a proportion of total mixture biomass had lower predictive power than models for legume biomass itself, but showed similar correlations with soil properties.

4 | DISCUSSION

Functional trait diversity can provide multiple benefits in agroecosystems (Martin & Isaac, 2015). For example, cover crop mixtures that include legumes can supply N while simultaneously providing other ecosystem functions (e.g., Schipanski et al., 2014). An emerging ecological framework for nutrient management has demonstrated increased N use efficiency in rotations with legume N sources, winter cover crops, and/or perennials (Blesh & Drinkwater, 2013; Drinkwater et al., 1998; Gregorich, Drury, & Baldock, 2001). Since winter cover crops can increase functional diversity without requiring major changes to crop rotations, the practice is applicable to a broad range of farms.

Building on evidence suggesting that functional diversity in cover crop mixtures predicts multifunctionality (Finney & Kaye, 2017), I tested the hypothesis that cover crop mixtures selected to leverage contrasts in plant traits—shoot N concentration, timing of peak growth, and BNF—would provide greater multifunctionality compared to cover crop monocultures and a no cover crop control across farms in southeastern

TABLE 4 Regression coefficients and *SEs* (in parentheses) for regression analysis of non-legume biomass in each cover crop treatment using baseline soil properties as predictors. Coefficients in bold font are significant, and the estimated model fit is indicated by the R^2 and adjusted R^2

Non-legumes										
Treatment:	**CC+RC+SW**	**CC+SW**	**RC+SW**	**SW**	**LN+YM+OA**	**LN+YM+OA**	**OA+WP+DR**	**OA+WP+DR**	**CV+CR**	**CR**
Species:	**SW**	**SW**	**SW**	**SW**	**YM**	**OA**	**OA**	**DR**	**CR**	**CR**
Intercept	−158.2 (932)	590·4 (1,005)	−842 (965)	160·3 (1,438)	−197 −1,267	−236 (455)	269 (202)	153.3 (553)	135.9 (2,071)	2,240 (2,469)
Clay (%)	−20.2 (22.9)	−24.9 (25)	−54.9* (23·6)	−38.1 (35.2)	41.1 −31	11 (11.1)	3.5 (4.9)	−20.9 (13.5)	**232*** (51)**	210** (60.5)
C:N free POM	21.1 (44.8)	−47.5 (48.4)	44.5 (46.4)	39.7 (69.2)	−28.4 −61	9.8 (21.8)	−11.2 (9.7)	6.8 (26.6)	−30.9 (99.7)	−143.1 (119)
fPOM pool	33.5 (20.9)	**114.1*** (22.6)**	**93.1*** (21.7)**	**104.7** (32.3)**	**126.2***** (28)	−9.25 (10.2)	6.1 (4.5)	**68.8*** (12.4)**	**−180.7*** 46.5**	−13 (55.4)
Bray-1 P	12.5* (5.6)	4.5 (6.2)	**25.4*** (5.9)**	12.3 (8.9)	−0.2 (7.8)	**7.6* (2.8)**	−0.9 (1.2)	−5.9 (3.4)	**70*** (12.8)**	**58.9*** (15.3)**
R^2	0.29	0.58	0.67	0.37	0.54	0.25	0.2	0.57	0.64	0.44
Adjusted R^2	0.19	0.52	0.63	0.28	0.47	0.14	0.08	0.50	0.58	0.35
N	32	32	32	32	32	32	32	32	32	32

SW, Spring wheat; YM, Yellow mustard; OA, Oat; DR, Daikon radish; CR, Cereal rye.
Significance: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

TABLE 5 Regression coefficients and *SEs* (in parentheses) for regression analysis of weed biomass in each cover crop treatment using baseline soil properties as predictors. Coefficients in bold font are significant, and the estimated model fit is indicated by the R^2 and adjusted R^2

Weeds									
Treatment:	**CC+RC+SW**	**CC+SW**	**RC+SW**	**SW**	**LN+YM+OA**	**OA+WP+DR**	**CV+CR**	**CR**	**Control**
Species:	**Weeds**	**Weeds**	**Weeds**	**Weeds**	**Weeds**	**Weeds**	**Weeds**	**Weeds**	**Weeds**
Intercept	4,646 (2,241)	3,630 (1,615)	5,169 (2,423)	4,730 (2,319)	1,857 (1,299)	4,339 (2,254)	3,315 (1,807)	1,572 (1,539)	2,516 (2,140)
Clay (%)	96.5 (55)	**87.4* (39.6)**	**189.2** (59.4)**	**220.2*** (57)**	**109.7** (31.8)**	110.5 (55.2)	44.7 (44.3)	**103.4* (37.7)**	**114.4* (52)**
C:N free POM	**−287* (107.8)**	**−254.2** (77.7)**	**−399.2** (116.6)**	**−329.4** (112)**	**−157.6* (62·5)**	**−284.9* (108)**	−211.5* (87)	**−171.9* (74)**	−195.1 (103)
fPOM pool	27.5 (50.3)	39.4 (36.3)	20.2 (54.4)	−68.3 (52)	−3.9 (29.2)	34.34 (51)	103.2* (40.5)	22.3 (34.5)	**134.1** (48)**
Bray-1 P	13.8 (13.8)	18.5 (9.98)	29.7 (14.9)	18.6 (14.3)	**22.5** (8)**	24.7 (13.9)	8.4 (11.2)	14.7 (9.5)	**37.7** (13)**
R^2	0.3	0.44	0.45	0.41	0.41	0.34	0.44	0.36	0.53
Adjusted R^2	0.2	0.36	0.37	0.32	0.32	0.24	0.36	0.26	0.46
N	32	32	32	32	32	32	32	32	32

Significance: $^{*}p < .05$; $^{**}p < .01$, $^{***}p < .001$.

Michigan. Soil types on the farms were all Alfisols and Mollisols, but fields varied in metrics of soil nutrient cycling capacity that reflected differences in management history and underlying soil texture. I therefore also tested the hypothesis that soil N availability from SOM pools would explain variation in legume biomass and BNF across farms. Understanding how abiotic conditions drive variation in functional trait expression and cover crop performance is a critical research gap that can inform management based on principles of functional ecology.

4.1 | Functional diversity of cover crop mixtures and ecosystem functions

Ecosystem functions varied widely along the farm gradient. The CR monoculture was the top performer for all individual ecosystem functions except for N supply from BNF, since it is a non-N-fixing species. CR is currently the most common winter cover crop grown in the region due to reliable establishment in the late fall after crop harvest and lower seed costs compared to legume species (Snapp et al., 2005). However, several mixtures were not significantly different from CR in terms of biomass production, N retention, and weed suppression, indicating opportunities for multifunctionality from mixtures that include legumes.

Across treatments and farms, cover crop biomass was positively correlated with other ecosystem functions (Figures 2, 3, and Figure S1). The relationships for N retention and weed suppression were only slightly weaker than similar relationships reported by studies conducted at a single research site (e.g., Finney, White, & Kaye, 2016). The larger scatter in the relationships for the cover crop treatments with legumes, compared to treatments with non-legumes only, is the result of greater variability in legume biomass compared to non-legume biomass.

The relationship between legume biomass and fixed N input was very strong (Figure 3), which corresponds with other studies (e.g., Unkovich, Baldock, & Peoples, 2010). The different slopes for different legumes indicated that WP was fixing N at the highest rate compared to other species, regardless of plant size. However, since legume biomass is a more important driver of total N supply than is % N from fixation (Crews et al., 2016), competitive interactions in mixtures may decrease fixed N inputs to agroecosystems if legume biomass is reduced.

4.2 | Multifunctionality

The relationship between the functional diversity index (Rao) of the treatments and multifunctionality was significant across farms, but was weaker than that reported in a study conducted at one experimental site (Finney & Kaye, 2017). This difference in findings may be due to the greater variation across multiple farm sites, the smaller number of species tested in the mixture treatments in this study, or perhaps because this experiment included several cover crops that have been less commonly studied and did not perform well on the farms. Expression of particular plant functional traits depends on the successful establishment and growth of different species in mixtures (e.g., the biomass-ratio hypothesis, Grime, 1998); however, cover crop mixtures are still rare on working farms, and their management has not been optimized for a broad range of conditions.

Here, I assessed multifunctionality at three thresholds (i.e., percentages of the maximum observed level of each function), which is preferable to using a single threshold value since the outcomes depend on the threshold chosen (Byrnes et al., 2014). Results supported the hypothesis that mixtures would simultaneously enhance more ecosystem functions than the CR monoculture; however, the difference was only statistically significant at the 30% threshold. For all treatments, the mean number of ecosystem functions provided decreased with increasing thresholds, indicating that there are trade-offs limiting the ability of cover crop mixtures to provide multiple functions at high levels (Finney & Kaye, 2017; Schipanski et al., 2014).

Table 1 translates the thresholds into absolute values for each function. Both the 30 and 50% thresholds provided substantial, management-relevant N input rates (46–77 kg N ha^{-1} $year^{-1}$), soil N retention in above-ground biomass (59–98 kg N ha^{-1}), and weed suppression (1,705–2,842 kg/ha of weed dry matter suppressed compared to a no cover crop control). One drawback of this approach is that it does not identify whether each function passes a threshold by a small or large amount (Byrnes et al., 2014). Assessment approaches like this one could be further developed together with farmers, to define relevant thresholds and manage functional diversity based on different goals.

Previous studies have explored legume-grass intercrops for simultaneously supplying and retaining N within agroecosystems (e.g., Ranells & Wagger, 1997; White et al., 2017). Mixtures of legumes and non-legumes commonly result in facilitation. For example, some of the N fixed by the legume can be directly transferred to the intercropped species through common mycorrhizal networks, or may indirectly increase N uptake by the non-legume via root exudation (and potentially priming effects), or root turnover (Høgh-Jensen & Schjoerring, 2001; Munroe & Isaac, 2014). Including estimates of fixed N transferred to intercropped species in the mixtures would likely increase their multifunctionality scores. Below-ground N inputs are another area of uncertainty in estimating BNF inputs, and the above-ground N inputs reported here are thus underestimates (Høgh-Jensen & Schjoerring, 2001).

4.3 | Do indicators of soil fertility and N availability predict variation in BNF across farms?

Given the critical role of legume biomass in determining the N supply from BNF, as well as the relevance of biomass to farm management, it is useful to understand drivers of variation in biomass across environmental conditions and management regimes. Legume biomass was higher in soils with lower N content in endogenous SOM pools, and increased with plant-available P concentration. POM pools are sensitive to management, and reflect differences in both the quantity and quality of organic matter inputs (Wander, 2004). The regression models were particularly strong for WP (R^2 = 0.76) and CC (R^2 = 0.54–0.67) biomass, which were negatively correlated with the amount of oPOM N, and with the total quantity of fPOM, both of which reflect N availability from mineralization from SOM. One legume, CV, showed an unexpected positive correlation with the fPOM pool, and, on average, the BNF rate and input for CV were much lower than for the overwintering legumes.

The models for BNF (shoot N from fixation; kg N/ha) corresponded with the results for legume biomass, though the fits tended to be weaker. These models indicated that legumes fix more N with increasing soil P concentrations, and, conversely, the % of legume shoot N from the soil was higher at lower soil P (i.e., when the % N from fixation was lower). Although WP and CC biomass were positively related to silt+-clay content, contrary to the hypothesis, the model for BNF (rather than biomass) indicated that CC BNF was negatively correlated with % clay.

The poorer fit for models of RC in CC+RC+SW may be partly due to the low biomass production by RC, which tended to be out-competed by CC and SW. However, Schipanski and Drinkwater (2011) did not find an inverse correlation between soil N availability and RC BNF across a farm gradient even with high RC biomass. In this study, the RC biomass in the RC+SW treatment was positively related to the C:N of the fPOM pool ($p < .0001$), indicating more biomass with lower quality POM.

Although fPOM pools tend to be larger on farms with a history of organic management (Marriott & Wander, 2006; Wander, Traina, Stinner, & Peters, 1994), the fPOM pool is also more ephemeral than oPOM and changes relatively quickly in response to organic matter inputs. The oPOM fraction turns over more slowly, and tends to be a more reliable indicator of longer-term changes in fertility due to management—making it possible to differentiate whether SOM stocks reflect background soil type versus management practices (Wander et al., 1994). I therefore expected to find stronger relationships with oPOM N, which was the case for CC and WP biomass. These findings contribute to ecological understanding of BNF within agroecosystems, and could inform development of management recommendations for farmers that provide estimates of BNF from mixtures to improve ecological N management.

4.4 | Implications for agroecosystem management

Data from this study suggest that cover crop mixtures designed with complementary plant traits could increase the multifunctionality of agroecosystems. However, there were trade-offs among functions in which increasing functional diversity enhanced some functions and decreased others. These findings highlight the need to better understand competitive interactions in mixtures as well as feedbacks with soil properties, since variation in species performance across farms affects trait expression and associated functions.

Linking soil characteristics to mixture performance could inform adjustments to cover crop seeding rates in different conditions. For instance, grasses and brassicas in the mixtures tested here were more competitive with increasing soil fertility; their biomass increased with both fPOM pool size and P availability. Since P was also limiting to legume biomass across farms (i.e., there was a positive relationship between P and legume biomass), farms in the early stages of ecological nutrient management may require supplemental P additions or a greater proportion of legume seeds within mixtures to increase the N supply from BNF. Legume biomass was strongly correlated with the N supply function across farms (Figure 3). Tools for farmers to predict biomass, along with models predicting mixture composition in different environmental conditions, could improve management recommendations based on functional ecology and ecological nutrient management frameworks.

Over time, regular use of legume N sources can increase labile soil N pools (Drinkwater et al., 1998; Schipanski & Drinkwater, 2011). Here, I found that the inverse relationship between soil N availability and BNF reported in more highly controlled conditions, often using synthetic N fertilizer, is also present on farms with organic nutrient management. These feedbacks would decrease BNF inputs at higher levels of N availability from SOM turnover, which corresponds with findings from on-farm research showing that legume N sources increase field-scale N use efficiency (Blesh & Drinkwater, 2013). Understanding how cover crop mixtures with complementary functional traits impact SOM pools over time could therefore inform adaptive management as soil properties change—improving management recommendations for farmers (e.g., selection of plant traits and appropriate mixture seeding rates) and reducing N surpluses that drive losses to surrounding ecosystems (Zhang et al., 2015). Research results from on-farm experimentation reflect realistic environmental and social contexts, and therefore have direct relevance to developing management systems that address critical sustainability goals.

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DATA ACCESSIBILITY

All data used in this article are available on Deep Blue Data, the University of Michigan's digital repository for research data https://doi.org/10.7302/z2wm1bk6 (Blesh, 2017).

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