Promoting enhanced ecosystem services from cover crops using intra- and interspecific diversity

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ABSTRACT

Farmers are increasingly looking to cover crops to provide ecosystem services to support yields, while reducing off-farm inputs. Research evidence in both unmanaged and agricultural ecosystems suggests that increased plant diversity can improve ecosystem service outcomes. The use of diverse cover crops as a practice to increase ecosystem service delivery has potential, though knowledge gaps remain regarding the types and levels of diversity that are most effective, and how environment may impact the desired outcomes. Using six species of legumes (hairy vetch, field pea, crimson clover) and of grasses (cereal rye, common wheat, ryegrass) along with multiple cultivars of each, we conducted an annual field experiment repeated twice during the winter fallow season. We tested the effect of a gradient of diversity in intraspecific mixtures, interspecific mixtures, and functional group (grass and legume) mixtures. We measured aboveground biomass production, weed biomass, soil-derived and fixed nitrogen in the shoots, and the C/N ratio of the aboveground biomass to evaluate corresponding ecosystem services relating to C and N cycling and weed suppression. Species mixtures, especially those composed of both grasses and legumes tended to have the most significant ecosystem service benefits suggesting that functional diversity is more significant than species richness. Where soil nutrients were less available, the diversity effect tended to be stronger, though this was significant in only two instances. These results lend some support to the stress-gradient hypothesis in that in more stressful conditions the diversity benefit was greater. Diverse mixtures may not consistently produce substantial service improvements, but there is little risk, and they may be most useful in marginal or depleted field conditions.

1. Introduction

Ecological intensification posits that increasing biodiversity can help to enhance ecosystem services reducing the need for anthropogenic inputs (Bommarco et al., 2013; Petersen and Snapp, 2015). As farmers seek to reduce their use of inputs without incurring yield reductions, interest is growing in managing biodiversity to foster ecosystem services. However, the low levels of diversity and ecosystem services in most agroecosystems present challenges for ecological intensification. Farmers are increasingly turning to cover crops as a means of diversifying their cropping systems, both spatially and temporally, which can help address the challenge of maintaining yields with reduced inputs (Dunn et al., 2016). Cover crops are not harvested, and are planted between periods of cash crop production when a field would typically be fallow (Carlson and Stockwell, 2013; Schipanski et al., 2014; Snapp et al., 2005). Cover crops can provide a range of ecosystem services, but farmers tend to prioritize certain ecosystem services over others, with a

heavy emphasis on soil health benefits and nutrient management, including symbiotic nitrogen fixation (SNF) by legumes, in addition to pest control (Wayman et al., 2016). Overall productivity of the cover crop is highly valued, especially as many of these ecosystem services are related to biomass production of the cover crop (Finney et al., 2016).

Productivity has also been used as one of the main measures of ecosystem function in unmanaged ecosystems. In these non-agricultural settings evidence suggests that diverse plant communities may improve the productivity and ecosystem service outcomes of these ecosystems (Hooper et al., 2005). These experiments tend to use species richness as the indicator of diversity and have used aboveground primary productivity as a proxy for ecosystem functioning (Cadotte et al., 2008, 2009; Cardinale et al., 2011; Grace et al., 2016). However, this relationship between plant species diversity and productivity is not always evident, and it is increasingly apparent that the diversity of functional traits within the community is often a better predictor of productivity than species richness alone (Cadotte et al., 2011). These traits, which are

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mainly related to resource utilization (light, nutrient and water capture), ultimately influence how the whole community functions (McGill et al., 2006). A more functionally diverse community has greater resource partitioning, potentially utilizing the available resources more efficiently and ultimately increasing overall productivity (Cadotte et al., 2011).

Identifying and then linking important functional traits to specific ecosystem functions is complex and challenging, which has led to the common use of simplified functional groups, especially in agroecosystems. The way a plant affects and responds to the full range of ecosystem processes is determined by a large number of functional traits, and only a subset of those have been identified as strong influences on specific ecosystem functions (Eviner and Chapin, 2003; Funk et al., 2017). Additionally, there are likely other key traits yet to be isolated or measured, making it difficult to fully account for and characterize species in terms of measurable functional traits (Cadotte et al., 2009). Functional groupings using single traits known to be major drivers of ecosystem processes (i.e. nutrient acquisition method, phenology, plant architecture) is a way to simplify the relationship between species identity and ecosystem function (Eviner and Chapin, 2003; Petchey and Gaston, 2002). This simplified approach of functional groups is commonly used in the management of agricultural systems, for example in rotations or companion planting, such as legumes or grasses, and winter or spring grains (Finney et al., 2016; Storkey et al., 2013). When constructing cover crop mixtures this simplification is quite useful as these groupings can help to easily characterize complementary species combinations. However, mixtures based on these simple groups do not acknowledge the complexity of plant functional and genetic diversity including the phylogenetic diversity of species within a functional group and the genetic diversity within species.

A common example of complementary functional traits in agriculture in order to provide multiple ecosystem services is the use of grass-legume mixes. These mixtures have a long tradition in agriculture, from forages to intercropped grain systems (Bateman and Keller, 1956; Boyd and Brennan, 2006; Cooper et al., 1960). Grass-legume mixtures are also a very appealing cover crop choice for farmers as they can provide a suite of ecosystem services that reflect the strengths of the these two functional groups (Baba et al., 2011; Bedoussac et al., 2015; Nyfeler et al., 2009; Osman and Diek, 1982). Legumes fix nitrogen (N) contributing to long term N supply, as well as a lower carbon to nitrogen (C/N) ratio providing N in the short term for the subsequent crop (Brainard et al., 2011). Alternately, grasses tend to produce greater biomass contributing more to organic matter accumulation as well as suppressing weeds better than legumes (Baraibar et al., 2018; Kruidhof et al., 2008). Grasses also excel at scavenging soil N, reducing losses of N from the field (Aronsson et al., 2016). Since farmers are rarely interested in one ecosystem service alone, and tend to prioritize weed suppression and N fixation equally, by mixing these two contrasting functional groups together it is possible to achieve multiple ecosystem-service goals while minimizing tradeoffs (Kaye et al., 2019; Schipanski et al., 2014; Wayman et al., 2016). In a grass-legume mix, grasses can suppress weeds while stimulating N fixation in the legumes through competitive N uptake, resulting in greater overall N content of residues, as well as equal or greater biomass production compared to grasses alone. However, if growing conditions favor grasses, they can outcompete the legumes, reducing their benefit. Consequently, managing the functional groups, and selecting appropriate species and cultivars for the given environmental conditions is a critical challenge.

Agricultural systems present the opportunity to draw on intraspecific diversity within species in functional groups, as cultivars, as well as to enhance cover crop performance and the delivery of ecosystem services, while increasing agroecosystem diversity overall. In agricultural systems, cultivars have been developed that exhibit significant variation in functional traits (Elzebroek, 2008; Fu, 2015). Cultivar mixtures, which increase spatial intraspecific diversity, have been successfully used to increase yield and other services, such as disease management, in cash

crops (Kaut et al., 2008; Kiær et al., 2009; Mundt, 2002; Reiss and Drinkwater, 2018). Given the accumulating evidence from a variety of ecosystems, increasing intraspecific diversity in cover crop mixtures could also prove to be advantageous. Intraspecific diversity in cover crops could be increased alone or in conjunction with species and functional group mixtures. As individual cultivars can vary substantially in their performance, even under common conditions, cultivar selection, for mixture or sole cultivation, can have a major influence on the outcome (Andrew et al., 2015; Duvick, 2005). For example, certain cultivars may excel under drought conditions, or be more competitive against weeds due to rapid early growth in addition to having differences in yields (Andrew et al., 2015). Increasing the attention paid to how cultivar identity and specific cover crop traits impact performance is a critical knowledge gap for optimizing cover crop use.

In addition to supporting ecological intensification and broadening ecosystem services provided by cover crop mixtures, diversity can also be a key tool for farmers looking to mitigate the effects of environmental variation. Environmental conditions can influence the diversity-ecosystem function relationship, with more stressful conditions accentuating the generally positive diversity effect, as described by the stress-gradient hypothesis (Baert et al., 2018; Dwivedi et al., 2016; He et al., 2013; Newton et al., 2010; Reiss and Drinkwater, 2018; Yu et al., 2015). In a comprehensive meta-analysis of cultivar mixtures, we found that more stressful environments tend to favor positive plant-plant interactions such as complementarity and facilitation over competition (Reiss and Drinkwater, 2018). For cover crops, this interaction between environmental conditions and diversity may have useful management implications in the future. Given the increased weather variability and greater frequency of extreme events due to climate change, well-designed cover crop mixtures will provide even greater benefits (Gaudin et al., 2015; Stott, 2016).

To build on this existing work, we conducted a field experiment to test several hypotheses about the relationship between cover crop community composition and provision of desirable ecosystem services. We included three species in each of two functional groups, grasses and legumes as well as treatment with multiple cultivars of each species. We measured aboveground cover crop biomass production, weed suppression, soil-derived and fixed N in the shoots, and the C/N ratio of the aboveground biomass to evaluate the corresponding ecosystem services (Table 1). We were interested in comparing the ecosystem service benefits resulting from intraspecific, interspecific, and functional diversity as well as the variation between cultivars of cover crop species in terms of the outcomes of the associated services. We expected species

Table 1

Measurements used as indicators for ecosystem services. Several metrics were used to characterize cover crop effects on ecosystem services derived from N cycling processes. Acquisition of soil N reflects the capacity of cover crops to scavenge soil N and reduce N losses through leaching and denitrification (Tonitto et al., 2006). We considered two different mechanisms that affect N supply. Symbiotic N fixation is a source of new N and increases N supply for the subsequent crop (short-term) while also adding to soil organic N reserves (long-term) (Brainard et al., 2011). The C:N ratio of the cover crop biomass is a secondary factor that influences the timeframe of net N mineralization and therefore plays a role in supplying N to the subsequent crop (Wagger et al., 1998). Weed biomass in the cover crop is a direct measurement of the capacity of the stand to suppress weeds. This is important from the farmer perspective because if weeds are allowed to grow along with the cover crop, they frequently produce seeds leading to increased weed pressure over the long term. Lastly, total cover crop biomass is one of several factors that govern SOM accrual and soil health improvements derived from cover cropping.

Collected metric	Ecosystem service
Soil N accrual in cover crop biomass	N retention
Shoot fixed N	N supply, long-term
C/N ratio	N supply short-term for the following crop
Weed biomass	Weed suppression
Cover crop biomass	C accrual

mixtures, and specifically those including both legumes and grasses, to provide a broader range of ecosystem service outcomes compared to less diverse plantings. We were also interested in testing if more subtle expansion of functional diversity though greater interspecific or intra-specific diversity would improve and/or reduce variation in the ecosystem service benefits of cover crops. Lastly, we hypothesized that more stressful conditions, such as those related to soil fertility, would strengthen the diversity effect on the delivery of these services.

2. Methods

2.1. Experimental site

Our research was conducted at Cornell University Musgrave Research Farm in Aurora, NY (42.73' N, 76.66' W). The region, in USDA plant hardiness zone 6a, has a humid temperate climate with a mean annual precipitation of 880 mm, and a mean annual maximum and minimum air temperature of 14 °C and 3 °C, respectively. The dominant soil types are a moderately well-drained Honeoye (fine-loamy, mixed, semiactive, mesic Glossic Hapludalfs) and Lima (fine-loamy, mixed, semiactive, mesic Oxyaquic Hapludalfs) silt loams.

We conducted identical experiments for two consecutive over-wintering cover crop growing seasons, which run from September to June when fields are normally fallow. Our experiments were established in fields that were less than 300 m from one another. For clarity, results from the 2013–2014 season are reported as from Field 1, and results from the 2014–2015 season are reported as from Field 2. Field 1 was primarily a Honeoye silt loam, whereas Field 2 was dominated by Lima silt loam. The two fields had been conventionally managed under a typical corn-soybean-wheat rotation following local recommendations for fertilizer applications and other management practices. While the management legacy was similar between the two fields, our experiments were established at differing points in the rotation. Field 1 was planted in soybeans for 2012 followed by winter wheat which was harvested in the summer of 2013. Field 2 was planted to corn in 2013 and then left fallow until we established our second experiment in September 2014. The two fields also differed in terms of soil N, which was higher in Field 2, and soil P, which was higher in Field 1 (Table 2).

2.2. Experimental design

We used a randomized, complete block, split-plot design with four blocks comprised of 46 cover crop treatments that varied from mono-cultures of a single cultivar to mixtures composed of six species and many cultivars (Table 3). Within each block, an additional three plots were designated as "controls" and were not planted with cover crops in order to assess the background weed pressure. These 49 main treatment plots (cover crop communities and controls) were repeated across four blocks giving 196, 2.4 m x 4.8 m main plots. To test the stress gradient hypothesis, main plots were split, and N fertilizer was applied to half (+N treatment) while the other half did not receive any added N (-N treatment) resulting in 98, 2.4 × 2.4 m sub-plots per block for a total of 392 sub-plots in the experiment (98 per block x 4 blocks).

We selected six annual cover crop species (3 legumes, 3 grasses) commonly used by farmers in the upstate New York region. In selecting

these two functional groups, the three species within each of these functional groups and multiple cultivars, our goal was to maximize the diversity in plant functional traits as much as possible. Thus, our treatments reflect a hierarchy of functional diversity at three distinct levels and multiple dimensions, starting with grasses versus legumes, which have profound differences in nutrient acquisition and shoot/root architecture. Functional diversity among legume or grass species is more modest and included differences in winter hardiness, potential biomass production and growth habit (Table S1). Lastly, the smallest contributions to functional diversity were derived from multiple cultivars of these species.

The species we used all shared the same life history (winter annuals) and are well-suited to the over-wintering fallow period in the region. These are planted in late summer/early fall and grow into late fall/early winter. In spring they break their winter dormancy and produce the majority of their biomass during April and May. The three legume species were hairy vetch (*Vicia villosa*), winter field pea (*Pisum sativa*) and crimson clover (*Trifolium incarnatum*). Cereal rye (*Secale cereale*), common wheat (*Triticum aestivum*), and ryegrass (*Lolium multiflorum*) constituted our three grass species.

We selected at least two cultivars from each species, and generally used at least one 'variety not stated' (VNS) line for each species and one named cultivar (Table S2). For vetch and wheat, we expanded the number of cultivars to five and four respectively. We used these two as focal species for our mixture treatment structure as these have a range of well-defined cultivars commercially available. Where trait information was available about cultivars, we attempted to select contrasting cultivars or lines, though this was difficult for many species that have not undergone intensive breeding. For example, cultivars with distinct trait combinations are plentiful in wheat due to a long history of dedicated breeding programs both private and public (i.e. Cornell Small Grains Program, the source of the trait information and seeds for the cultivars used in this study). However, while there are multiple commercially available cultivars of vetch, there is limited support for vetch breeding, limiting the trait information and differentiation in cultivars. (Several commercial vetch cultivars 'Purple Bounty' and 'Purple Prosperity' originated as part of the breeding efforts of the USDA-ARS Sustainable Agriculture Systems Laboratory based in Beltsville, MD led by Thomas E. Devine.) Nevertheless, to the extent possible we chose cultivars that differed in traits such as flowering time and winter hardiness for legumes and growth potential and fall vigor for grasses.

Using these species and cultivars, we constructed treatments to assess the effects of these three levels of functional diversity along a gradient of mono-specific, single cultivar treatments to a maximum diversity comprised of six species represented by a combined total of 17 cultivars (Table 3). These treatments can be grouped into three categories (species composition) consisting of (A) species monocultures, (B) species bicultures composed of one legume species and one grass species, and (C) three or more species with two subcategories each (cultivar composition).

For mixtures of legumes and grasses together, each functional group composed 50% of the mixture by number of seeds. Where there were multiple species in one functional group, each species was seeded equally. Actual species richness differed in some plots from the planted species richness, likely due to winter mortality, but as fall emergence

Table 2

Field and environmental conditions for the two experiment fields. For p value results, "ns", not significant; "nt", not tested.

Field	Inorg N (mgN/kg soil)	PMN (mgN/kg soil/week)	N (%)	C (%)	C:N	pH	P (ppm)	K (ppm)	CEC	Sand (%)	Clay (%)	Soil Textural Class	Precip. (cm)	GDD (0 °C base temp.)
1	5.8 (1.1)	10.9 (3.6)	0.16 (0.01)	2.1 (0.1)	13.5 (0.80)	7.7 (0.1)	23.5 (3.5)	73.8 (10.3)	13.7 (1)	42.7	23.7	Loam	66.4	2068
2	11.0 (1.4)	8.8 (2)	0.18 (0.02)	2.0 (0.1)	11.0 (0.69)	7.7 (0.1)	16.5 (1)	75.8 (8.4)	14.8 (0.6)	44.6	22.7	Loam	60.8	1770
p value	0.0013	ns	0.0271	ns	<0.0017	ns	0.0086	ns	ns	nt	nt	nt	nt	nt

and establishment was not assessed, it is not possible to confirm this. Actual species richness was used in all analyses.

2.3. Plot establishment

We used a replacement series design with a consistent seeding rate of 285 live seeds/m2 (adjusted for germination rate certified by the seed source) in all plots. This design is consistent with the biodiversity-ecosystem function work in unmanaged ecosystems, where plant density is held constant so that changes in plant community composition can be assessed independently of density (Tilman et al., 1996). This design is different compared with other cover crop diversity research in agroecosystems, where a proportional replacement design, or additive design, is more commonly used (Bybee-Finley et al., 2016; Finney et al., 2016; Poffenbarger et al., 2015). These alternate designs often use agronomically appropriate seeding rates on a kg/ha basis instead of plant density. With six species of different seed sizes and growth habits, it was not realistic to achieve relevant seeding rates for all species while maintaining the same density. Instead, we pegged our target seeding density to the recommended seeding rate in kg/ha for wheat, one of our focal species (Table S3) (Clark, 2007; Thomas, n.d.).

Due to seed size, some species were over-seeded (e.g. the larger seeded legumes), whereas others were under-seeded compared to recommended rates. This is because agronomic seeding rates for legumes tend to be lower, perhaps due to higher seed costs. Despite these differences in seeding rate on a kg/ha basis, we generally achieved our objective of consistent plant density in the plots. The overall harvest densities were on average at least a third of the seeded densities, suggesting that seeding density may have had minimal impact on final plant densities at harvest (Fig. S1).

We applied appropriate bacterial inoculant to all legume species unless they were pretreated by the supplier (N-Dure brand, Verdesian, Cary, NC). We planted all treatments in mid-September (September 14–15 in 2013, September 15 in 2014). After the field was disked, plots were broadcast seeded by hand, and then the whole field was rolled with a cultipacker. We applied 80 kg N/ha as pelletized ammonium nitrate fertilizer by hand to the soil surface of the fertilizer sub-plots during the first week of May in both 2014 and 2015. The experiment plots received no fertilizers or pesticides other than this application and were rainfed. Actual precipitation was 664 mm and 608 mm for the duration of the experiment (planting to sampling) in the two years respectively.

Additionally, the growing degree days (GDD) were 2068 and 1770 for the two experiments using 0 °C as the base temperature (Table 2).

2.4. Sample collection and analytical methods

2.4.1. Plants

We sampled the plants at the same physiological stage in both years. When the vetch monoculture plots were at approximately 50% flowering, we harvested aboveground biomass for all treatments (June 16–19 2014, June 9–10 2015). At this developmental stage vetch is unlikely to regrow after field incorporation, yet viable seeds have not set so farmers prefer to terminate cover crops at this stage to ensure mortality of the cover crop, and prevent introduction of new seeds from the cover crop which might become problematic. The grasses were all post-anthesis and some were starting grain-fill. The pea and clover were also at peak or just post-peak flowering.

Cover crop stand establishment varied between the two years. In Field 1, winter mortality resulted in patchy stands in some plots. We cannot be certain of the reasons for this variable winter survival; it may be due to the minimal snow cover in Year 1, and could have been exacerbated by differences in drainage across this field. The aging tile drainage systems at the Musgrave Research Farm are not fully functional and created uneven drainage in this field. Given the possibility that the patchy stands reflected a condition that farmers would not tolerate, we decided to focus our sampling on the surviving patches in order to have the fullest possible representation of our diversity gradient. To better sample these patchy plots, we used two quadrats (0.125 m² each) in plots that consisted of patchy stands, with the total area equal to the larger quadrat (0.25 m²) used in the remaining plots. Areas for sampling with the smaller quadrats were selected to represent the planted composition of the plot and combined into a composite sample for analysis. For the second experiment, we specifically requested a field that did not have drainage problems. Furthermore, snow cover during the second winter was more consistent, reducing mortality from desiccation in susceptible species such as winter pea. As a result, in Field 2, where the tile drainage was functioning more evenly and plants protected by winter snow cover, stands were very consistent across the field, and all plots were sampled with the standard 0.25 m² quadrat.

Biomass was cut 9 cm above the soil surface to minimize contamination from soil and very small plants (Bybee-Finley et al., 2016). As we removed shoot biomass, we recorded the number of individual plants for

Table 3

Overview of treatments used to create our experimental diversity gradient. A) Species monoculture treatments were planted as single cultivars of each species (A1-A17) or as cultivar mixtures of each species (A18-A23). B) Grass/legume bicultures consisted of one legume/one grass species with only a single cultivar of each (B1–9) or all cultivars for each legume and grass species (B10-B13). C) Each treatment with three or more species was planted either with single cultivars representing each species (C1, C3, C5, C7, and C9) or as a species mixture with all cultivars for each species (C2, C4, C6, C8, and C10). Lastly, each block (4 total) included three control plots where no cover crops were planted and weed pressure was evaluated (D1–3, not shown here).

A) Species monocultures		B) Grass/legume bicultures		
Trt. ID	*Cultivars by species*	*Trt. ID*	*Grass in mixture*	*Legume in mixture*
A1-A5	Vetch cultivars 1–5	B1-B9	Full 3 × 3 cross of 3 wheat & 3 vetch cultivars	
A6-A7	Winter pea cultivars 1–2	B10	Rye (all cultivars)	Pea (all cultivars)
A8-A9	Crimson clover cultivars 1–2	B11	Wheat (all cultivars)	Pea (all cultivars)
A10-A11	Rye cultivars 1–2	B12	Rye (all cultivars)	Vetch (all cultivars)
A12-A15	Wheat cultivars 1–4	B13	Wheat (all cultivars)	Vetch (all cultivars)
A16-A17	Ryegrass cultivars 1–2			
A18-A23	Cultivar mixtures (all) by species			
C) Three or more species				
Trt. ID	*Treatment description*	*Treatment contents*		
C1	Legume species mix	Vetch 1	Pea 1	Clover 1
C2		Vetch (all cultivars)	Pea (all cultivars)	Clover (all cultivars)
C3	Grass species mix	Rye 1	Wheat 1	Ryegrass 1
C4		Wheat (all cultivars)	Rye (all cultivars)	Ryegrass (all cultivars)
C5	All legumes, 1 grass	Vetch 1	Pea 1	Clover 1	Wheat 1
C6		Vetch (all cultivars)	Pea (all cultivars)	Clover (all cultivars)	Wheat (all cultivars)
C7	1 Legume, all grasses	Vetch 1	Rye 1	Wheat 1	Ryegrass 1
C8		Vetch (all cultivars)	Rye (all cultivars)	Wheat (all cultivars)	Ryegrass (all cultivars)
C9	All species	All species (1 cultivar of each)		
C10		All species (all cultivars of each)		

each cover crop species (individual cultivars could not be separated) and the number of weeds present in each quadrat. We separated this biomass by cover crop species, and weeds (all weed species were kept together as a single biomass sample from each quadrat), which was then oven-dried for at least 48 h at 60 °C before weighing to the nearest 0.01 g. Biomass from weed control plots was sampled in the same way as in the cover crop plots.

To determine total N acquisition from soil, C/N ratio and total N from symbiotic N fixation, aboveground biomass samples from each species were first ground to 2 mm (Thomas Wiley Mill, Thomas Scientific, Swedesboro, New Jersey, USA). However, because we did not need to analyze all these samples for ^{15}N, there were some small differences in the methods used to analyze samples of legume and grass biomass from different mixtures. Grass cover crop samples from all treatments were analyzed for total C and N on combustion using a LECO TruMac CN analyzer (Leco Corporation, St. Joseph, Missouri, USA). To estimate symbiotic N fixation by leguminous species, legume shoots and a subset of grass monoculture samples that were to be used as references for soil ^{15}N signatures were processed for mass spectrometer isotopic analysis. Specifically, subsamples of shoot material (legumes and reference grasses) were further ground to < 0.5 mm with a propeller mill (Cyclotec™ Sample Mill, Foss, Hillerød, Denmark). These samples were analyzed for ^{15}N natural abundance, total N content and total C content using a continuous flow Isotope Ratio Mass Spectrometer (Stable Isotope Facility, UC Davis).

We used the ^{15}N natural abundance method, to estimate symbiotic N fixation by legumes in monocultures and mixtures grown in unfertilized sub-plots (Shearer and Kohl, 1986). For this method, we used the ^{15}N signatures of grasses from unfertilized monoculture sub-plots as the reference plants, for the following calculation:

$$\%N \text{ from fixation} = 100 \times \left(\frac{\delta^{15}\text{Ngrass} - \delta^{15}\text{Nleg}}{\delta^{15}\text{Ngrass} - B} \right)$$

The *B* value, which is used to account for the isotopic fractionation of the N during fixation in the legumes, was determined by growing each cultivar of legume and each seed lot, in cases where we obtained seeds from different sources in years one and two (Table 2S) in a greenhouse experiment. We grew plants in an N-free, autoclaved, calcined clay media. Seeds were sterilized with hypochlorite (Parr, 2010; Somasegaran and Hoben, 1994), and inoculated with the same inoculant used for the field experiment plus native rhizobia present in soil from Field 1 (Unkovich et al., 1994). We prepared the soil slurry by combining a representative sample of approximately 300 ml of 2 mm sieved soil in 6.5 L of distilled and deionized water. Both inoculants were added at planting, and the soil slurry applied again one week after planting. Nitrogen addition from the slurry was negligible. Plants were grown to the same physiological point as they were harvested in the field, and then cut and dried as in the field experiment. Overall, the *B* values we obtained in this experiment were within accepted literature values (Parr, 2010; Unkovich et al., 2008).

In Field 1, we used the average of the three reference grass species in each block as the reference value to calculate the %N from fixation for each legume sample. In this field a few samples (less than 5%) were greater than 100%, and we adjusted those samples to 100% for a realistic result (Unkovich et al., 2008). When we used this same approach in Field 2, where there was considerable spatial variability in total N and ^{15}N signatures, we found that 31 of 157 samples had negative rates of SNF. We attempted to resolve these negative results in a number of ways including using actual field δ^{15}N values instead of the greenhouse *B* values, which had no effect on the total number of negative fixation results (Brainard et al., 2012). Ultimately, we developed a systematic approach to improve our confidence in the true estimate of SNF across the field. First, we used spatial statistics, specifically, Ordinary Kriging, to estimate the reference ^{15}N signature more accurately across the field. Then, for samples that had negative N fixation rates after using the

kriged reference values, we used reference values from adjacent grass monocultures if they were available. We then had increased confidence in our estimates of SNF and concluded that the remaining samples with negative ^{15}N values (n = 28) did indeed reflect down regulation of SNF due to the elevated levels of soil N present in this field. For these samples, we adjusted to 0% N from SNF. We also estimated the spatial distribution of plant available soil N across Field 2 by using the shoot N concentration of grass monocultures as a proxy for available soil N. Using a z-distribution, we standardized the %N in the biomass within each of the three grasses growing as monocultures. With these known standardized %N values, we used Ordinary Kriging to estimate the plant available N across the field, which we then used to help explain down-regulation of SNF in legumes.

2.4.2. Soils

To characterize soil conditions, we collected composite soil samples (ten, 2.25 cm diameter soil cores to 20 cm depth) from each of the four blocks prior to planting in the fall and stored at 4 °C in plastic bags to retain moisture. Within 24 h a subsample of each composite sample was sieved to 2 mm and used to determine total inorganic N (Inorg N) and potentially mineralizable N (PMN) as in Drinkwater et al. (1996). Briefly, duplicate aliquots of sieved, field moist soil were immediately extracted for total inorganic N (Inorg N) with 2 M KCl and another set of duplicates were incubated anaerobically for seven days and extracted with KCl (Drinkwater et al., 1996). Total NH_4^+ and NO_3^- were analyzed via a colorimetric microplate technique (Ringuet et al., 2011). Gravimetric water content of each sample was determined after being dried in the oven for 7 days at 65 °C. Air dried soil samples were sieved to 2 mm and analyzed for total C and N on combustion (Leco Corporation, St. Joseph, Missouri, USA) and pH (water), CEC, Mehlich buffer lime requirement, and for P, K, Mg, Ca, Zn, Cu, and S by the Mehlich 3 (ICP) test, and particle size (Agricultural Analytical Services Laboratory, Penn State University, University Park, PA, Table 2).

2.5. Data analysis

2.5.1. Ecosystem service and cover crop performance calculations

Total biomass N per m^2 was calculated for all plants as %N x g of cover crop biomass/m^2. Nitrogen derived from the atmosphere through SNF in legumes was partitioned using the %N from fixation result from the ^{15}N natural abundance method. The grams of fixed N in legumes was calculated as %SNF x total N. The soil N accrual for legumes was calculated as total N minus fixed N. For grasses, all biomass N is derived from the soil so soil derived N is equal to total N. These calculations were only done for unfertilized sub-plots as the natural abundance method of fixed N estimation is not applicable when fertilizer is applied.

C/N ratio was calculated as the sum of the cover crop biomass C content (gC/m^2) divided by the sum of biomass N content (gN/m^2) for all the cover crop species present in a plot. For consistency with N accrual and long-term supply, these calculations were also only done for unfertilized sub-plots.

The land equivalent ratio (hereafter designated as LER$_T$, to indicate total LER) is a concept often used to evaluate the benefits of intercropping systems, and quantifies the relative land area required under sole cropping (monoculture) to produce the same yield as under intercropping (usually species mixtures) (Weigelt and Jolliffe, 2003). In our case, we compared monocultures of grasses and monocultures of legumes (including treatments with multiple cultivars or species of either grasses or legumes together) to grass-legume mixtures (grass-legume mixtures of cultivars and species).

The LER$_T$ is calculated as:

$$\text{LER}_T = \sum Y_{pi} / Y_{mi}$$

Where Y_{pi} is the biomass of grass or legume in grass-legume mixtures, and Y_{mi} is the biomass of grass-only or legume-only stands. By

comparing the LER$_T$ of a mixture to 1, the productivity of grass-legume mixtures can be compared to either grasses or legumes grown alone. Values >1 indicate that biomass production in the mixture is greater than for grasses and legumes grown separately, while values <1 indicate reduced production in the grass-legume mixture.

To determine the performance of grasses versus legumes in the mixtures, we compared their partial LERs to 0.5 (since our mixtures were planted used a 50:50 mix of grass and legume seeds resulting in a planting density that was 50% of the planting density pure grass and legume stands). Evaluating the partial LER is useful for assessing the effects of cover crop stand composition on competition dynamics. For example, different LER$_T$ of mixtures can be attributed to increases in biomass from one component or the other, as indicated by the partial LER.

2.5.2. Statistical analysis

Mixed models were used to test the effects of diversity on ecosystem service outcomes. In all models, all possible interactions of field (1 and 2) and fertilizer (+N/-N) and the main model factor (e.g. cultivar richness, species richness) were tested, and included in the model if significant. If the interaction was not significant, data were pooled across fields and fertilizer treatments. Block was nested in field and included as a random effect. Where evaluation of model residuals indicated non-normality, or heterogeneous variance was observed data were transformed as noted in figures. In particular, weed biomass data were

transformed using natural log after a constant (1) was added in order to meet model assumptions. Untransformed data is presented in figures unless otherwise noted. Full statistical results are reported in Supplemental Information.

To avoid pseudoreplication when analyzing species effects, treatments of the same diversity level composed of different cultivars (i.e. cultivar monocultures), were pooled and averaged within block before further analysis. For example, though vetch has five cultivar monocultures and pea only two, there is no influence of this imbalance in the analysis comparing cultivar monocultures to the cultivar mixture. All cultivar monocultures for a species are averaged within block first, so that each species only has one data point for cultivar monocultures for each block.

Tukey's honestly significant difference (HSD) at $\alpha = 0.05$ was used to test for differences between multiple levels (i.e. species richness), while two sample t-tests were used to compare low and high diversity levels (i.e. cultivar monoculture vs. cultivar mixture) and fertilizer effects (+N versus –N sub-plots). One sample t-tests were used to assess differences from reference values for total and partial LER (1 and 0.5 respectively). All analyses except Ordinary Kriging (Bivand et al., 2013; Gräler et al., 2016; Pebesma, 2004; Pebesma and Bivand, 2005; R Core Team, 2016) were conducted using JMP v.11 software (SAS Institute Inc. Cary, NC).
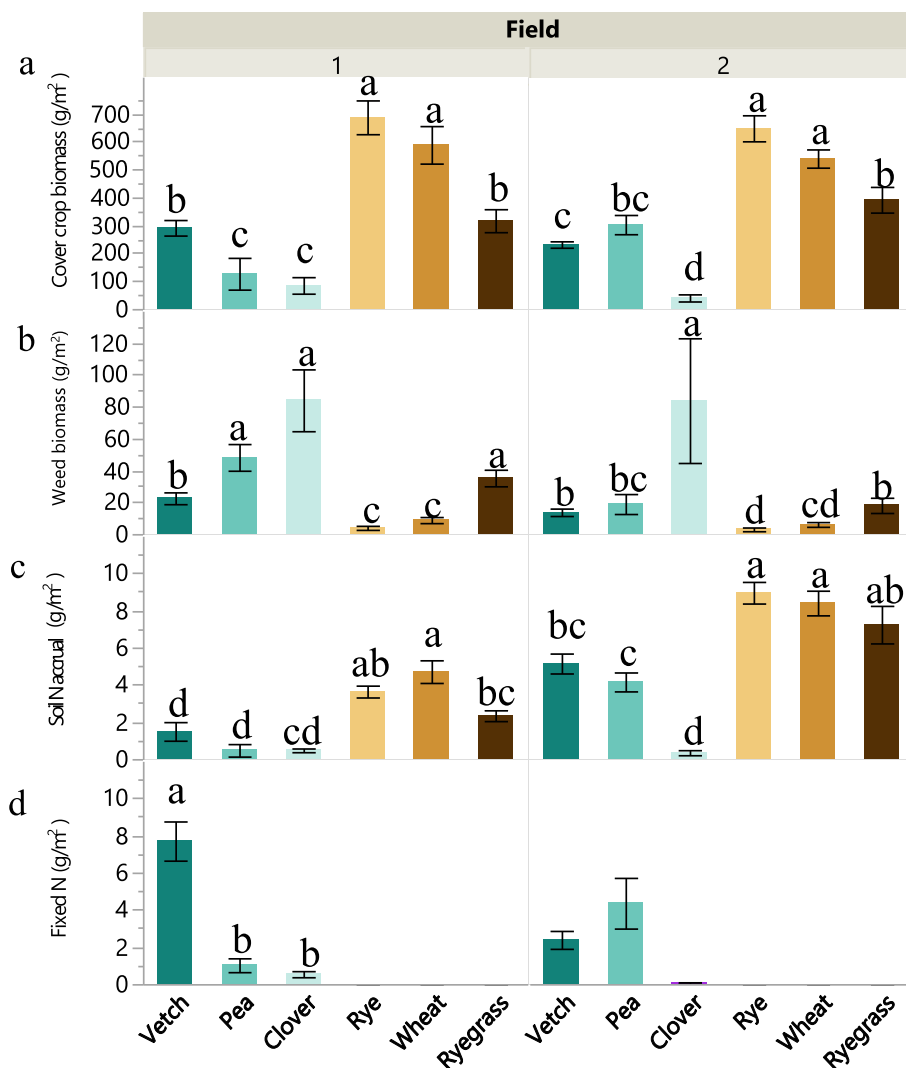

Fig. 1. Average (a) aboveground cover crop biomass (b) weed biomass (c) soil N accrual and (d) fixed nitrogen of species monocultures for six species in Field 1 and Field 2. Different letters within an ecosystem service and field indicate significant differences between species (p < 0.05, Tukey HSD analysis on transformed data (ln for weed biomass; Square root for biomass, soil N accrual, and fixed N), but untransformed data presented). Both fertilized treatments are pooled for a and b with no interactions, while unfertilized data presented for c and d due to methodology. Error bars represent one standard error from the mean.

3. Results

The fields we used for this two-year series of experiments were located on the same soil type within the Cornell University Musgrave Research Farm, and we expected weather-related variability to be the primary source of environmental variation between the two years the experiment was conducted. However, while there were small differences in precipitation and temperature, weather conditions were relatively similar with only slightly less total precipitation over the cover crop growth period for Field 2 as well as about 300 fewer GDD (Table 2).

In contrast, there were significant differences in soil fertility and weed pressure between the fields used in the two years. Field 1 had greater extractable P, but lower total soil N and standing inorganic N pools in the spring suggesting that overall plant available N was lower in Field 1 compared to Field 2 (Table 2). Background field weed biomass and weed density were both lower in Field 2 (Field 1 vs. Field 2: 128 g/m^2 vs. 58 g/m^2, p = 0.0028 compared to 371 plants/m^2 vs. 39 plants/m^2, respectively, p = <0.0001).

3.1. Summary of variation among species and cultivars

We found considerable variation across the six species for several of the ecosystem services we evaluated (Fig. 1, Tables S5 and S7). As expected, rye and wheat generally produced greater biomass in conjunction with greater soil N uptake compared to ryegrass and the legume species (Fig. 1, Table S7). Crimson clover survival was substantially impaired by harsh winters in both years, decreasing biomass production and, as a result, the ecosystem services derived from shoot biomass were also reduced. Plant density at harvest for clover in monoculture was typically less than half the average density of other species for both Field 1 and 2 (Fig. S1).

Compared to these striking species differences, intraspecific variability was rather limited. We observed statistically significant differences in biomass among wheat and vetch cultivars, although these differences were generally small, and rarely relevant from an agronomic perspective (Figs. S2 and S3 and Table 8S). Within-species weed suppression and soil N accrual were not significantly different across cultivars (with one exception for weed suppression in Field 2 where 'Ernst Vetch' was significantly less effective than the highly weed suppressive 'Purple Bounty').

3.2. Intraspecific diversity effects

The effect of increased intraspecific diversity was inconsistent across the ecosystem services we measured. While we observed a significant increase in weed suppression by cultivar mixtures compared to the average cultivar monoculture performance, we detected no other statistically significant intraspecific diversity driven responses for the other ecosystem services evaluated (Fig. 4S, Tables S6 and S10). While there was a general trend for greater weed suppression in cultivar mixtures across all six species, significant differences were only detected in three species, vetch, pea, and ryegrass (Fig. S4b, Table S10).

To analyze the effects of intraspecific diversity more fully, we compared the performance of cultivar mixtures to each individual vetch or wheat cultivar monoculture. The results of these comparisons reinforced our findings from the comparison using averages across monocultures where we found no instances where the cultivar mixture differed from all the cultivar monocultures (Fig. S2 and Table S8).

3.3. Increasing species richness and functional diversity

The ecosystem service response to increased species richness was considerably greater compared to intraspecific diversity, though again, it was strongest for certain outcomes including biomass production, weed suppression and the rate of N fixation. We observed a significant increase in biomass production with increasing species richness in grass/
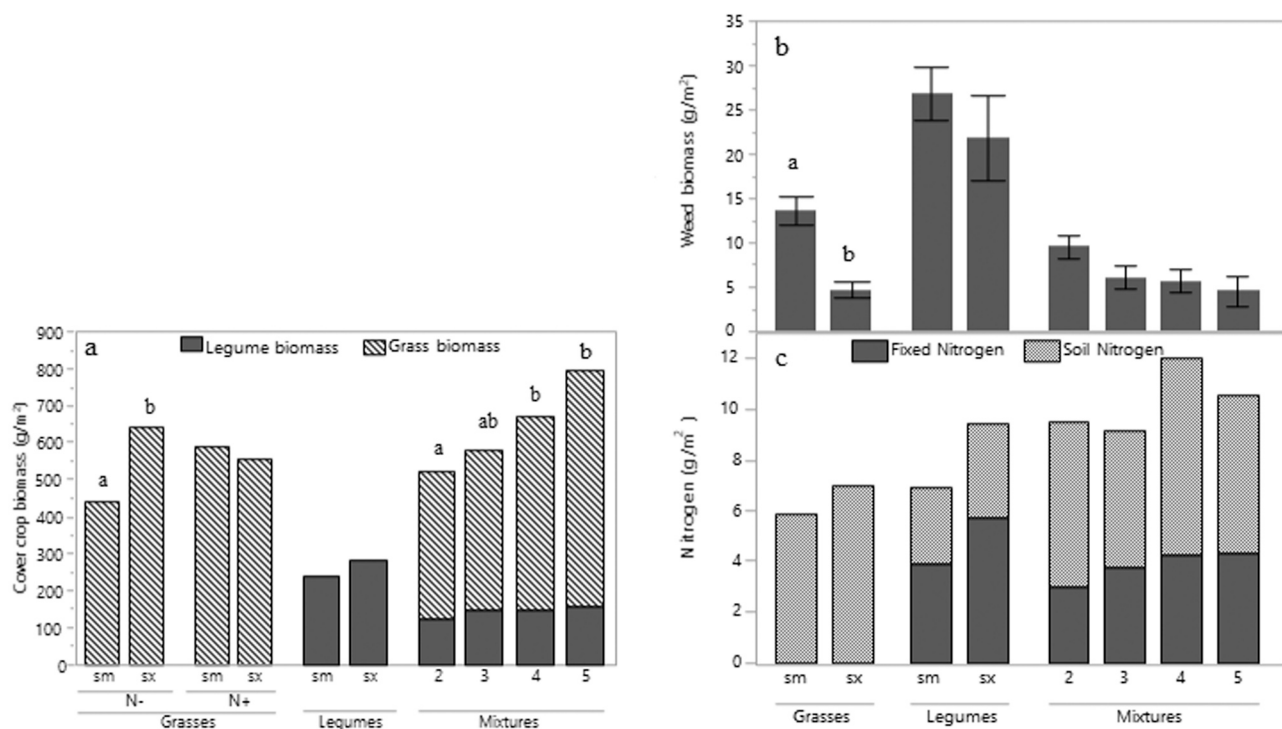

Fig. 2. Average effect of interspecific diversity on cover crop biomass. Comparison of species monocultures (sm) and species mixtures (sx) for single functional group treatments (grasses and legumes) and of species richness levels (2−5) for grass/legume mixture (Mean +/- SE for b). Both fields and fertilizer treatments are pooled and presented except for grasses, which is presented for both fertilizer treatments (+/- N applied) due to an interaction. Different letters within functional group categories indicate significant differences between interspecific diversity levels (p < 0.05, Tukey HSD analysis on transformed data (square root for cover crop biomass, soil N and fixed N, ln for weed biomass), but untransformed data presented.) See table 9 S for more details.

legume mixtures, and for grass species mixtures compared to their monocultures under unfertilized conditions (Fig. 2 and Table S9). While biomass was influenced by species richness, the weed biomass levels were low for all the grass-legume mixtures, with no significant differences between the different species richness levels (Fig. 2b and Table S9). However, increasing the species richness of grass mixtures did result in a significant reduction in weed biomass. Increased species richness did not have a significant effect on total N or on soil N accrual (Fig. 2c and Table S9). Compared to legume only plots, the rate of SNF increased for both vetch and peas in grass/legume mixtures (5–11% in both fields and species, Table S6). However, the reduced legume biomass was a larger driver on total SNF in grass-legume mixtures. As a result, the increased proportion of fixed N did not compensate for the smaller legume biomass in these mixtures and total SNF was not significantly increased in grass-legume mixtures.

3.4. Relationship between cover crop biomass, ecosystem services and environmental conditions

Weed biomass in the cover crop plots had a weak negative correlation with cover crop biomass, but the strength of this correlation varied with functional group composition and field (Fig. 3a). Notably, shoot biomass explained somewhat less of the variation in weed biomass for the grass-legume mixtures compared to grasses and legumes alone, and this trend was most pronounced in Field 2. This suggests that the increased functional trait diversity in these grass-legume mixtures may be contributing to the weed suppression beyond increased cover crop biomass.

Total N, soil N, and fixed N in plant biomass were all correlated with aboveground biomass, though the relationships among these N metrics varied also across functional diversity levels and field. In contrast to grasses, biomass total N for legumes and grass-legume mixtures was not affected by the differences in soil N availability between Fields 1 and 2 (Fig. 3b-e). However, the relative contributions of soil N and N fixation changed as legumes acquired more soil N and down-regulated N fixation under the increased soil N fertility conditions in Field 2 (Fig. 3b-e). In general, total cover crop biomass was correlated with the amount of N fixed, but, in Field 2 there was no relationship between biomass and fixed N in the mixtures. This was likely due to the lower amounts of fixed N in the mixtures where the highest values were ~5 g SNF/m^2 compared to 10–15 g SNF/m^2 in the legumes in both fields and the mixtures in Field 1. We also observed very consistent performance by legumes and the grass-legume mixtures across the two soil N fertility environments for C/N, while grasses growing without legumes had lower C/N in Field 2 compared to Field 1 (Fig. 3d). Interestingly, biomass explained less of the variation in C/N for legumes and mixes compared to grasses, suggesting an additional mechanism may be driving this other than biomass alone. As grasses only have one source of N, overall N content in biomass is strongly influenced by site differences. The ability to source N from soil and the atmosphere allows the legumes and mixtures to compensate for lower soil N though increased SNF.

We found that grass N acquisition varied with location in Field 2 indicating that soil N availability was spatially heterogeneous across the field. We used this variation to examine the response of pea and vetch N fixation in grass/legume mixtures to variations in soil N availability. Both species down-regulated their N fixation in response to higher levels of available soil N (Fig. S5). Due to the small number of points for each treatment, we were not able to detect cultivar or intra/interspecific diversity effects on this relationship. Removal of the data points with undetectable N fixation did not change the regression equation or significance of this relationship (data not shown).

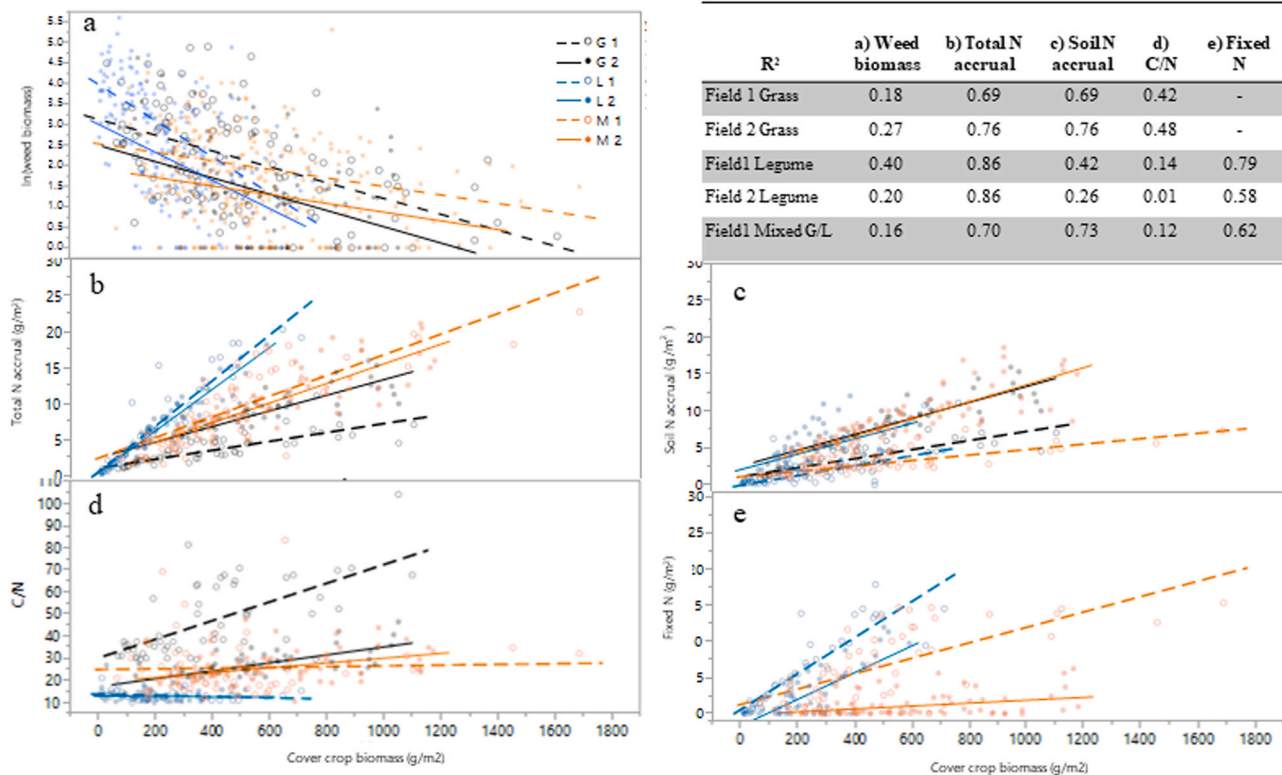We used the land equivalent ratio (LER) to evaluate the possible

R^2	a) Weed biomass	b) Total N accrual	c) Soil N accrual	d) C/N	e) Fixed N
Field 1 Grass	0.18	0.69	0.69	0.42	-
Field 2 Grass	0.27	0.76	0.76	0.48	-
Field 1 Legume	0.40	0.86	0.42	0.14	0.79
Field 2 Legume	0.20	0.86	0.26	0.01	0.58
Field 1 Mixed G/L	0.16	0.70	0.73	0.12	0.62

Fig. 3. (a−e) Relationships between cover crop biomass by (a) weed biomass (natural log transformed) (b) total aboveground biomass nitrogen accrual (c) soil N accrual (d) C/N ratio of biomass and (e) fixed nitrogen in biomass for each field (1,2) and plot composition (G, grass only; L, legume only; M, grass/legume mix). Only data from unfertilized plots presented for b-e, and no interaction with fertilizer for weed biomass (a). R^2 values for regression models on transformed data. Data transformed where necessary (Square root for total N uptake and soil N accrual; natural log for weed biomass; Log10 for C/N). $P < 0.05$ for cover crop biomass for all fields, compositions and ecosystem services.
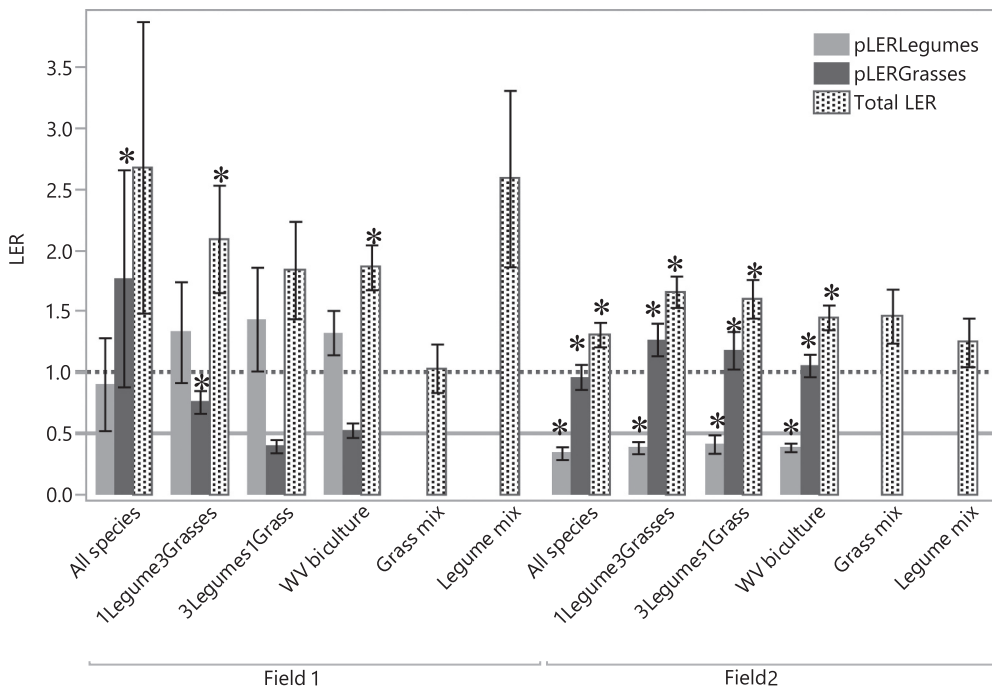
Fig. 4. Total and partial LERs of cover crop mixtures by field; pooled fertilizer treatments. Significant difference from reference indicated by *(p < 0.05, *t*-test on log transformed data, but untransformed data presented). Reference is 1 for total LER (dashed line) and 0.5 for partial LER (solid line). All species, all six species (legumes: vetch, pea, clover; grasses: rye, wheat, ryegrass) together; 1Legume3Grasses, vetch and the three grasses; 3Legumes1Grass, the three legumes and wheat; WV biculture, wheat and vetch mixtures; Grass mix, the three grasses only; Legume mix, the three legumes only. Error bars represent one standard error from the mean.

benefits, of growing multiple cover crop species and grass-legume mixtures on a land area basis compared to monocultures or cover crops consisting of only grasses or legumes. Nearly all the species mixtures had average total biomass LERs greater than 1 in both fields indicating that increased species richness significantly increased biomass production compared to monocultures. There were a few exceptions, with species mixtures composed of either legumes or grasses and the 3Legumes1Grass mix in Field 1 showing no significant increase in shoot biomass compared to single species stands (Fig. 4 and Table S11). Partial LERs for legumes and grasses indicate that while the increased rates of productivity in grass-legume mixtures occurred in both years, the relative contributions of grasses versus legumes varied between fields. Under the higher N fertility conditions in Field 2, LERs greater than 1 were largely due to the success of the grasses, while in the less fertile Field 1 legumes were more dominant. In fact, in Field 2, the partial LERs for grasses were all significantly higher than 0.5, while legumes were all significantly lower.

There were several instances where diversity effects were statistically significant under low N fertility, but not under high N fertility conditions. First, we observed a significant increase in aboveground biomass with grass species mixtures only under unfertilized conditions (Fig. 2). Second, in Field 1 we found a significant reduction in the proportion of mixtures exhibiting transgressive overyielding (where mixture yield is greater than the most productive monoculture component) in fertilized sub-plots (32%) compared to 47% in unfertilized sub-plots (Fisher's Exact Test p = 0.0440, data not shown). However, there were no other differences in occurrence of transgressive overyielding in the other fields or fertilizer treatments, with approximately 50% of the plots showing transgressive overyielding overall. Though not significantly different, species richness accounted for more of the variation in biomass under unfertilized conditions, and this was more pronounced in the lower fertility Field 1 (Fig. S6a). Finally, in both fields the fertilized sub-plots had lower average LERs than the unfertilized sub-plots, though this difference is also not significant (Fig. S6b).

4. Discussion

An extensive literature demonstrates that incorporating cover crops into conventional crop rotations where cash crops normally alternate

with bare fallows is an effective way to increase diversity in agroecosystems, and provide ecosystem services beyond improvements in cash crop yield (Davis et al., 2012; King and Blesh, 2018; McDaniel et al., 2014; Smith et al., 2008; Tonitto et al., 2006). Our study shows that diverse cover crop plantings have the potential to extend these benefits even further using cover crop mixtures composed of functionally diverse species and cultivars. We found that mixtures with the greatest functional diversity within grasses and legumes provided the greatest increases in measured ecosystem services. These mixtures minimized the tradeoffs of using grasses or legumes alone and lessened the effects of variation in soil N availability on productivity. Compared to the effects of species richness, increased intraspecific diversity had a muted effect on ecosystem services. In some cases, reduced available soil N increased the strength of the diversity effect on biomass production, lending some support to the stress-gradient hypothesis (He et al., 2013).

4.1. Intraspecific diversity: limited benefits for ecosystem services

In general, greater intraspecific diversity had limited effects on the ecosystem services we measured, with the notable exception of weed suppression. In choosing cultivars and populations, we attempted to select contrasting cultivars to expand functional trait diversity in each mixture. Functional trait diversity has been shown to be highly correlated with ecosystem function in unmanaged ecosystems (Cadotte et al., 2011; Flynn et al., 2011; Roscher et al., 2012). However, there were four barriers to achieving this goal of selecting contrasting cultivars to expand intraspecific functional diversity. First, detailed information about the characteristics of cultivars and populations of the species used in our experiment was not always available. Second, for several species there had been little or no effort to develop cultivars. For example, there has been very little cultivar selection for vetch, resulting in only few recognized cultivars (Maul et al., 2011), two of which we did use ('Purple Prosperity' and 'Purple Bounty'). Third, out-crossing species such as vetch tend to have significant variation within populations further reducing the potential increase from adding another cultivar or line (Maul et al., 2011; Yeater et al., 2004). Lastly, for four of the six species we used, our cultivar/population mixtures consisted of only two different lines/cultivars. Our meta-analysis found that significantly greater benefits accrue with four or more cultivars, consequently this

may have been a key limiting factor for species with fewer populations or cultivars growing in mixtures (Reiss and Drinkwater, 2018).

While the relationship between greater intraspecific diversity and expanded ecosystem services in this study was restricted to only a few instances, the responses we did detect (e.g. enhanced weed suppression, greater winter survival) are important and highly desired by farmers (Wayman et al., 2016). The considerable literature investigating the capacity for cultivar mixtures to expand ecosystem services suggests that this strategy could be a low risk, valuable component of a multi-faceted approach to using restored biodiversity to reduce dependence on purchased chemical inputs(Grettenberger and Tooker, 2015; Kiær et al., 2009; Newton et al., 2009; Smithson and Lenne, 1996; Swanston et al., 2005).

4.2. Species richness and functional diversity enhance ecosystem service provision

The grass/legume mixtures in our study, even at low species richness levels, were comparably or more productive than either grasses or legumes grown alone. The clear expansion of functional traits in grass-legume mixtures compared to both monocultures and species mixtures comprised of only one of these functional groups likely drives the gains in ecosystem services we observed with species mixtures. Our results support the generally held view that functional diversity is a better predictor of ecosystem functions compared to species richness, which is more straightforward to quantify. This is true in both unmanaged and agricultural ecosystems, particularly for communities on the lower end of species richness (Cadotte et al., 2011; Ebeling et al., 2014; Finney et al., 2016; Martin and Isaac, 2015; Roscher et al., 2012).

In our study, grass/legume mixtures tended to be more productive and the greater productivity of these mixtures was driven largely by increased grass biomass. When grown in mixtures with legumes, the grasses take up the bulk of available soil N while legumes, which are less competitive for soil N are able to maintain their productivity through SNF (Bedoussac et al., 2015; Temperton et al., 2006). In this case, the differences in N acquisition are the most important functional traits driving biomass production, though other differences within these functional groups may also play a role, such as growth habit, height and root architecture differences (Eviner and Chapin, 2003; Roscher et al., 2012).

The fact that legumes can down-regulate their SNF in response to available soil N also has important implications for how legumes can moderate the effect of environmental variability, as well as the tradeoffs of legumes or grasses alone. In contrast to studies with artificial application of N or multiple site conditions, we used the large amount of in situ plant-available soil N heterogeneity across Field 2 to investigate the relationship between soil N fertility and SNF in these three legume species. More than a quarter of the variation in N fixation rates for vetch and pea grown in grass/legume mixtures was explained by variation in plant available soil N. In these mixtures, the legumes clearly down-regulated N fixation when plant available soil N was high. The tendency for legumes to downregulate of SNF with increasing levels of soil nitrate has been documented across sites with different soils or in treatments receiving different rates of inorganic fertilizer application (Blesh, 2019; Kiers et al., 2003; Schipanski et al., 2010; Unkovich et al., 2008; Waterer and Vessey, 1993). For example, in a long-term cropping systems experiment, soybeans and clover down-regulated their rates of N fixation (15% and 19% respectively) in soils with a history of compost application and consequently higher inorganic soil N levels (Snapp et al., 2017). From a cover crop management perspective, it would be advantageous to capitalize on this self-regulation by selecting legume species and cultivars that can prioritize soil N uptake and retention when N is available, and still fix N when it is less available (Blesh, 2019; Rengel, 2002).

Ultimately, the grass/legume mixtures successfully buffered the variation in the soil environment such as soil N fertility. While the difference in available soil N between Field 1 and 2 reduced total N uptake in grass only stands by $> 50\%$ (3.6 vs. 8.2 g N m^{-2}, respectively), total N uptake was not significantly different for mixtures (Field 1 vs. Field 2: 9.6 vs. 10.4 g N m^{-2}, respectively). Instead, the composition of N shifted and the greater SNF in mixtures growing in Field 1 compensated for the lower soil N fertility. For example, N fixed by vetch in grass-legume mixtures tended to be 3-fold greater in Field 1 compared to Field 2.

While the soil environment effects on SNF were generally much greater than those of cover crop community composition, we did find significant differences in SNF between legume only stands and grass-legume mixtures and these patterns were consistent across the diversity gradient. The proportion of fixed N increased for legumes growing in mixtures with grasses compared to legume monocultures and legume species/cultivar mixtures. However, this increase in the contribution of fixed N to total N acquired did not compensate for reductions in legume biomass that occurred in Field 2. Overall, the amount of fixed N m^{-2} did not increase in mixtures. This trend for increased N fixation rates accompanied by no change or reductions in total N fixed compared to legume monocultures is a common outcome in grass-legume mixtures. The consistent upregulation of SNF by legumes grown in mixtures with grasses compared to legume only stands regardless of soil N background suggests that this is a robust response to grass competition. However, the outcome of the total amount of fixed N is dependent on field conditions and the effect of competition between legumes and non-legumes on total SNF is difficult to predict. Additional studies to quantify interactions among seeding density, soil fertility and grass-legume competition would be useful for fine tuning grass-legume seeding rates so that both N retention and N supply can be enhanced.

The C/N of the mixtures was also stabilized in grass-legume mixtures and was not affected by the differing soil conditions in the two fields. The grasses efficiently took up any available soil N, while the legumes compensated when soil N was low through fixation, maintaining overall N in the aboveground biomass (White et al., 2017). It is also worth noting that for legumes and grass-legume mixtures aboveground biomass and C/N were not strongly correlated; both had relatively low C/N at small and large biomass levels, a valuable consideration in terms of organic matter contribution and nutrient availability. In contrast, biomass production in grasses was significantly correlated with greater C/N, increasing the risk that N immobilization could negatively impact crop growth following incorporation of a large stand of cover crop biomass dominated by grasses (Jensen, 1997).

The value of the moderate 22:1 C/N of the mixtures is also important for subsequent crop nutrition. When net N mineralization is not well synchronized with crop acquisition, the potential for environmental N losses is greater (Quemada and Cabrera, 1995; Schweizer et al., 1999). When relying on cover crops or other organic matter as a primary crop nutrient source, synchronizing N availability is critical (Poffenbarger et al., 2015). Grasses, with average C/N of 33:1 might immobilize N when the crop needs it, while legumes have a very narrow C/N, commonly about 10:1, which decomposes quickly before the crop's peak demand (Snapp et al., 2005; Wagger et al., 1998). In addition to the consistency of C/N in the mixtures and across biomass levels, the 22:1 ratio of the mixtures is more appropriate for adequate nutrient release for a subsequent crop compared to either grasses or legumes grown alone (Ruffo and Bollero, 2003). The consistency of an appropriate C/N in the functional group mixtures across the two fields suggests that these mixtures can help to synchronize N mineralization with crop uptake and are a robust choice across very different soil conditions.

Finally, weed suppression was generally good across all the treatments, regardless of composition, with nearly two thirds of plots having < 5 g/m^2 of weed biomass. At low levels of cover crop productivity (< 200 g biomass/m^2), there was a tendency for grass-legume mixtures to have reduced weed biomass compared to stands composed of only grasses or legumes. Similarly, while cultivar mixtures of vetch and ryegrass did not have significantly greater shoot biomass compared to

single cultivar stands, weed control was significantly greater in these mixtures.

Our results support the idea that multiple mechanisms contribute to weed suppression. Weed suppression by cover crops is generally attributed to direct competition for resources and the resulting increase in biomass production by the cover crop and reduced growth in weeds (Christensen, 1995; Kruidhof et al., 2008; Masilionyte et al., 2017). However, certain cover crop species also produce allelochemicals, which can inhibit the growth of weeds beyond just resource competition (Lawley et al., 2012). While it is challenging to tease apart the relative contribution of different mechanisms, it is likely that allelopathic effects from vetch, rye, and ryegrass contributed to the weed suppression (Mennan et al., 2020). This is especially evident for the increased weed suppression in cultivar mixtures of vetch and ryegrass. Increased weed suppression in cultivar mixtures did not correspond with greater cover crop productivity, suggesting that there may be variation across these cultivars in the production of allelopathic compounds.

Our study included only a few plots with five species growing together (n = 14). In unmanaged ecosystems when overall species richness is low the consensus is that the addition of each species has a substantial impact on overall functioning (Cardinale et al., 2011). While the low species richness in our experiment reflects conditions that are typical for agricultural systems and agriculture research plots, it may have restricted our ability to detect a strong relationship between species richness and ecosystem services. We found a significant increase in certain ecosystem services such as carbon accrual and weed suppression, which was related to species richness (Fig. 2). For other services, a sizable portion of the variation in ecosystem services was better correlated to cover crop biomass (Fig. 3). Expanding species richness and increasing the representation of mixtures with greater diversity may be necessary to achieve/detect ecosystem service impacts.

Our approach to seeding rate and density, while consistent with our objectives to evaluate the effect of plant community diversity on ecosystem services, may also have influenced our results and outcomes. Stand density can substantially effect total biomass as well as the performance of the components in that community (Connolly, 1986). If we had selected a different seeding density, one that corresponded with lower legume rates for instance, the grass/legume mixtures we evaluated may have been even more dominated by grasses. Of course, there are many factors other than initial seeding density or proportions that determine ultimate stand composition, even in short lived annuals, such as time of seeding and the environmental conditions during germination (Mirsky et al., 2017). Plant architecture and growth habits (e.g. viney legumes and tillering small grains) can also contribute to both stand composition through competition as well as overall biomass outcomes. At a given seeding density, a tillering grass may be able to exploit open space and produce high biomass, when seeded at a lower rate in mixtures. Finally, while we did not consider seed costs while developing our seeding rates, economics likely influence the recommended rates for cover crops. Cheaper grass seed is generally favored over more expensive legume seed, resulting in skewed proportions that may not be best suited to the given desired outcomes.

4.3. Enhanced diversity effect under more stressful conditions

In some instances, the effect of species richness was increased (though not always significantly) under less favorable conditions, providing some support for the stress-gradient hypothesis (SGH) under nutrient stress conditions. The SGH suggests that the greater facilitation between plants in high stress environments accentuates the diversity effect in these conditions, while under lower stress the diversity benefit is not as important or detectable (He et al., 2013). Many studies of the SGH have looked at physical stressors such as temperature, salinity, and moisture, while relatively few studies investigated the impact of the specific resource stress of N availability or even nutrients in general. One study in Glacier Bay, Alaska suggested that variations in nutrients across

the landscape could shift the balance towards facilitation and away from competition between a conifer and a N-fixing tree (Callaway and Walker, 1997). Interestingly, the strongest evidence in our study for the SGH was in the grass monocultures and mixtures, where there was no change in biomass in the species mixtures when fertilized, but a significant increase in biomass when unfertilized. For mixtures with grasses and legumes we also observed small increases in biomass under lower fertility conditions, although these differences were not always significant (Fig. S6).

Complementary rooting structures, or preferences for different soil N forms (ammonia vs. nitrate) may explain some of the facilitation allowing the species mixtures to perform better than expected under these more nutrient limited conditions (Bedoussac et al., 2015; Bukovsky-Reyes et al., 2019; Hauggaard-Nielsen and Jensen, 2005; Kiær et al., 2013). Additionally, in agricultural systems, legumes and grasses have been shown to facilitate growth beyond basic nutrient uptake differences, through cultivation of beneficial microbial communities (Duchene et al., 2017). Both grasses and other non-woody species tend to be more competitive under lower stress conditions compared to trees and shrubs, but can shift dramatically towards facilitation with increased stress (He et al., 2013). These characteristics make annual cover crop species ideal for responding to variations in resource stresses across fields and farms.

Lower fertility conditions interacted with plant community diversity to drive several ecosystem service outcomes we measured. Our imposed +/- N fertilizer treatments had different effects on ecosystem service outcomes between the two fields demonstrating how the experimental design interacts with environmental conditions to influence diversity effects on ecosystem functions. Some recent cover crop diversity experiments have found inconsistent outcomes from more diverse mixtures in terms of transgressive overyielding (Finney et al., 2016; Smith et al., 2014; Wortman et al., 2012). This may be explained by generally good conditions and management practices in small-scale experiments conducted on research farms. Differences in general in environmental conditions on the dynamics of the mixtures may also account for these inconsistent results (Reiss and Drinkwater, 2020). In our experiment alone, we found inconsistent results for transgressive overyielding, with increased rates in one field and fertility treatment, but not others (See Section 3.4). Additionally, while our experimental design included a basic plus/minus fertilizer treatment to test the effect of N availability on these cover crop monocultures and mixtures, many more studies of environment-cover crop interactions are needed. In particular, treatments designed to specifically test how soil fertility and other stressors influence the diversity-ecosystem function relationship need to be included in research aimed at optimizing cover crop mixtures. Ultimately, more targeted experimental trials could help expand the stress-gradient hypothesis into agroecosystems, for example by identifying relevant thresholds where nutrient stress conditions begin to impact the diversity response. Additional research would also help to develop reliable mixtures for a wide range of environmental conditions.

5. Conclusions and practical outcomes

We observed significant benefits from increasing species richness, especially when greater species richness led to substantial increases in functional diversity. Specifically, species mixtures had stronger effects on biomass production compared to intraspecific mixtures. When considering the broader applications for agricultural systems, it is important to note that the seeding rates used are not typical for on-farm plantings of cover crops. Furthermore, as with all agricultural experiments, factors such as management constraints or cost-limitations will impact farmer adoption decisions. Mixtures of grasses and legumes, which contained the greatest functional diversity, were particularly effective at increasing ecosystem service performance and buffering environmental variation such as soil fertility. Consequently, diverse mixtures may be especially useful in poorer soils or in fields where there

is substantial soil spatial heterogeneity. Lastly, as climate change increases the frequency of extreme weather events such as drought and excessive rainfall, the resulting unpredictable growing conditions will increase the need for planting cover crops composed of species that tolerate these differing conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2021.107586.

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