Winter Cover Crop and Fall-Applied Poultry Litter Effects on Winter Cover and Soil Nitrogen

R. Seman-Varner,* Jac J. Varco, and M.E. O’Rourke

ABSTRACT

Increasing diversity in monoculture production through inclusion of cover crops (CC) and reducing chemical fertilizer inputs by application of animal manures could increase multiple ecosystem services and agroecosystem function. This study was designed to quantify selected ecosystem services from legume, grass, and biculture CC and poultry litter (PL) in strip-tilled corn (Zea mays L.). Treatments included hairy vetch (Vicia villosa Roth), cereal rye (Secale cereale L.), vetch-rye biculture, and winter fallow (WF) all with and without fall-applied PL. Prior to CC termination and corn planting, aboveground dry matter, N content, and C/N ratios of CC and weed biomass were measured; soil inorganic N (ΣNO₃⁻ + NH₄⁺) was analyzed every 2 wk following CC termination. Biculture transgressively over-yielded in 2014 but not in 2015, accumulated levels of biomass N similar to vetch treatment (Vetch), suppressed weeds as effectively as ryegrass treatment (Rye), and maintained soil inorganic N levels similar to Vetch by the second year of study. Overall, CC yield increased 20 to 30% with fall applied PL. Vetch C/N ratio decreased 14% with PL in 2015, while Rye C/N ratio was unaffected. Average weed C/N ratios were 24.5 with Rye and 16.2 with vetch + poultry litter treatment (VetchPL). Cover crop systems with Vetch showed the greatest soil inorganic N, while Rye inorganic N levels were similar to WF. Winter annual weeds were suppressed at least 92% by CC, and most effectively when Rye was present. Ultimately, optimization of cropping systems including CCs and PL will require consideration of interspecific interactions and variable responses among species.

Core Ideas

• Cover crop yield increased 20 to 30% with fall applied poultry litter.
• Biculture cover crop optimized yield, nitrogen content, weed suppression, and soil nitrogen.
• Carbon/nitrogen ratios of vetch and weeds were affected by poultry litter and cover crop treatments.
• Apparent poultry litter nitrogen recovery was greatest when a legume was present.
• Soil inorganic nitrogen increased with vetch and poultry litter.

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Conventional monoculture cropping systems designed to optimize productivity with high chemical fertilizer and pesticide inputs often result in simplified, low-functioning agroecosystems that may not sustain an optimum level of productivity (Tilman, 1999). In contrast, conservation agriculture focuses not only on specific provisioning ecosystem services (providing food, fiber, and fuel), but includes regulating (e.g., water quality) and supporting (e.g., soil health, soil conservation, weed suppression, and nutrient cycling) services as well (Braat and de Groot, 2012). Agroecosystem function increases in response to conservation practices that minimize soil disturbance, maintain cover, increase organic residues, and diversify crop rotations (Duiker and Thomason, 2013).

Cover crops are a fundamental conservation practice used to enhance the sustainability of agricultural systems (Dabney et al., 2010). Cover crops have long been recommended to reduce soil erosion and improve soil quality (Langdale et al., 1991; Dapaah and Vyn, 1998). Additionally, water quality is positively affected by CCs as they can absorb and assimilate residual nutrients and supplement nutrient requirements of cash crops (Shipley et al., 1992; Dabney et al., 2010). Cover crops can also reduce herbicide inputs when managed to suppress in-season weeds that compete with row crops, especially when CCs are mechanically terminated (Yenish et al., 1996; Creamer and Dabney, 2002). Cover cropping is also used to suppress winter annual weeds; thereby reducing the need for fall applied herbicides (Krausz et al., 2003). Furthermore, agroecosystems that maintain CC residues with reduced tillage increase soil organic matter and support soil biological activity and nutrient cycling (McDaniel et al., 2014; Tiemann et al., 2015).

Combining two or more CC species that offer complementary benefits or exploit contrasting functional niches may theoretically provide ecosystem service benefits beyond single-species cover cropping (Storkey et al., 2015); however, these benefits may not materialize in the field depending on species composition (Appelgate et al., 2017). Species functional traits drive...
differences in nutrient retention, nutrient cycling, biomass production, and weed suppression (Díaz et al., 2013; Finney et al., 2016). Grass cover crops typically provide the greatest soil conservation, nutrient retention, and weed suppression benefits, but may decrease crop yields due to N immobilization (Meisinger et al., 1991; Tonitto et al., 2006). In contrast, legume CCs enhance soil N availability through the introduction of biologically fixed N, and although net N availability can be variable (Frye et al., 1988), it is quantifiable and substitutable for commercial fertilizer N (Varco et al., 1999; Miguez and Bollero, 2005). However, legume CCs may not suppress weeds or conserve soil as effectively as grasses due to slower growth during fall establishment and more rapid decomposition of ground cover residues following termination (Clark et al., 1995; Ranells and Wagger, 1996; Daniel et al., 1999; Bärberi and Mazzoncini, 2001). With multiple species of CCs, there may be tradeoffs in functionality due to competition or facilitation including biomass yield, N content, residual nutrient recovery (especially N), decomposability and N release, and weed suppression (Quemada and Cabrera, 1995; Hayden et al., 2014; Poffenbarger et al., 2015).

These tradeoffs may alter residue production relative to original planting densities or affect residue quality, which could directly affect ecosystem services such as N mineralization and weed suppression (Finney et al., 2016). Carbon, N, and lignin content of CC residues drive N mineralization rates and availability to a following cash crop (Quemada and Cabrera, 1995). Additionally, weed suppression is most effective in cover cropping systems with residues with wider C/N ratios, which have slower decomposition rates (Creamer et al., 1997). Conversely, inclusion of a legume species may narrow the overall C/N ratio, which could speed decomposition and N release from the resulting residues (Collins et al., 1990).

Coupling CCs with animal manures may further enhance the sustainability of agricultural systems if the CCs recover and recycle nutrients from the manure while maintaining other ecosystem services (Drinkwater et al., 1998). Utilization of PL in cropping systems in the mid-southern United States, primarily applied in the fall due to convenience, can improve soil properties by adding organic matter and nutrients, and increasing aggregate stability (Adeli et al., 2009). However, overwinter losses of N may be great due to mild temperatures and abundant rainfall, and growers generally assume 0 N credit for the next cropping season (Adeli et al., 2011). Previous studies have explored the effects of CC mixtures or animal manures on crop production, but they do not provide a comprehensive picture of the impacts of coupling CC mixtures with animal manures to maximize supporting and regulating services (Singer et al., 2008; Adeli et al., 2011).

In this study, we compared either a monoculture legume or grass as well as a biculture mix of the two to a WF control all with and without PL. We examined CC biomass productivity and N content, C/N ratio, weed suppression, and early season soil inorganic N for all combinations of CCs and PL to quantify the effects on selected ecosystem services. We hypothesized PL would increase biomass yield and decrease C/N ratios of rye and hairy vetch residues in monoculture and biculture. We also expected the biculture would produce more total and individual species biomass, while we did not expect an effect on C/N ratios of individual species. Additionally, we hypothesized weed biomass would be lowest for the rye and biculture systems, and did not anticipate a change in weed C/N ratio. Finally, we expected soil inorganic N to be greatest with hairy vetch and PL following CC termination and to increase during the growing season and the second year of the study.

MATERIALS AND METHODS

Study Site

The study was conducted at the W.B. Andrews Agricultural Research Systems Farm at Mississippi State, MS (33°28´ N, 88°45´ W). Soils at the experimental site are mapped as a Marietta fine sandy loam (fine-loamy, siliceous, active, thermic Fluvic Haplargids; 89.9% of experimental area) and a Leeper silt clay loam (Fine, smectitic, nonacid, thermic Vertic Epiaquents; 10.1% of experimental area). The local climate is characterized by cool winters (January average high 11.9°C and low –0.7°C) and hot summers (July average high 33.1°C and low 21.5°C) with average annual rainfall of 1402 mm distributed somewhat evenly throughout the year (Diamond et al., 2013). Temperatures during the experiment were normal, but precipitation deviated from normal throughout (Fig. 1).

Experimental Design

The experimental design was a randomized complete block of 12 treatments and four replicates. The field study included four CC treatments in factorial combination with and without fall applied PL. Cover crop treatments included ‘Elbon’ rye, hairy vetch, hairy vetch plus rye biculture, and WF. Poultry litter broadcast was broadcast applied to plots following CC establishment in the fall. All CC × PL treatment combinations are referred to hereafter, as Vetch, VetchPL, Rye, RyePL, VetchRye, VetchRyePL, WF, and WFPL (lowercase “vetch” and “rye” in text refer to the species, plant component, or residue, rather than the treatment). Fertilizer N rate treatments were included in the design to compare soil inorganic N levels of fertilizer and CC × PL treatments. Fertilizer N rates ranged from 0 to 224 kg N ha⁻¹ in 56 kg N ha⁻¹ increments as NH₄NO₃ applied broadcast following corn planting. Fertilizer N rate treatments were managed as WF and were randomly assigned within blocks along with CC × PL treatments. Each plot measured 3.86 × 12.19 m and included four rows of corn at a spacing of 0.97 m. The study was replicated across 2 yr using the same treatment design and treatment plot locations (October 2013 to September 2014 and October 2014 to September 2015).

Field Methods

Cover crops

Cover crops were planted with a grain drill on 10 Oct. 2013 and 21 Oct. 2014. Prior to fall planting, hairy vetch seed were inoculated with rhizobacteria (N-Dure, INTX Microbials, LLC, Kentland, IN). Seeding rates were 30 kg ha⁻¹ for Vetch, and 60 kg ha⁻¹ for Rye in both years of the study, and 15/30 kg ha⁻¹ VetchRye in 2014 and 25/25 kg ha⁻¹ VetchRye in 2015, adjusted for 90 and 85% germination rates for hairy vetch and rye, respectively. Cover crops were terminated with glyphosate [A-phosphonomethyl (glycine) as the K salt at 1.54 kg a.e. ha⁻¹ on 4 Apr. 2014 and 9 Apr. 2015, 2 to 3 wk prior to corn planting.

Poultry litter

Pelletized, composted PL was obtained from MightyGrow Organics Inc. (Fruitdale, AL). Based on annual laboratory
analysis, PL average nutrient concentrations were \( N = 3.33 \text{ g kg}^{-1} \), \( P = 1.80 \text{ g kg}^{-1} \), and \( K = 3.18 \text{ g kg}^{-1} \). Average moisture content was 16.7 g kg\(^{-1}\), with a range from 11 to 22 g kg\(^{-1}\). Poultry litter was broadcast applied on 30 Oct. 2013 and 21 Nov. 2014 at a rate of 2 Mg ha\(^{-1}\) on a dry-weight basis or approximately 60 kg N ha\(^{-1}\). To isolate N effects of PL, treatment plots without PL were amended with approximately equivalent amounts of P and K as concentrated superphosphate \([\text{Ca(H}_2\text{PO}_4], 0–46–0\) and muriate of potash (KCl 0–0-60) at rates of 32 kg P ha\(^{-1}\) and 42 kg K ha\(^{-1}\) in 2014 and 26 kg P ha\(^{-1}\) and 50 kg K ha\(^{-1}\) in 2015.

**Field management**

The field site was strip-tilled prior to corn planting each year. Soil pH was determined annually using a 1:2 w/v (soil/deionized water) equilibrated and measured with a Fisher Scientific Model 25 Accumet pH meter (Denver, CO). Dolomitic lime was applied at a rate of 2240 kg ha\(^{-1}\) as needed in 2012 and 2013 after corn harvest in individual plots with pH values ≤ 6.0. For a more complete description of the site history see Seman-Varner et al. (2017).

**Sampling Methods**

**Cover crops and weeds**

Immediately prior to termination, four randomly selected 0.25-m\(^2\) square quadrat samples of CCs and winter annual weed aboveground biomass were harvested from each plot. Samples were cut with pruning shears approximately 1.0 cm above the soil surface. Samples were separated by CC species and general weed species, oven-dried at 65°C, and weighed. Dried samples were ground through a Wiley mill to pass a 0.43-mm screen. Ground samples were re-dried for another 24 h and sealed in vials. Samples were weighed in duplicate and analyzed for total C and N analysis using an automated Carlo Erba NA 1500 NC dry combustion analyzer (Carlo Erba, Milan, Italy). Nitrogen and C concentrations were used to calculate the C/N ratios of each plant component biomass (hairy vetch, rye, weeds). Weighted C/N ratios for CC × PL treatments were calculated by multiplying the C/N of each individual plant species biomass by its proportional biomass and adding each plant species present in the treatments.

**Soil**

To characterize soil test nutrient levels prior to initiation of CC and PL treatments, soil samples were collected in the fall of 2013 (prior to any lime application) by compositing eight 2.5-cm diameter core samples collected from the 0- to 15-cm depth of each plot. Soil samples were air-dried, crushed to pass a 2-mm sieve, and then analyzed for extractable nutrients using the Mississippi Soil Test Method (Rasberry and Lancaster, 1977). Initial average soil test results and ratings were: pH = 6.25; \( P = 127.5 \text{ mg kg}^{-1} \) (very high), \( K = 127.3 \text{ mg kg}^{-1} \) (high), \( Mg = 70.6 \text{ mg kg}^{-1} \) (high), \( Ca = 2050.5 \text{ mg kg}^{-1} \), and \( CEC = 10.9 \text{ cmol c kg}^{-1} \). Soil samples were collected at 2, 4, and 6 wk following CC termination. Soil samples were collected using a composite of eight 2.5-cm diameter soil cores per plot at depths of 0- to 15-cm, 15- to 30-cm, and 30- to 60-cm on 24 Apr., 8 May, and 22 May in both 2014 and 2015. Soil sampling dates in 2014 corresponded to 20, 34, and 48 d following CC termination, and 3, 17, and 31 d following corn planting. In 2015, soil sampling dates corresponded to 15, 29, and 43 d following CC termination, and 2, 16, and 30 d following corn planting. Soil samples were placed on ice in the field, and then stored frozen at –18°C until extracted. Defrosted, crushed, and mixed soil was extracted for

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**Fig. 1.** Line represents the deviation from the normal average monthly precipitation (mm, solid line) and temperature (°C, dashed line) for the duration of the experiment (September 2013 to October 2015) in Starkville, MS, based on Climate data from the National Centers for Environmental Information (Diamond et al., 2013).
Statistical Analyses

A general linear mixed model ANOVA was used to determine treatment effects on selected response variables. Year, CC, and PL were fixed effects; replicate was random. Year was analyzed as a fixed effect due to the possibility of residual effects of treatments applied to the same plot area each year. The distributions of all data were examined, and normality and variance were evaluated. Data that failed to meet the assumption of normality were log transformed (plant N, weed biomass, weed C/N ratio, and total inorganic N). Data that failed to meet the assumption of equal variance (plant biomass and weighted C/N ratio) were transformed using the square root function. All treatment means are reported after back-transformation, unless otherwise noted. Fisher’s LSD was used for mean separation among treatments. For C/N ratios of separated plant species (i.e., hairy vetch and rye) residues, paired comparisons were determined with a t test.

RESULTS

Total Cover Crop Biomass and Nitrogen Content

Total biomass yield of CC treatments depended on the year (CC × Yr, p = 0.006) and PL (p = 0.02). All CC treatments produced less yield in 2015 compared to 2014 possibly due to variations in planting and termination dates; CCs were planted 11 d earlier in October and terminated 5 d earlier in April in 2014 than in 2015. Averaged across PL treatments, the yearly trends in dry matter production of the CC treatments are shown in Fig. 2a. In 2014 transgressive over-yielding occurred in the VetchRye treatment, but in 2015 VetchRye yielded similarly to Rye. Overall, PL increased CC yield by 33%, or 479 kg ha⁻¹ and the response did not depend on year or CC.

The effects of CC and PL treatments on total N content of residues were similar to the dry matter results as the response to CC depended on the year, the PL effect was significant, and there were no interactions with PL. Overall, Vetch and VetchRye resulted in the greatest N contents both years (Fig. 2b). The effect of PL on CC N content was an overall increase of 34% or 10.4 kg N ha⁻¹ (p = 0.03). Based on an applied PL N rate of 60 kg ha⁻¹ y⁻¹ and CC N contents, the calculated apparent Vetch PL N recovery [(N contents of VetchPL – Vetch)/PL N] across the 2-yr was 41.8% with VetchPL compared to 15.6% for RyePL, 33.7% for VetchRyePL, and 4.9% for WFPL.

Winter weed suppression

Winter annual weeds were effectively suppressed by all CC treatments compared to WF (Table 1). Cover crop treatments reduced winter annual weeds by at least 92% compared to WF (p < 0.0001). Dominant species were henbit (Lamium amplexicaule L.), chickweed (Stellaria media (L.) Vill.), and annual
bluegrass (*Poa annua* L.). The main effect of PL was significant and fall-applied PL increased winter weed biomass by 88% when averaged across all CC treatments (*p = 0.03*). Weed biomass was reduced by 81% with Rye and 61% with VetchRye treatments, compared to Vetch.

**Plant carbon/nitrogen ratio**

To explore the dynamics of the individual CC species and N dynamics within CC communities, paired comparisons of biomass and C/N ratio between CC treatments with and without fall applied PL were made for the separated vetch and rye residues (Table 2). The biomass of both rye and vetch components of the VetchRye treatment in 2014 increased due to the addition of PL by 50 and 70%, respectively. This likely contributed to the overall yield increase and transgressive over-yielding of the VetchRye treatment in 2014. Although there appeared to be a decrease in the C/N ratio of the vetch residues due to PL, the difference was only significant in the Vetch treatment in 2015.

The weighted C/N ratio of total plant biomass, which included CC residues and weeds, for CC × PL treatments differed among CC treatments including WF (Table 3). Both years, the Rye treatment had the greatest weighted C/N ratio and Vetch the lowest, while the VetchRye treatment fell in between the two. VetchRye and VetchRyePL weighted C/N ratios were similar to the weighted C/N of weeds in the WF and WFPL treatments. Winter annual weeds growing in the VetchPL treatment may have benefited from the presence of vetch and PL as evidenced by the lower C/N ratio of the weed fraction in 2014 and 2015. Due to a lack of two-way interactions for weed C/N ratios, the three-way interaction may be a spurious result and the result of alternating high and low C/N values each year between the PL treatments depending on the CC treatment.

**Soil inorganic nitrogen**

Initial samples taken for soil inorganic N prior to ammonium nitrate fertilization [20 (2014) and 15 (2015) d after winter cover termination] showed no residual differences compared to WF (Fig. 3a and b). During the soil sampling period following fertilization, there were precipitation events greater than 12.7 mm on 4, 5, and 20 d after fertilizer application in 2014 and 1 and 24 d after fertilizer application in 2015. The rapid increase in soil inorganic N both years as a result of fertilization was obvious with elevated soil inorganic N levels observed for the fertilizer treatments at 2 wk following application, 34 and 29 d after CC termination. By 2015, the first sampling for soil inorganic N was slightly elevated for VetchPL and VetchRyePL compared to the 224 kg N fertilizer treatments. By the last sampling in 2015 (43 d after CC termination), VetchPL demonstrated soil inorganic N levels similar to the 112 kg N ha⁻¹ rate, while other CC treatments that received the PL treatments depending on the CC treatment.

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**Table 2.** Average biomass (kg ha⁻¹) and C/N ratios of above ground rye and vetch components immediately prior to termination for eight cover crop (CC) × poultry litter (PL) treatments across 2 yr, Mississippi State, MS. Paired comparisons between CC treatments with and without PL are included.†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biomass C/N</th>
<th>Biomass C/N</th>
<th>Treatment</th>
<th>Biomass C/N</th>
<th>Biomass C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye</td>
<td>1795 31.6</td>
<td>– –</td>
<td>Vetch</td>
<td>1508 30.9</td>
<td>2268 30.9</td>
</tr>
<tr>
<td>RyePL</td>
<td>2702 32.1</td>
<td>NS NS</td>
<td>VetchRye</td>
<td>1670 31.0</td>
<td>740 11.3</td>
</tr>
<tr>
<td>Vetch</td>
<td>1105 31.0</td>
<td>2331 9.8</td>
<td>VetchRyePL</td>
<td>2318 31.3</td>
<td>1645 12.6</td>
</tr>
<tr>
<td>VetchPL</td>
<td>NS NS</td>
<td>1485 10.2</td>
<td>VetchRyePL</td>
<td>2322 30.1</td>
<td>22.7 bc</td>
</tr>
</tbody>
</table>

† Abbreviations: Vetch, vetch treatment; VetchPL, vetch + poultry litter treatment; Rye, rye treatment; RyePL, rye + poultry litter treatment; VetchRye, vetch and rye biculture treatment; VetchRyePL, vetch and rye biculture + poultry litter treatment; NS, not significant.

*Paired comparisons are significant at < 0.05 based on a t-test.

**Table 3.** Average weighted C/N ratio by treatment and weed and weed component C/N ratio and ANOVA probability values for the fixed effects of year, cover crop (CC), and poultry litter (PL) for eight treatments during 2014 and 2015, Mississippi State, MS.†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>df</th>
<th>Weighted C/N</th>
<th>Weed C/N</th>
<th>Yr × CC</th>
<th>PL</th>
<th>Yr × PL</th>
<th>CC × PL</th>
<th>Yr × CC × PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vetch</td>
<td>3</td>
<td>11.3 c‡</td>
<td>19.6 c</td>
<td>&lt; 0.001</td>
<td></td>
<td>0.018</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td>VetchPL</td>
<td>3</td>
<td>9.9 c</td>
<td>18.0 d</td>
<td></td>
<td></td>
<td>0.802</td>
<td>0.515</td>
<td></td>
</tr>
<tr>
<td>Rye</td>
<td>3</td>
<td>31.5 a</td>
<td>31.6 a</td>
<td></td>
<td></td>
<td>3.19 a</td>
<td>22.6 a</td>
<td></td>
</tr>
<tr>
<td>RyePL</td>
<td>3</td>
<td>31.9 a</td>
<td>22.6 bc</td>
<td></td>
<td></td>
<td>1.7 c</td>
<td>18.9 abc</td>
<td></td>
</tr>
<tr>
<td>VetchRye</td>
<td>3</td>
<td>23.3 b</td>
<td>23.8 b</td>
<td></td>
<td></td>
<td>1.98</td>
<td>19.4 abc</td>
<td></td>
</tr>
<tr>
<td>VetchRyePL</td>
<td>3</td>
<td>23.2 b</td>
<td>22.7 bc</td>
<td></td>
<td></td>
<td>1.97 c</td>
<td>18.0 bc</td>
<td></td>
</tr>
<tr>
<td>WF</td>
<td>3</td>
<td>24.9 b</td>
<td>24.9 b</td>
<td></td>
<td></td>
<td>1.94 c</td>
<td>19.4 abc</td>
<td></td>
</tr>
<tr>
<td>WFPL</td>
<td>3</td>
<td>24.9 b</td>
<td>24.9 b</td>
<td></td>
<td></td>
<td>22.1 bc</td>
<td>22.1 ab</td>
<td></td>
</tr>
</tbody>
</table>

† Abbreviations: Vetch, vetch treatment; VetchPL, vetch + poultry litter treatment; Rye, rye treatment; RyePL, rye + poultry litter treatment; VetchRye, vetch and rye biculture treatment; VetchRyePL, vetch and rye biculture + poultry litter treatment; df, degrees of freedom; NS, not significant.

‡ Different letters represent significant differences using Fisher’s LSD test at *α = 0.05*.
included vetch had soil inorganic N levels similar to the 56 kg N ha⁻¹ rate. Both the Vetch and VetchPL treatments tended to have the greatest soil inorganic N levels for CC treatments followed by the VetchRye, PL, and VetchRyePL treatments in 2014. In 2015, PL application resulted in a 34 and 21% respective increase in soil inorganic N for the Vetch and VetchRye treatments by the third sampling, though the differences were not significant at α = 0.05. However, by the last sampling date in 2015, soil inorganic N of VetchPL was significantly greater than VetchRye, Rye, RyePL, and WF. Soil inorganic N for the Rye and RyePL treatments did not differ either year and were similar to the non-fertilized WF control for most sampling dates, except at the last sampling of 2014 when soil N was significantly lower.

**DISCUSSION**

This field study quantified the tradeoffs and benefits of winter rye, hairy vetch, and biculture CCs and fall-applied PL for selected ecosystem services including biomass productivity, biomass N accumulation, C/N ratio, weed suppression, and soil inorganic N. The biculture of VetchRye was at least as good as the most productive species grown in monoculture at producing biomass (Rye in 2015), N content (Vetch), and suppressing weeds (Rye). Other ecosystem services varied by CC species with PL which suggests additional interspecies effects.

Our results support the hypothesis that combining functionally diverse species, such as leguminous and grass CCs, can stabilize biomass productivity, possibly by maximizing different resource niches (Silvertown, 2004; Hooper et al., 2005). Although overall CC yield in 2015 was less than in 2014, the reduced rye yield in 2015 when grown in combination with vetch compared to monoculture may have been partially due to the lower seeding rate of rye (25 vs. 30 kg ha⁻¹) and a greater vetch seeding rate (25 vs. 15 kg ha⁻¹) used in 2015 (Clark et al., 1994; Hayden et al., 2014; Poffenbarger et al., 2015). In this study, the VetchRye mix produced greater total yield compared to either species grown in monoculture in 2014, but not in 2015. Transgressive over-yielding in mixed CCs has been investigated with varying results (Sainju et al., 2005; Wortman et al., 2012; Appelgate et al., 2017; Wendling et al., 2017). Further research on interspecific effects between multiple species of CCs grown in mixtures on biomass, N content, and C/N ratio would clarify trends and mechanisms to design optimal systems.

The addition of PL to CCs in the fall increased CC yield across the 2 yr of this study. Cover crop biomass productivity and variation in productivity between years tended to increase with fall application of PL, even though the applied PL nutrient rates were relatively low (approx. 60 kg N, 30 kg P, and 45 kg K ha⁻¹). The probability and magnitude of a response to fall-applied PL is likely dependent on seasonal rainfall and temperature effects on mineralization, N loss mechanisms, CC establishment and other factors affecting growth, such as observed disease pressure (Phytophthora sp.) on vetch in 2015.

Nitrogen content was predictable for some factors in this experiment, while other factors produced unexpected results. Total N content and C/N ratio differences observed for vetch in the Vetch and VetchRye treatments suggest a greater effect of PL on the vetch component than the rye component as demonstrated by apparent PL N recovery of 41.8 and 15.6%, respectively. Typically, grass CCs have been shown to have greater scavenging ability in fertilized systems (Shipley et al., 1992; Ranells and Wagger, 1997). Vetch responded to fall-applied PL N both in N content and in a potential for increasing biomass yield from PL (significant at α = 0.1). The minimal apparent PL N recovery by WF or volunteer weeds of 4.9% suggests the importance of planting a winter CC when PL is applied in the fall to minimize potential N losses. Any additional effect of PL on biological N₂ fixation in the Vetch may be minor, especially in high fertility soils, but it is also a consideration. Previous work exploring the relationship between soil fertility and biological N₂ fixation on legumes have come to varied conclusions (Smeltekop et al., 2002; Schipanski and Drinkwater, 2012; Wortman and Dawson, 2015). Future research that assesses the combined effects of mixed species CC interactions and PL on N assimilation and N₂ fixation may help illuminate mechanisms.

Winter annual weed suppression was effective across all CC treatments compared to WF, thus reducing the need for fall-applied herbicides (Krausz et al., 2003). The increase in weed biomass in response to PL suggests that there exists a potential for enhancing winter annual weed growth with fall-applied PL unless a CC is planted. Residue productivity is often correlated with weed suppression (Finney et al., 2016), and CC mixtures may over-yield (Clark et al., 1997) compared to monocultures. However, other recent studies have found no difference in...
in-season weed suppression between mixed CC and the most productive monoculture species (Smith et al., 2014; Wortman et al., 2012; Appelgate et al., 2017). Additionally, weed suppression and legume proportion have been shown to have an inverse relationship in a study comparing biculture mixtures with seeding rate increments of 17% (Hayden et al., 2014). This study illustrates that CCs including at least 50% Rye are more effective in winter annual weed suppression compared to 100% Vetch CC.

Lower residue C/N ratio of VetchPL in 2015 suggests an effect of PL on vetch residue quality, but no consistent interspecific effect of biculture on the C/N ratio of the vetch component was found. The consistent and relatively fixed C/N ratio of rye residues among treatments suggests the stability of rye biomass production and residue quality, and partially explains the popularity of winter rye in conservation systems as a residue builder (CTIC Report–National Cover Crop Survey; CTIC, 2017). In contrast, an older study including a hairy vetch-rye biculture with later CC termination dates, showed a decrease in C/N ratio for rye grown in association with vetch (Sullivan et al., 1991). Understanding differences in residue quality and quantity of individual species in mixtures and with PL, along with regionally optimized planting dates, termination dates, and seeding rates, will aid in managing CC C/N ratios and consequently the effect on nitrogen cycling in these systems.

Altering the overall C/N ratio of CC biomass through the modification of the ratio of grass to legume composition could more effectively utilize residual sources of soil inorganic N, while maintaining other residue benefits such as weed suppression and water quality (Clark et al., 1997). While PL and inclusion of a legume may not lower the C/N ratio of the rye component, the overall biomass C/N ratio of the biculture would be lower than Rye monoculture resulting in quicker decomposition and N release of the combined residues (Collins et al., 1990). Additionally, the effects of a legume CC and PL on the C/N ratio of the weed fraction suggests that the weed biomass may respond to applied manure or may benefit from legume-derived N. Differences observed in the C/N ratios and tissue N concentration of the CC and PL systems are useful for the prediction of net effects on soil mineral N dynamics (Trinsoutrot et al., 2000).

VetchPL provided greater levels of soil inorganic N compared to other treatments by the end of the sampling season, albeit at much lower levels relative to fertilized plots. Slightly elevated soil inorganic N resulting from the biculture with a vetch seeding proportion of 33% (15 kg ha\(^{-1}\)) was found in 2014, while extractable soil inorganic N with a vetch seeding proportion of 50% (25 kg ha\(^{-1}\)) was comparable to a fertilizer rate of 112 kg N ha\(^{-1}\) in 2015 at the 4-wk sampling. While these results are based on seeding proportions by weight, this suggests corroboration of prior recommendations of at least 40% hairy vetch residue in proportion to rye to increase availability of N from residues (Kuo and Sainju, 1998). The greater concentration of extractable inorganic N resulting from the mixture in the second year likely reflects residual benefits from repeated CC and PL applications and the effect on soil organic matter. These results are also in agreement with a meta-analysis of grass, legume, and grass-legume-biculture CCs in corn production systems where greater fertilizer N rates were needed to optimize corn yield with a grass or no CC, while the greatest benefit of a legume CC existed in low fertilizer N scenarios (Miguez and Bollero, 2005). Essentially, high fertilizer N application rates can mask the effects of the N-supplying ability of the legume CC. Interestingly, biculture systems included in the meta-analysis showed a tendency for intermediate fertilizer N response and provided indications for non-N benefits of the biculture residues, supporting the intermediate soil N availability between Rye and Vetch monocultures in this study. Vetch and the VetchRye residues resulted in net N mineralization and 2-yr average biomass C/N ratios of 11.5 and 23.2, respectively, supporting the conclusion that crop residues with C/N ratios less than 24 result in net N mineralization (Trinsoutrot et al., 2000). Addition of PL showed evidence of improving N availability when used in combination with Vetch alone or in biculture. Corn yield and N content response at this site is consistent with the soil inorganic N results shown here (Seman-Varner et al., 2017).

**CONCLUSIONS**

These results aid in the selection of hairy vetch, rye or biculture and PL combinations to target specific ecosystem services, while considering complex interspecific and management interactions. Addition of PL resulted in about 30% greater yield and N for CCs. Biomass N content of biculture CC was not significantly different from Vetch monoculture. Under low N (non-fertilized) systems, Vetch CC demonstrated the greatest efficiency in utilization of fall-applied PL suggesting both good scavenging ability and possibly a growth response to some other PL derived benefit. The C/N ratio was generally stable for rye under all CC × PL treatments, lower for vetch with PL, and varied by treatment for the weed fraction. Additionally, VetchPL resulted in greater soil inorganic N concentrations than other CC treatments without a significant increase in weed pressure. Rye was most effective at suppressing weeds, although all CC treatments reduced weeds compared to WF. Based on soil inorganic N observations and compared to WF, Rye may decrease productivity if the depressing effect of it on soil N availability is not accurately accounted for with an increase in fertilizer N input (Varco et al., 1999; Seman-Varner et al., 2017). Soil N availability following biculture CC was not consistently affected by PL, but ultimately resulted in similar soil N availability compared to the Vetch monoculture with and without PL, while suppressing weeds as effectively as Rye.

Prioritizing multiple provisioning (providing food, fiber, feed, and fuel), regulating (e.g., water quality) and supporting (e.g., soil health, soil conservation, weed suppression, and nutrient cycling) ecosystem services will ultimately result in more sustainable production practices. Cover cropping and the incorporation of animal manures are fundamental conservation practices that, when managed appropriately, enhance ecosystem services and function. A consolidation of field data that includes multiple CC species with and without animal manures and additional ecosystem services, including evaluation of N losses, would be useful in designing diverse systems that meet economic and environmental priorities.

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