THE ROLE OF COVER CROPS IN AGROECOSYSTEM FUNCTIONING

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Abstract

Current interest in cover cropping is focused on enhancing ecosystem services beyond soil conservation. Cover crop (CC) species function uniquely in their effects on ecosystem services when grown in monoculture or mixtures. This research integrated field experiments and a literature synthesis to evaluate the role of cover crops in improving nitrogen (N) management and simultaneously providing multiple ecosystem services. Legume CC fertilized with poultry litter (PL) could replace 101 to 117 kg N ha^{-1} of fertilizer in corn (*Zea mays* L.) production. Rye (*Secale cereale* L.) CC fertilized with PL had a negligible effect on corn production. Biculture fertilizer equivalence ranged between -12 to +75 kg N ha^{-1}. Fertilizer equivalence of legume-containing treatments increased across time. Without CC, fall-applied PL failed to supply N to corn. Ecosystem services of CC and PL illustrate complex species functions. Bicultures produced more total biomass than monocultures in year 1 but less than rye in year 2. Bicultures were as effective in suppressing weeds as rye, produced corn yield similar to legume, and by the second year had similar amounts of available soil N as the legume. Poultry litter effects and interspecific effects cover crop species biomass differed. Rye yield increased, while legume yield decreased slightly in biculture. Poultry litter increased legume N content and a decrease in legume C:N, while rye N content and C:N were unaffected. The synthesis corroborates that mixed and biculture cover crops yield more than the individual component species. Overyielding was transgressive in 60% of cases studied. Mixture effects varied by species: rye and brassica yield increased, while legume decreased in mixtures. The effect of mixed CC on crop yields varied by crop species and management practices, though generally crops increased 8 to 18% overall. This work can be applied to the design of complex CC and PL systems that optimize individual species functions to enhance ecosystem services.

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Current interest in cover cropping is focused on enhancing ecosystem services beyond soil conservation. Cover crop (CC) species function uniquely in their effects on ecosystem services when grown in monoculture or mixtures. This research integrated field experiments and a literature synthesis to evaluate the role of cover crops in improving nitrogen (N) management and simultaneously providing multiple ecosystem services. Legume CC fertilized with poultry litter (PL) could replace almost half of the inorganic fertilizer required by spring corn (*Zea mays* L.) production. Rye (*Secale cereale* L.) CC fertilized with PL had a negligible effect on corn production. Fertilizer equivalence of legume-containing treatments increased across time. Without CC, fall-applied PL failed to supply N to corn. Bicultures produced more total biomass than monocultures in year 1 but less than rye in year 2. Bicultures were as effective in suppressing weeds as rye, produced corn yield similar to legume, and by the second year had similar amounts of available soil N as the legume. Poultry litter effects and interspecific effects cover crop species biomass differed as well. Rye yield increased, while legume yield decreased slightly in biculture. Poultry litter increased legume N content and a decrease in legume C:N, while rye N content and C:N were unaffected. The synthesis corroborates that mixed and biculture cover crops yield more than the individual component species. Mixture effects varied by species: rye and brassica yield increased, while legume decreased in mixtures. The effect of mixed CC on crop yields varied by crop species and management practices, though generally crops increased 8 to 18% overall. This work can be applied to the design of complex CC and PL systems that optimize individual species functions to enhance ecosystem services.

Dedication

This work is dedicated to the memory of my mother, Cherrie Jones Seman, who taught me perseverance, independence, passion for life, and to be true to myself.

And for Kylie Erin Gillette
1997-2014

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> Long, blue, spiky-edged shadows crept out across the snow-fields, while a rosy glow, at first scarce discernible, gradually deepened and suffused every mountain-top, flushing the glaciers and the harsh crags above them. This was the alpenglow, to me the most impressive of all the terrestrial manifestations of God. At the touch of this divine light, the mountains seemed to kindle to a rapt, religious consciousness, and stood hushed like devout worshippers waiting to be blessed.
>
> -John Muir

Table of Contents

List of Figures

Chapter 2

Chapter 3

Chapter 4

List of Tables

Chapter 2

Chapter 3

Chapter 4

CHAPTER 1 – The role of cover crops in sustainable agriculture

1.1 Introduction

The current rise in interest in cover cropping has been fueled by environmental issues associated with agricultural production, including ground water contamination (Spalding and Exner, 1993), hypoxic zones of bays, gulfs, inland seas and oceans (Diaz and Rosenberg, 2008), and by increasing interest in sustainable production. Cover cropping became a popular practice in the mid 20th century after the Dust Bowl illustrated the critical interaction of climate, weather, agriculture, agricultural policy, and human needs. Cover crops were used primarily to conserve soil and moisture when productive fields were fallow, but have since been managed for more complex ecosystem services including nutrient retention and reducing the use of inorganic fertilizers.

Cover cropping can help to mitigate nutrient losses and pollution. Recoupling of C and N cycles through the application of organic inputs has increased total N systems recovery by 30 to 42% (Gardner and Drinkwater, 2009). The use of cover crops as nutrient-rich green manures may reduce the risk of N losses compared to chemical fertilizers. Recovery of residual soil N from fall planting through spring, as well as microbial-mediated mineralization of organic N from decomposing residues, reduces the quantifty of soil N susceptible to loss (Hansen et al., 2002; Ekholm et al., 2005; Pimentel et al., 2005). Cover crops, especially grass species like rye (*Secale cereale* L.), will scavenge residual N in soil and thereby reduce nitrate leaching. Rye fertilized with vetch green manure or $(NH_4)_2SO_4$, showed consistently less NO_3^- leachate with rye compared to fallow and NH_4^+ fertilizer alone (McCracken et al., 1994). Grass cover crops have shown reductions in NO_3^- concentrations in leachate of 20% to 80% when compared to non-cover cropped systems (Meisinger et al., 1991). Shipley and others (1992) used ^{15}N labeled fertilizer and found that winter rye recovered 45% of fall-applied N, while hairy vetch recovered 10%. The recovery of fertilizer N and N immobilization by high C:N residues may result in a "tightening" of the N cycle and long-term storage of N (McSwiney et al., 2010).

Cover crops may increase nutrient concentrations in soil and be used as a partial substitute for inorganic N in row crop production (Varco et al., 1999). Hairy vetch (*Vicia villosa* Roth) grown as a winter cover crop has been shown to increase grain yield, plant tissue N concentration, and inorganic soil N compared to rye (Ebelhar et al., 1984; Utomo et al., 1990). Nitrogen recovery by corn from hairy vetch was 40 to 45 kg N ha^{-1}, or 30% to 36% of the total N content of this cover crop, while rye immobilized N, conserved soil moisture, and resulted in greater fertilizer N use efficiency during a dry year (Wagger, 1989). The greatest cover crop dry matter, but a slightly lower amount of N released (108 kg N ha^{-1}), occurred with a biculture of rye and hairy vetch compared to a hairy vetch monoculture, which had greater cover crop N content (154 kg N ha^{-1}) and greater N release (132 kg N ha^{-1}; Ranells and Wagger, 1996). In Virginia, N availability was greater with a vetch cover crop than with rye or vetch plus rye, but timing of desiccation and the incorporation method were important to synchronize N release with corn N requirements (Vaughan and Evanylo, 1988). In a recent meta-analysis, non-legume cover crops did not significantly reduce yield, but a reduction in NO_3-N leaching of 70% on average was reported, while legume cover crops reduced leaching by 30% and produced comparable yield to conventional production when at least 110 kg N ha^{-1} was accumulated in the cover crop residue (Tonitto et al., 2006).

Cover crops are also used for weed management as they compete for nutrients, moisture, and light. In addition, winter rye may also have allelopathic effects on weeds (Smith et al., 2011). In tilled versus no-tilled systems of corn production systems, a rye cover crop resulted in a 54% to 99% reduction of weeds, while legumes reduced weeds by 20% to 65% at the time of planting (Bàrberi and Mazzoncini, 2001). Weed suppression is affected by cover crop species functional group, season of cover cropping, and weed species.

Cover crops increase soil biological activity, and therefore nutrient cycling, by providing habitat. Total fungal and bacterial populations and activity were highest in a system with crimson clover (*Trifolium incarnatum* L.) cover crop, followed by rye compared to no cover (Reddy et al., 2003). Using phospholipid fatty acid profiles, soil respiration rates, and ^{13}C-cycling, organic cover cropped soil had the largest and most heterogenous microbial populations, compared to animal manure or no cover systems (Wander et al., 1995). Nematode enrichment indicator groups, including bacterial and fungal feeders associated directly with N mineralization, were greater in legume and grass-legume cover crop mixtures compared to a grass cover crop or winter fallow (Dupont et al., 2009).

Like cover crops, animal manures provide nutrients and other ecosystems services to soil, but can also be a potential non-point source of pollution (Edwards and Daniel, 1993; Coufal et al., 2006). The combination of poultry litter and cover crops can be managed to conserve soil, provide nutrients, and reduce leaching and runoff in a variety of cropping systems. Winter rye grown in a cotton-cotton-corn rotation was shown to scavenge residual N two years after poultry litter was applied; thus, effectively decreasing overall fertilizer N input and NO_3^- leaching (Nyakatawa et al., 2001). Fall-applied poultry litter and rye did not affect cotton yield, but reduced NO_3^- leaching by an average of 50% (Adeli et al., 2011). High-N demanding crops may benefit from diversified nutrient management systems that incorporate green manure cover crops and animal manures with more sustained soil N supply (Johnson et al., 2012).

The management of biculture and diverse mixed species of cover crops requires an understanding of functionally diverse species interactions and the resulting differences in ecosystem services. Several studies have compared the effects of mixed rye and hairy vetch on a variety of ecosystem services or disservices (Ledgard and Steele, 1992; Rosecrance et al., 2000). While individual studies suggest mixtures produce greater biomass, or “overyield” (Creamer et al., 1997; Wortman et al., 2012; Schipanski and Drinkwater, 2012; Halde et al., 2014), increased cover crop residue may negatively impact crop yield and inorganic N availability, while positively affecting weed suppression and N retention (Finney et al., 2015). Mixing complementary species can affect both the residue quantity and quality, which consequently affects associated ecosystem services.

Complex agroecosystems that combine diverse cover crops with poultry litter to provide multiple ecosystem services have not be extensively studied. This research was designed to explore the ecological and management impacts of mixed species of cover crops and poultry litter. Research reported in Chapter 2 was specifically designed to quantify fertilizer N equivalence of cover crop × poultry litter treatments. Chapter 3 explores ecosystems services of cover crop × poultry litter systems, including cover crop biomass and N content, soil available N, weed suppression and corn grain yield. Chapter 4 is a synthesis of the literature that examines the effect of biculture and mixed cover crops on cover crop biomass and cash crop yield. This dissertation provides a novel examination of cover cropping systems with fall-applied poultry litter in corn production, and applies an ecological lens to cover crop interactions and organic fertilizer sources in sustainable agriculture. These studies summarize the ecological and production impacts of using cover crops and poultry litter in sustainable production and can be applied to similar systems across the country.

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CHAPTER 2 - Nitrogen benefits of cover crop and fall-applied poultry litter to corn

2.1 Abstract

Nitrogen from manures and fertilizers requires careful management to maximize efficiency and minimize losses. Cover crops may conserve nutrients from fall-applied manure and cycle the N to corn (*Zea mays* L.). To quantify N benefits from winter cover crops (CC) and poultry litter (PL) under conservation-tillage, fertilizer N equivalence (FNEQ) was quantified. Cover crop treatments included rye (Rye; *Secale cereale* L.), legume (Legume; crimson clover *Trifolium incarnatum* L. and/or hairy vetch *Vicia villosa* Roth), and LegumeRye with and without fall-applied PL. Controls consisted of winter fallow (WF) with and without PL. Five fertilizer N rates from 0 to 224 kg N ha^{-1} provided an FNEQ index. Cover crop N content, corn plant N content, grain yield and grain N content were measured. Grain yield and N were used to calculate FNEQ for each treatment. Nitrogen content of legume residue was greater with PL. Without a legume CC, fall-applied PL failed to result in fertilizer N credit. Fall-applied PL coupled with a legume CC resulted in a fertilizer N credit that increased across 3-y of the study from 25 to 117 kg N ha^{-1}. Rye FNEQ was negligible or negative for the duration of the study. For biculture, FNEQ was variable and ranged between a deficit of -12 to a positive credit of 75 kg N ha^{-1}. The FNEQ of legumes, legumes with PL, and biculture with PL increased across time. By the third year of the study, legume CC in combination with PL provided a substantial N credit to corn.

2.2 Introduction

Managing N resources for reduced leaching and greater crop and soil recovery in row crop production is critical to improving long-term agricultural sustainability. Animal manures play an important role in the recycling of plant nutrients when properly utilized in row crop production systems and can serve as a substitute for inorganic fertilizer inputs. As with fertilizers, manure derived N and P can become environmental pollutants when availability is asynchronous with crop demand or rates are excessive (Andraski et al., 2000; Sharpley et al., 2007; Adeli et al., 2011). Current row crop production practices are heavily reliant on inorganic fertilizer sources (Hera, 1995), which have experienced fluctuations in pricing and an overall increase in cost in recent years causing growers to seek other nutrient source options such as manure. In the mid-south, PL applications may occur following crop harvest in the fall, as it is a period which is operationally favorable and more easily coordinated with logistics of trucking and spreading. Growers are primarily interested in PL benefits of added organic matter, P, and K, while assuming zero credit for PL derived N due to potential overwinter N losses, especially by leaching (Adeli, 2011).

Coupling C and N in animal and green manures tightens nutrient cycling (Drinkwater, 1998). Non-legume CC such as rye (*Secale cereale* L.) are known to reduce leaching losses of NO_3^--N derived from organic sources such as manures and legumes, as well as from inorganic fertilizers (Adeli et al., 2011; Ditsch et al., 1993; Staver and Brinsfield, 1998). Studies on N dynamics in legume cover crop systems have indicated the importance of the associated C in immobilizing legume N in the organic matter fraction of soil (Ladd et al., 1981; Varco et al., 1993; Harris et al., 1994; Seo et al., 2006). In contrast, inorganic fertilizer N application generally results in lower immobilization compared to legume N (Azam et al., 1985). Coupling C and N dynamics by integrating organic N sources in cropping systems has been shown to increase total recovery of applied N in the crop and soil by 30 to 42%, compared to inorganic fertilizer N sources alone (Gardner and Drinkwater, 2009).

Cover crop benefits vary among species and can be selected based on ecosystem service preferences. Proper selection can help achieve various production and nutrient stewardship goals (Schipanski et al., 2014). Typically, leguminous cover crop species are selected to supplement N through biological N_2 fixation and subsequent decomposition and mineralization. A reduction in fertilizer N requirements by a row crop following a legume cover crop has been widely demonstrated across crops and environments (Touchton et al., 1982; Hargrove, 1986; Sullivan et al., 1991; Varco et al., 1999; Seo et al., 2000). In some instances, the combined effects of using a legume cover crop plus fertilization have been shown to result in the greatest yield (Ebelhar et al., 1984; Decker et al., 1994). Legume N may be important to long-term organic matter maintenance (Janzen et al., 1990; Ladd et al., 1981), and combining legumes with fertilizer N management may be an important contribution to reducing inorganic fertilizer and to long-term agricultural sustainability (Seo et al., 2006). In contrast, grass species of CC are touted for their greater scavenging ability of residual nutrients, especially mobile nutrients such as NO_3^--N, compared to legumes (Shipley et al., 1992; Ranells and Wagger, 1997; Thorup-Kristensen, 2001; Dabney et al., 2001).

Bicultures of legume and grass species have been studied to optimize multiple ecosystem services in addition to N_2 fixation, including: controlling erosion, improving water relations, recovering residual nutrients, and building soil organic matter. Tradeoffs in residue quantity (Sainju et al., 2005), quality, crop yield (Miguez and Bollero, 2005), and biological N_2 fixation rates (Wortman and Dawson, 2015) must be considered when selecting mixtures. In many instances, these trade-offs can be predicted from the characteristics and proportions of individual component species. For example, total N accumulation of biculture residues and winter rye tissue N concentration have been positively correlated with legume proportions in rye/hairy vetch (*Vicia villosa* Roth) bicultures (Hayden et al., 2014). Recovery of residual soil NO_3^--N by bicultures has been shown to be intermediary between rye and legumes alone (Ranells and Wagger, 1997). However, other functions of cover crop mixtures may not always be directly predicted by component species. Studies have shown that biological N_2 fixation by legumes may be reduced or enhanced when grown with grass species, with stronger correlations in perennial systems (Brainard et al., 2011; Schipanski and Drinkwater, 2012).

Coupling CC with manure has been suggested as a way to retain applied nutrients in row crop production systems (Singer et al., 2008). Legume, grass, and biculture cover crop systems fertilized with PL may conserve PL N by uptake and retention until spring row crop establishment with later release of nutrients following cover crop termination. However, limited research exists examining fall applied animal manures coupled with CC and subsequent impacts on crop yield and inorganic fertilizer needs. Adeli and others (2011) noted that although rye sequestered PL N applied in the fall and reduced NO_3^- leaching, there was little effect on cotton (*Gossypium hirsutum* L.) yield. Rye production responded to increasing levels of residual soil N derived from fertilizer and manure, but generally a subsequent row crop the following season did not benefit from grass-manure systems (Staver and Brinsfield, 1998; Singer et al., 2008). In contrast, legume-manure systems have been shown to potentially decrease the economic optimum N rate for corn to as low as zero (Andraski et al., 2000).

Fertilizer N equivalence (FNEQ) has been used to interpret the crop response to animal manures and CC residues (Ebelhar et al., 1984; Decker et al., 1994). The objective of this study was to quantify the FNEQ of legume, grass, and biculture winter CC combinations with and without fall-applied PL to examine the potential to reduce inorganic N inputs to conservation-tilled corn systems. We expected PL to increase the N availability from CC residues across species. We also expected the greatest FNEQ to be from the legume monoculture and the legume rye biculture FNEQ to be intermediate between legume and rye monoculture. This specific research is critical for developing a better understanding of nutrient dynamics and crop productivity of systems that take a multi-process approach to nutrient management by integrating manures, CC, and inorganic fertilizers.

2.3 Methods

Study Site

The study was conducted at the W.B. Andrews Agricultural Research Systems Farm at Mississippi State in Oktibbeha County, MS, USA (33°28’ N, 88°45’W). Alluvial soils at the experimental site are mapped primarily as a Marietta fine sandy loam (fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) and minimally as a Leeper silty clay loam (Fine, smectitic, nonacid, thermic Vertic Epiaquepts). Prior to installation of the study plots, soil samples from the 0- to 15- cm depth were collected and air-dried for determination of pH in deionized water (1:2 soil:d.i. water) and the Mississippi soil test (Raspberry and Lancaster, 1977) for extractable nutrients. Initial soil test results were: average plot pH, 6.25; pH range from 5.27 to 7.32; P = 127.5 mg kg^{-1} (very high), K = 127.3 mg kg^{-1} (high), Mg = 70.6 mg kg^{-1} (high), Ca = 2050.5 mg kg^{-1} and approximate CEC = 10.9.

Experimental design

The experimental design of this study was a randomized complete block in a 4 by 2 factorial arrangement of cover crop treatments. Cover crop treatments included rye (variety “Elbon”), legume, legume/rye, and winter fallow (hereafter, Rye, Legume, LegumeRye and WF). In 2013, the Legume treatment was a combination of hairy vetch and crimson clover (*Trifolium incarnatum* L.). In 2014 and 2015, only hairy vetch was used in the Legume and Legume/Rye treatments due to its winter hardiness. Cover crop seed was broadcast in 2013 and planted with a grain drill in 2014 and 2015. Prior to planting, legume seed was inoculated with rhizobacteria (N-Dure, INTX Microbials, LLC, Kentland, IN, USA), with seeding rate calculations corrected for any seed coating. Pelletized, minimally composted poultry litter (PL) was obtained from MightyGrow Organics Inc. (Fruitdale, AL, USA). Based on lab analysis, PL average nutrient concentrations were: N = 3.33%; P = 1.80%; and K = 3.18%. Average moisture content was 16.7%, with a range from 22% to 11%, which was analyzed annually prior to application rate calculations. All cover crop treatments including the WF control, were grown with and without PL (treatments with PL hereafter denoted as RyePL, LegumePL, LegumeRyePL, and WFPL) broadcast applied within four weeks of fall cover crop planting (2 Mg ha^{-1} on a dry-weight basis or approximately 60 kg N ha^{-1}). There were four replicates per treatment. Seeding rates and planting dates are shown in Table 1.

Table 1- Cultural practices and dates for cover crops and for corn production for the experimental period from October 2012 to August 2015.

	Experimental Years													
Methods	**1**						**2**				**3**			
CC Planting Termination	25 Oct 2012 10 April 2013						10 Oct. 2013 4 April 2014				21 Oct. 2014 9 April 2015			
CC Seeding Rates	Rye	Legume		Legume Rye Mix			Rye	Legume	Legume Rye Mix		Rye	Legume	Legume Rye Mix	
Species		Crimson Clover	Hairy Vetch	Crimson Clover	Hairy Vetch	Rye		Hairy Vetch	Hairy Vetch	Rye		Hairy Vetch	Hairy Vetch	Rye
Seeding Rates (kg ha^{-1})	60	18	22	9.2	11	34.5	60	30	15	30	60	30	25	25
PL, P, & K application date	25 Oct. 2012						30 Oct 2013				21 Nov 2014			
Tillage	No-till into stale seedbed						Strip-till				Strip-till			
Corn Planting date	18 April 2013						21 April 2014				22 April 2015			
Fertilizer application date	9 May 2013						24 April 2014				24 April 2015			
Corn Harvest	11 Sept. 2013						3 Sept. 2014				28 Aug. 2015			

Experimental Year 1 defined as Oct 2012- August 2013. Year 2 defined as October 2013 to September 2014. Year 3 defined as October 2014 to August 2015. CC = Cover Crop, PL = Poultry Litter, P = Phosphorus, K = Potassium

All treatments that did not include PL were amended, based on litter analysis, at approximately equivalent amounts of P and K, in the form of concentrated superphosphate [$Ca(H_2PO_4)$, 0-46-0] and muriate of potash (KCl, 0-0-60) at rates of 18 kg P ha^{-1} and 33 kg K ha^{-1} in 2013, 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. Dolomitic lime was applied at a rate of 2240 kg ha^{-1} as needed each fall to individual plots with fall-measured pH values of less than 6.0.

Plots consisted of four rows with a spacing of 0.97 m and a length of 12.19 m. Seedbeds were initially prepared by a seedbed finisher run across bedded rows in the fall of 2012 and with no subsequent tillage in 2013. Prior to the experimental period, the field had been managed with conventional tillage. Cover crop seed was broadcast onto the minimally tilled beds on 25 October 2012, and drilled into untilled corn residue on 10 October 2013 and on 21 October 2014. Cover crops were terminated with glyphosate [N-phosphonomethyl) glycine] as the potassium salt at 1.54 kg a.i. ha^{-1} on 10 April 2013, 4 April 2014, and 9 April 2015 and immediately following corn planting each year. Immediately prior to termination, four 0.25 m^2 samples of cover crop and annual weed above-ground biomass was harvested, dried at 65°C, ground in a Wiley mill to pass a 0.425 mm sieve and then analyzed for total N content with a Carlo Erba NC 1500 dry combustion analyzer (Carlo Erba, Milan, Italy). Remaining residues were returned to the experimental plots.

In preparation for corn planting in 2014 and 2015, field plots were strip-tilled followed by a bed roller to smooth and firm beds. Corn (Pioneer hybrid 33-N-58 in 2013, Pioneer hybrid 1395 in 2014 and Pioneer hybrid P1637YHR in 2015) was planted with a vacuum planter at a rate of 74,000 kernels ha^{-1} on 18 April 2013, 21 April 2014, and 22 April 2015. Five N rate treatments from 0 to 224 kg N ha^{-1} in 56 kg N ha^{-1} increments as ammonium nitrate were broadcast after corn planting on 9 May 2013, 24 April 2014, and 24 April 2015 to create a response function for the calculation of FNEQ for all cover crop and PL treatment combinations. For preemergence weed control, a tank mix of Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) at 2.34 kg a.i. ha^{-1}, Mesotrione (2-[4-methylsulfonyl)-2-nitrobensoyl]cyclohexame-1,3-dione) at 0.16 kg a.i. ha^{-1}, and Metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(1-methoxypropan-2-yl)acetaminde) at 1.22 kg a.i. ha^{-1} was applied following planting. A postemergence application of glyphosate at 1.54 kg a.i. ha^{-1} was made in 2013 and 2015. At physiological maturity, a 1-m length of whole plants per plot was harvested on 8 August 2014 and 10 August 2015, and stover and grain were separated prior to drying at 65°C. Corn grain was also harvested from the center two rows the whole length of each plot using an automated plot combine (see Table 1 for harvest dates). Whole plot and 1-m corn grain and stover N contents were analyzed using the dry combustion analysis described above. All reported grain yield was adjusted to 15.5% moisture content. Apparent FNEQ was determined for the cover crop and PL treatments based on grain yield and grain N content harvested from the center two rows of the whole experimental plots.

Fertilizer N Equivalence (FNEQ)

The experiment included five N rate treatments that were used to calculate a response function between N rate and grain yield or grain N content, which was in turn used to calculate fertilizer N equivalence of the CC × PL treatments. Although manure P and K availability was accounted for by applying fertilizer sources to non-manure treatments, other treatment effects on soil structure or disease, weeds, or other pests would be implicitly included in this FNEQ value, though not directly measured (Hargrove, 1986; Balkcom and Reeves, 2005).

Statistical methods

We compared corn plant N content (stover plus grain from 1-m harvest), grain yield, and grain N across treatments and years. The distribution of all data was first examined visually and utilizing a Shapiro-Wilk test for normality. Due to non-normality of the cover crop total N data, data was log transformed. With the normality assumption met, we used the General Linear Models procedure in SAS (SAS Inst. 2003) to conduct ANOVA. When differences among treatments were detected, LSD was used to separate means. All tests were performed at $\alpha = 0.05$. Log-transformed means and standard errors were back-transformed before reporting. Linear regression of the N rate response in whole plot grain yield and grain N yield were calculated with PROC REG in SAS. Estimated FNEQ of CC × PL treatments were based on the predicted linear regressions. Because the FNEQ values are based on whole plot grain yield and N content, the statistical analysis of treatment effects on FNEQ is equivalent to the analysis of treatment effects on grain yield and N content.

2.4 Results

Cover crop N

Cover crop N content was greatest in Legume PL, LegumeRye PL and Legume, intermediate in LegumeRye, and lowest in Rye treatments (Table 2). There was a significant decrease in CC N content from 2013 to 2015 ($p = 0.0002$) and no year interactions with CC and PL. In 2014 and 2015, Legume N content was 30% greater with the application of PL than Legume without PL but not significantly different in 2013. Three-year average suggests 22% or 16.1 kg N ha^{-1} greater residue N content in Legume PL compared to Legume without PL. Based on the 3-y average of N content of legume residues, legume recovered 27% of PL N. Rye recovered 22% of PL N, based on the 3-y average N content of Rye and RyePL residues. LegumeRye N content was greater with the application of PL by 18.5 kg N ha^{-1}, on average, and greater by 19% to 52%

from 2013 to 2015. LegumeRye apparently recovered 29% of applied PL N. Winter fallow, which included biomass that remained from annual weeds, captured about 5% of the PL N applied in fall for the two years that weeds were monitored (2014 and 2015; data not shown).

Table 2- Yearly and 3-year average cover crop N contents as influenced by cover crops and PL prior to termination.

		CC N Content **-------------------(kg N ha^{-1})------------------**		
Treatment	**2013**	**2014**	**2015**	**Average**
Legume	80.2	79.8	49.7	74.2
Legume PL	85.9	112.4	70.5	90.3
Rye	38.2	25.8	25.6	30.3
Rye PL	58.6	39.6	32.5	44.2
LegumeRye	64.4	61.6	47.2	58.6
LegumeRye PL	78.9	93.6	56.1	76.3
ANOVA		**P>F**		
CC	0.0242	<0.0001	<0.0001	<0.0001
PL	0.2389	0.0034	0.0065	0.0054
CC*PL	0.4334	0.5242	0.3074	0.9502
Contrasts		**P>F**		
L v LPL	0.8001	0.0240	0.0071	0.0766
R v. RPL	0.1259	0.3052	0.3211	0.1229
LR v. LRPL	0.4582	0.0267	0.2049	0.0545
L v. LR	0.2954	0.1837	0.7144	0.0863
LPL v. LRPL	0.9492	0.1685	0.0470	0.1192

CC = cover crop and PL = poultry litter. Treatments are Legume (L), LegumePL (LPL), Rye (R), Rye PL (RPL), LegumeRye (LR), LegumeRye PL (LRPL), Winter Fallow (WF), and Winter Fallow PL (WFPL).

Plant N content

Overall, plant total N content (grain plus stover N content at physiological maturity from 1-m subplot) was greater for all cover crop PL treatments in 2015 than in 2014 ($p < 0.0001$; Table 3), with significant effects of CC and PL both years (2014: CC $p < 0.001$ and PL $p = 0.047$; 2015: CC and PL $p < 0.001$). Overall, the effect of year on plant total N content was significant ($p < 0.0001$) and N content was greater in 2015 than in 2014. Plant total N content for the LegumePL treatment was 24 kg N ha^{-1} greater in 2014, and 31 kg N greater in 2015 compared to the Legume treatment. LegumeRye and LegumeRyePL were not significantly different either year at $\alpha = 0.05$, but PL increased plant total N content of biculture treatments by 8% and 18%. In both years, plant total N content of Rye, RyePL, and WFPL did not differ significantly.

Stover N content was significantly affected by CC and PL both years and on average ($p < 0.007$), while CC affected grain N both years and on average ($p < 0.002$), but PL increased grain N content only in 2015 ($p = 0.003$). Year was a significant factor showing an increase in stover and grain N from 2014 to 2015 ($p < 0.0001$). Fall-applied PL increased stover N following Legume CC by 28% on average and by 9.2 in 2014 and 8.5 kg N ha^{-1} in 2015 ($p < 0.04$). Similarly, LegumePL grain N was 33% greater than Legume in 2015 ($p = 0.008$).

Table 3 - Yearly and 2-year average grain, stover, and total plant N content for cover crop/poultry litter combinations at physiological maturity.

	2014			**2015**			**2 Yr Avg**		
	Grain N	**Stover N**	**Total N**	**Grain N**	**Stover N**	**Total N**	**Grain N**	**Stover N**	**Total N**
	----------------------	----------------------	----------------------	----------------------	kg N ha^{-1}	----------------------	----------------------	----------------------	----------------------
Legume	45.3	26.2	71.4	70.3	36.6	106.9	57.8	31.4	89.2
LegumePL	60.0	35.4	95.4	93.3	45.1	138.4	76.6	40.2	116.9
Rye	30.2	17.3	47.5	41.6	22.0	63.6	35.9	19.7	55.6
RyePL	31.0	22.0	53.0	50.4	29.1	79.5	40.7	25.6	66.3
LegumeRye	37.8	24.4	62.3	64.3	32.9	97.2	51.0	28.7	79.7
LegumeRyePL	40.4	27.8	68.2	78.4	39.1	117.5	59.4	33.4	92.8
WF	30.0	24.3	54.2	56.8	30.8	87.7	43.4	27.5	70.9
WFPL	26.0	23.8	49.8	40.9	28.1	69.0	33.4	25.9	59.4
ANOVA					**P>F**				
CC	0.0007	0.0035	0.0003	<0.0001	<0.0001	<0.0001	0.0016	0.0002	0.0006
PL	0.1684	0.0066	0.0470	0.0029	0.0028	0.0011	0.0598	0.0040	0.0244
CC*PL	0.3614	0.7194	0.3203	0.4420	0.9053	0.5486	0.5407	0.6731	0.5633
Contrasts					**P>F**				
L vs. LPL	0.0585	0.0386	0.0222	0.0081	0.0316	0.0061	0.0558	0.0175	0.0342
R vs. RPL	0.9073	0.2771	0.5723	0.2662	0.0658	0.1325	0.6008	0.0968	0.3811
LR vs. LRPL	0.7320	0.0656	0.5463	0.0829	0.1062	0.0598	0.3726	0.1717	0.2875
L vs. LR	0.3213	0.6745	0.3494	0.4440	0.3184	0.3479	0.4705	0.4208	0.4329
LPL vs. LRPL	0.0149	0.4941	0.0107	0.0703	0.1151	0.0542	0.0776	0.0571	0.0613

CC = cover crop and PL = poultry litter. Treatments are Legume (L), LegumePL (LPL), Rye (R), Rye PL (RPL), LegumeRye (LR), LegumeRye PL (LRPL), Winter Fallow (WF), and Winter Fallow PL (WFPL).

Whole Plot Grain Yield and N Content

Treatment effects of cover crops on whole plot grain yield were significant each year (2013 $p = 0.048$, 2014 and 2015 $p < 0.0001$), but PL was only significant in 2015 ($p = 0.03$; Table 4). An increasing trend was observed in grain yield among treatments from 2013 to 2015 ($p < 0.0001$). As with cover crop N content, grain yield followed the same trends with the greatest production in Legume treatments, followed by LegumeRye, and the lowest in Rye treatments. In 2014 and 2015 and through examination of the 3-y average, LegumePL produced more grain than Legume without PL by 26%, 20%, and 28% respectively. Poultry litter did not significantly increase grain yield in Rye or LegumeRye treatments. While differences were not significant, Rye and RyePL yield was less than or equal to WF and WFPL across years.

Trends in whole plot grain N content were similar to grain yield with respect to treatments. Overall, cover crop treatments had a marginal effect on grain N content the first year, but greater differences observed the 2nd and 3rd years (Table 4). The overall effect of PL on grain N content was significant by 2015 ($p = 0.025$). Among treatments, grain N content also increased across time ($p < 0.0001$). In 2014 and 2015, LegumePL had 23% and 20% more grain N than Legume, respectively. RyePL yielded only an insignificant amount of grain N content greater than the grain N of WFPL and WF treatments in 2014 and 2015; with Rye, grain N was lower than WF each year and on average. Three year average grain N content was 15.1 kg N ha^{-1} greater for LegumePL treatments than for Legume, and only 2.9 kg N ha^{-1} greater for RyePL than Rye. The application of PL to LegumeRye and WF showed a negative effect on grain N equal to a difference of -1.7 kg N ha^{-1} for LegumeRye and -3.6 kg N ha^{-1} for WF.

Table 4. Yearly whole plot grain N content and yield as influenced by cover crop and poultry litter treatments.

	Grain Yield				**Grain N Content**			
	--------------------Mg ha^{-1}-----------------------				**--------------------kg N ha^{-1}--------------------**			
Treatment	**2013**	**2014**	**2015**	**3 Yr Avg**	**2013**	**2014**	**2015**	**3 Yr Avg**
Legume	3.5	4.9	5.6	4.7	43.4	52.2	69.0	54.9
Legume PL	4.3	6.6	7.0	6.0	56.0	67.7	86.2	69.9
Rye	1.9	2.9	3.6	2.8	25.4	35.7	46.2	35.8
Rye PL	1.9	3.0	4.0	3.0	25.4	37.8	52.7	38.6
LegumeRye	4.4	3.8	5.0	4.4	51.6	45.5	61.8	53.0
LegumeRye PL	2.6	3.9	6.1	4.2	35.6	41.8	76.4	51.3
WF	3.3	3.6	4.2	3.7	42.6	37.4	50.8	43.6
WFPL	3.0	3.3	4.0	3.4	35.1	34.8	49.4	39.8
ANOVA					**P>F**			
CC	0.0475	<0.0001	<0.0001	0.0001	0.0670	0.0005	<0.0001	0.0010
PL	0.5185	0.1563	0.0250	0.4251	0.6615	0.4504	0.0251	0.4437
CC*PL	0.3359	0.0867	0.2346	0.2831	0.4332	0.2637	0.3354	0.3609
Contrasts					**P>F**			
L v. LPL	0.4002	0.0064	0.0192	0.0486	0.3233	0.7359	0.8609	0.0721
R v. RPL	0.9360	0.8296	0.4239	0.7937	0.9960	0.0479	0.0360	0.7222
LR v. LRPL	0.892	0.7556	0.870	0.7719	0.2105	0.7795	0.4005	0.8302
L v. LR	0.3794	0.0505	0.3372	0.6623	0.5174	0.3811	0.3582	0.8154
LPL v. LRPL	0.0960	<0.0001	0.0993	0.0100	0.1149	0.0021	0.2145	0.6548
WF v. WFPL	0.7607	0.5430	0.7507	0.6657	0.5530	0.6192	0.0712	0.6392

CC = cover crop and PL = poultry litter. Treatments are Legume (L), LegumePL (LPL), Rye (R), Rye PL (RPL), LegumeRye (LR), LegumeRye PL (LRPL), Winter Fallow (WF), and Winter Fallow PL (WFPL).

Fertilizer N Equivalence

Fertilizer N equivalence based on grain yield compared the N response among CC × PL treatments (Fig. 1). Regression equations modeled the response of grain yield to fertilizer N rates under winter fallow conditions to characterize CC × PL effect on corn, i.e. FNEQ (Table 5). In 2013, the FNEQ of LegumePL was almost four times the 6.7 kg FN ha^{-1} of Legume without PL based on grain yield (FN = fertilizer N based on FNEQ). The remaining three treatment combinations (LegumeRyePL, Rye, and RyePL) had negative FNEQ based on grain yield. In 2014, the FNEQ value for LegumePL was 84.2 kg FN ha^{-1} and Legume without PL was equal to 32.9. kg FN ha^{-1} . Results suggest a synergistic effect of the combined Legume PL treatment, as FNEQ was 82% greater than the added FNEQ values of Legume and PL treatments. This effect is at least partially due to the negative FNEQ of uncoupled PL (-18 kg FN ha^{-1}). LegumeRyePL produced grain yield equivalent to 3 kg FN ha^{-1}, while the remaining treatments (LegumeRye, RyePL and Rye) had negative FNEQ values. In 2015, similar trends in FNEQ were observed among treatments based on grain yield. The greatest FNEQ value of 117 kg FN ha^{-1} was in the LegumePL treatment, which was nearly twice Legume without PL. There was again a synergistic effect of LegumePL FNEQ compared to the additive FNEQ of Legume and PL. By the third year of the study, the LegumeRye combinations both had positive FNEQ and the maximum FNEQ of biculture with PL was 67% of LegumePL and 50% greater than biculture without PL. Rye and RyePL FNEQ based on grain yield was negative in 2015 as in other years. The range of FNEQ values was greater by the third year of the study; in 2015, LegumePL had the greatest FNEQ (117 kg FN ha^{-1}) and the deficits for Rye and RyePL were greater as well (-30 kg FN ha^{-1}). Similar trends across the 3-year study were observed in calculating FNEQ based on grain N, but with a maximum value equal to 101 kg FN ha^{-1} for LegumePL and a minimum value of -5 kg FN ha^{-1} for Rye.

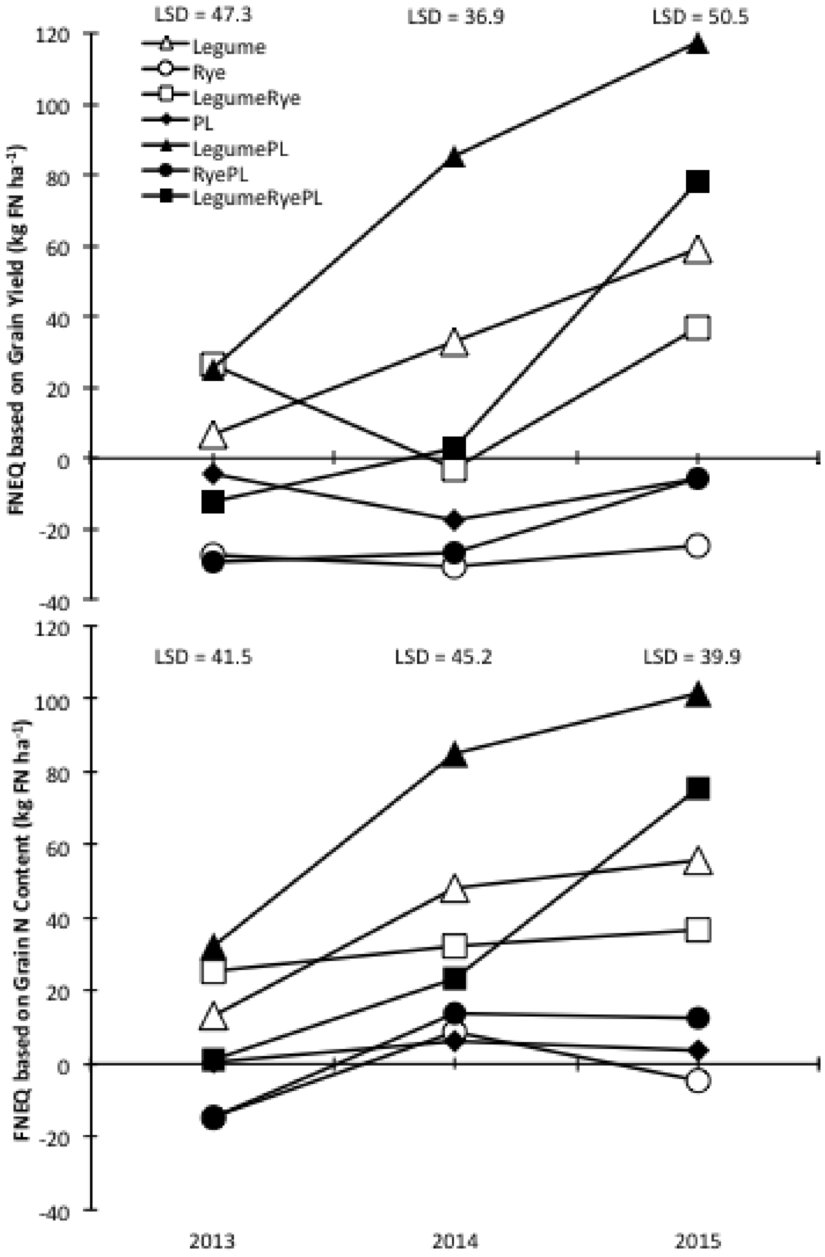

Figure 1 - Three-year trends in FNEQ based on corn grain yield and grain N content for cover crop/PL management schemes.

Table 5 - Regression equations and r^2 values for N rate plots used to develop the FNEQ index based on grain N and grain yield response.

Year

$989 - (6.410\ Block)$ 0.74

Fertilizer N Equivalence values based on grain N content focused on the N contribution of the CC × PL combination and showed similar trends as the FNEQ values based on grain yield. The range of FNEQ values based on grain N was from -15 to 101 kg FN ha^{-1}, while the range of FNEQ values based on grain yield was from -31 to 117 kg FN ha^{-1}. In 2013, based on grain N content, LegumePL treatment was equivalent to 32 kg FN ha^{-1}, followed by LegumeRye (25 kg FN ha^{-1}), and Legume (13 kg FN ha^{-1}, Fig. 1). LegumeRyePL and WFPL had negligible FNEQ. Combining PL with legume resulted in FNEQs that were more than twice the FNEQ of legume alone. The Rye and RyePL treatments both yielded less corn grain than WF and resulted in negative FNEQ values. In 2014 all CC × PL treatments had positive values of FNEQ based on grain N content. As in 2013, the greatest FNEQ in 2014 was observed for LegumePL. Poultry litter without any cover crop (WFPL) resulted in an FNEQ of 6 kg FN ha^{-1} and had a synergistic benefit when combined with legume cover crop, which resulted in 44% greater FNEQ compared to the additive FNEQ values of PL and Legume in 2014. LegumeRye, LegumeRyePL, Rye, and RyePL FNEQ values were all positive in 2014. In 2015, the range in FNEQ was greater than in previous years. LegumePL had the greatest FNEQ equal to 101 kg FN ha^{-1}, and once again almost twice that of Legume alone. Again in 2015, PL resulted in a synergistic effect on FNEQ when comparing Legume (56 kg FN ha^{-1}) and LegumePL FNEQs, partially due to the negative FNEQ value for WFPL (-6 kg FN ha^{-1}). Poultry litter also increased the FNEQ of LegumeRye by 51% compared to LegumeRye alone. RyePL resulted in an equivalence of 13 kg FN ha^{-1}, a slight but positive effect compared to the negative FNEQ observed the first year. As in 2013, Rye resulted in less grain N content than WF, and a negative FNEQ in 2015.

The general effect of PL on cover crop treatments varied when comparing the FNEQs. LegumePL treatments resulted in the greatest FNEQ, which increased across the 3-y and was

78% to 148% greater than for Legume alone, based on N content. Poultry litter did not significantly increase grain yield, grain N content or FNEQ for Rye any of the years of this study. When legumes were grown in biculture with rye, the effect of the PL was reduced and variable. The effect of PL on the biculture FNEQ fluctuated from as much as an 84% decrease the first year to 105% increase in the last year compared to biculture without PL. However by 2015, LegumeRye PL FNEQ was 78 kg FN ha^{-1} based on grain yield and 75 kg FN ha^{-1} based on grain N content, and neither value differed from LegumePL FNEQ values.

2.5 Discussion

Fall-applied PL and cover crop management schemes have the potential to reduce spring inorganic fertilizer N inputs. Uncoupled PL (applied without cover crop) did not supply spring N to corn, even though approximately 60 kg N ha^{-1} was applied each fall. Cover crop N, corn total N content, grain yield, grain N content and FNEQ show fall-applied PL coupled with a legume cover crop resulted in a fertilizer credit of approximately 100 kg FN ha^{-1} or more by the third year of this study. Fertilizer credit attributable to rye was negligible across the 3-y period, which is in agreement with previous work on rye (Clark et al., 1997, Huntington et al., 1985). Cover crop N content, grain yield and FNEQs of the biculture were variable across the 3-y period, but by the end of the study FNEQ was not significantly different from the maximum FNEQ value, which occurred with the LegumePL treatment. The FNEQ of Legume, LegumePL and LegumeRyePL cover crop systems increased across the experimental period, suggesting a buildup of residual effects on soil N.

Based on grain yield and grain N, the application of PL to winter fallow suggests some residual N availability, but not enough to provide a fertilizer N credit. There was no difference between the annual winter weed N yield (data not shown), stover N yield, grain yield, grain N yield, or FNEQ of the PL treatment (WFPL) compared to WF. It is not known from this study if the PL N was lost by leaching, denitrification or both, nor how much was immobilized in soil organic matter and resistant to re-mineralization (Jung Ho et al., 2006). Without the potential to couple PL N with cover crop C, the risk of nutrient loss is high with fall-applied litter (Meisinger et al., 1991) and there was zero or minimal fertilizer credit based on FNEQ. Poultry litter N applied in the fall to winter fallow was essentially lost from the system. Although the application rate of PL in this study was below rates typically applied to dispose of waste and meet N requirements, the application rate was reasonable for fertilizing CC, coupling C and N, lowering the risk of N loss by leaching and avoiding P buildup in soils (Brink et al., 2008).

Fall-applied PL had a synergistic effect on the FNEQ of the legume cover crop treatment. Each year the FNEQ of LegumePL was greater, by as much as 9 times, compared to the additive FNEQ of Legume and PL treatments, partly due to the negative FNEQ values of uncoupled PL. Increased corn grain productivity of legume treatments was correlated with an increase in total cover crop N yield, which was 41% greater with the application of PL. There was no significant effect of PL on legume cover crop residue quantity ($p = 0.71$, data not shown); however, PL did increase the N content. Therefore, greater residue quality or N content of legume with PL, greater N mineralization rates compared to rye and biculture (Kuo and Sainju, 1998), and possibly greater synchrony of nutrient release with crop uptake (Stute and Posner, 1995), may have contributed to increased grain productivity.

The effects of PL on rye became obscured by the effect of the rye cover crop on grain productivity and FNEQ. The lowest FNEQ, based on grain N content, occurred the first year of the study (-15 kg N ha^{-1}) when residues were greatest (3200 to 5200 kg ha^{-1}), and corn stand was reduced by 26% for the RyePL treatments (data not shown). However, even with lower residue quantities (max 2700 kg ha^{-1}), strip-tillage in 2014 and 2015, and resulting excellent corn stands, FNEQ of rye treatments ranged between -5 to 14 kg N ha^{-1}. The cover crop N yield of RyePL was greater than Rye without PL by 27% to 54% across the 3-y period, but the effect on grain production was detrimental or only minimally positive. These results confirm prior research in which rye biomass production and N uptake increased quadratically with up to 9 Mg of PL, with no effect on cotton lint (Adeli et al., 2011). Immobilization of N is likely an overriding factor due to the high C:N of rye residues (Allison 1966; Clark et al. 1997), but other factors may also be involved such as those related to reduced stand, decreased soil moisture content, or allelopathy of rye residues (Burgos et al., 1999). It is possible that rye residues were increasing overall N use efficiency of this system by increasing SOM, scavenging residual and PL N, and potentially creating a “slow-release” N source (McSwiney et al., 2010), but a fertilizer N credit could was not apparent within the 3-y study period. Although grain yield for both Rye and RyePL significantly increased by 2015, the greatest yield was only 4 Mg ha^{-1}, or about 30% of the maximum yield in this study. Longer experimental studies may reveal greater N benefits from the Rye-PL combination.

The effect of PL on cover crop bicultures was variable across the 3-y period. Biculture residue N was not consistently affected by PL application. Previous studies have suggested that mixed cover crop N yields do not necessarily follow the same over-yielding pattern as shown in biomass yield because N content is directly related to legume proportion of a mix (Sainju et al. 2005, Smith et al. 2014). There were also inconsistent trends in grain productivity for the biculture treatments across the 3-y period. However, the biculture residue N, grain yield, stover N, and grain N tended to fall between those values for Legume and Rye and ultimately resulted in FNEQ similar to FNEQ of LegumePL by year three. While grass-legume biculture with PL may not consistently have as high an FNEQ as LegumePL, there are benefits and tradeoffs when managing bicultures including N retention, weed suppression, and management costs (Brainard et al., 2011; Hayden et al. 2014; Seman-Varner, 2016).

The full benefits of these cover crop and PL management schemes may take several years to be realized. The greatest positive trend emerged in the LegumePL treatment by the third year of the study. There was also a positive effect on grain yield and FNEQ for Legume and LegumeRyePL across the experimental period. This suggests a cumulative effect on organic N and supply of plant available N from the legume residues in this low disturbance system (McSwiney et al. 2010). These results are consistent with previous work on FNEQ of other legume CC, which also show increasing trends (Reeves et al., 1993; Torbert et al. 1996). The effects of Rye and RyePL on grain yield also increased across time, but the FNEQ was negligible. Inclusion of rye in production systems has resulted in prediction of greater fertilizer N rates to realize economically optimum yield compared to no winter cover crop (Varco et al., 1999). Comparing the rate of development of benefits from legumes, bicultures, and rye CC in this experiment, it appears that increasing C:N of CC residues may result in a longer period of time to maximize FNEQ.

2.6 Conclusions

The effects of legume, grass, or biculture winter CC on crop productivity have been studied in many agronomic systems, but the combined effect of fall-applied PL coupled with these CC has not. Our results suggest that inclusion of a legume cover crop fertilized with PL can improve grain yield productivity and suggests substitutability for inorganic fertilizer N up to 117 kg N ha^{-1} for minimally tilled corn. Rye with and without PL did not result in a substantial fertilizer N credit to corn within the three years of this study. Compared to monocultures, the biculture resulted in an intermediate and variable FNEQ based on grain N content and yield, but ultimately resulted in a substantial fertilizer N credit similar to legume with PL. Furthermore, the FNEQ of legume, legume with PL, and biculture with PL increased substantially across the 3-y period, while the FNEQ of rye with and without PL and biculture without PL did not. Additionally, the application of PL to winter fallow resulted in zero N credit, but when coupled with CC, nearly 30% PL N was assimilated in CC residues. Our results suggest the combination of a legume CC with fall-applied PL can result in a synergistic effect and supply substantial fertilizer N credits to corn. The long-term effects and N stability of management schemes that combine organic and inorganic N sources warrant further study.

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CHAPTER 3 - Ecosystem services of cover crops coupled with poultry litter in conservation-tilled corn

3.1 Abstract

Conventional monoculture agricultural production is ecologically low-functioning and focused on a single provisioning ecosystem service. Increasing the temporal diversity of monoculture field crops by adding cover crops and reducing chemical fertilizer inputs by utilizing animal manures could increase ecosystem services. This study quantified multiple ecosystem services provided by legume, grass, and biculture cover crops fertilized with poultry litter (PL) in a conservation-tilled corn (*Zea mays* L.) system. Treatments included rye (*Secale cereale* L.), legume (hairy vetch *Vicia villosa* Roth), legume/rye biculture, and winter fallow with and without fall-applied PL. Biomass and N content of cover crops and weeds, post-termination soil NO_3^- and NH_4^+, and corn yield were measured. Biculture produced more total biomass than monocultures, suppressed weeds as effectively as rye, produced corn yield similar to legume and greater than rye, and by the end of the second year had similar available soil N as legume. The interspecific effects of biculture on cover crop biomass differed; rye yield increased, while legume yield decreased. Poultry litter also differentially affected cover crops; legume N content increased and C:N decreased, while rye N content and C:N was unaffected. Annual differences in precipitation may have affected cover crop biomass and soil N speciation. Ultimately, the selection of grass, legume, or biculture with or without PL depends on the prioritization of ecosystem services, but all combinations consistently enhanced weed suppression, soil available N, and corn yield compared to winter fallow.

3.2 Introduction

Conventional monoculture production is typically designed to maximize productivity with fertilizer and pesticide inputs and can result in simplified, low-functioning agroecosystems (Tilman, 1999). Excessive N fertilization and N losses from conventional production have been linked to negative environmental consequences including hypoxic zones and groundwater contamination (Rabalais et al., 2010; Spalding and Exner, 1997). In contrast, conservation agriculture focuses on not only provisioning ecosystem services (food, fiber, fuel, or feed), but also regulating (e.g., water quality) and supporting (e.g., soil conservation and nutrient cycling) services (Millennial Ecosystem Assessment 2015). Conservation techniques, which include minimizing soil disturbance, maintaining cover, increasing organic residue, and diversifying crop rotations including cover crops, can increase agroecosystem function and decrease pollution risks (Duiker and Thomason, 2014).

Originally promoted to reduce soil erosion, cover crops are now managed to also conserve residual nutrients, suppress weeds, and enhance microbial habitat (Dabney et al., 2001). Water quality is positively affected by cover crops because they assimilate residual nutrients and can supplement nutrient input requirements of cash crops (Dabney et al., 2001; Chapter 2). When managed to suppress weeds, especially when mechanically terminated (Creamer and Dabney, 2002), cover crops can reduce herbicidal inputs (Yenish et al., 1996). Agroecosystems that maintain cover crop residues with reduced tillage also support soil organism diversity and nutrient cycling (McDaniel et al., 2014; Tiemann et al., 2015).

Among the commonly recommended plant species grown as cover crops, there are several functional groups that differentially affect ecosystem services. Species functional traits drive differences in nutrient retention, nutrient cycling, biomass production, and weed suppression (Diaz et al., 2013; Finney et al. 2015). Grass species typically provide greater protection from erosion, retain residual nutrients, and suppress weeds, but may or may not decrease crop production due to N immobilization (Meisinger et al., 1991; Tonitto et al., 2006). In contrast, legumes mineralize more N than grasses due to lower C:N and usually increase crop yield, but may not suppress weeds as well as grasses (Barberi and Mazzoncini, 2002; Clark, 1995; Ranells and Wagger, 1996). With biculture mixtures of two functional groups, there are proportional tradeoffs of biomass, N content, winter survival, weed suppression, and economic costs (Hayden et al., 2014). Previous research indicates that cover crops mixtures that exploit functional contrasts among species can increase ecosystem services (Storkey et al., 2015).

Optimal cover crop system design could be further informed by examining residue production, nutrient dynamics, and weed suppression of individual species in monocultures and mixtures. Interactions between cover crop species in mixtures may alter residue composition, which directly impacts post-termination nutrient dynamics. Carbon, N, and lignin content of residues, as well as the soil temperature, aeration, and moisture, drive mineralization rates (Quemada and Cabrera, 1997). Management practices, including timing of planting, and timing and method of termination, also affect residue quantity and quality (Dabney et al., 2010). Additionally, residues that are more resistant to decomposition may suppress weeds more effectively (Creamer et al., 1997).

Coupling functionally diverse cover crops and animal manures has not been extensively studied, especially within the context of supporting and regulating ecosystem services. The few studies that exist do not provide a comprehensive picture of the impacts on ecosystem services. For example, in a cotton system, winter rye (*Secale cereale* L.) sequestered fall-applied PL N, which resulted in reduced NO_3^- leaching compared to no cover, but had little effect on cotton

(*Gossypium hirsutum* L.) yield (Adeli et al., 2011). In another study, coupling a rye-oat (*Avena sativa* L.) cover crop with liquid dairy manure injection reduced the cover crop stand at the injection site and increased N, P, and K uptake compared to unfertilized cover crop, and there was no effect of cover crop with or without manure on corn grain yield (Singer et al., 2008). While many studies have explored specific aspects of cover crop species, combinations, and effects on nutrient loss or crop production, the delivery of multiple services within complex management systems has not been thoroughly examined.

To better understand tradeoffs of ecosystem services in complex cover crop systems, the effects of hairy vetch (*Vicia villosa* Roth), rye, and vetch/rye biculture coupled with fall-applied PL were examined. The effect of cover crops and PL combinations on cover crop biomass productivity, weed suppression, available soil N, and corn productivity was measured. We hypothesized that PL would increase the biomass and N content of cover crop species. We expected both extractable soil NH_4^+ and NO_3^- availability of cover crop residues post-termination and corn yield to increase with PL and legume proportion. Furthermore, it was hypothesized that weed suppression would be greatest with rye monocultures. Spider plots were used to illustrate tradeoffs in ecosystem services.

3.3 Methods

Study Site

The study was implemented at the W.B. Andrews Agricultural Research Systems Farm at Mississippi State University in Oktibbeha County, Mississippi, USA (33°28’ N, 88°45’ W). Soils at the experimental site are mapped as a Marietta fine sandy loam (fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) and partially a Leeper silty clay loam (Fine, smectitic, nonacid, thermic Vertic Epiaquepts). The local climate is characterized by cool winters (January average high 11.9 and low -0.7 °C) and hot summers (July average high 33.1 and low 21.5 °C) with average annual rainfall of 1402 mm distributed somewhat evenly throughout the year (National Climate Data Center, National Oceanic and Atmospheric Administration, 2016).

Experimental design

The experimental design was a randomized complete block with a 4 by 2 factorial arrangement of cover crop and PL treatments. Cover crop treatments included Rye (*Secale cereale* L., variety "Elbon"), Legume (*Vicia villosa* Roth, hairy vetch), Legume/Rye, and Winter Fallow (hereafter, Rye, Legume, Legume/Rye and WF). Poultry litter was broadcast applied at a rate of 2 Mg ha^{-1} on a dry-weight basis or approximately 60 kg N ha^{-1} and treatment was either present or absent (hereafter, RyePL, LegumePL, Legume/RyePL, and WFPL). Also included were fertilizer N rates from 0 to 224 kg N ha^{-1} in 56 kg N ha^{-1} increments applied as broadcast ammonium nitrate. Fertilizer N treatments were used to compare extractable soil N levels among all treatments. There were four replicates with each plot consisting of four rows with 0.97 m spacing by 12.19 m in length. The study was repeated twice across 2-y using the same treatment design and plot layout (October 2013 – September 2014 and October 2014 – September 2015).

Field methods

Cover crops

Cover crops were planted with a grain drill on 10 October 2013 and 21 October 2014. Prior to fall planting, vetch seed was inoculated with rhizobacteria (N-Dure, INTX Microbials, LLC, Kentland, Indiana, USA). Seeding rates were 60 kg ha^{-1} for Rye, 30 kg ha^{-1} for Legume, and 15/30 kg ha^{-1} in 2014 and 25/25 kg ha^{-1} in 2015 for Legume/Rye mixture, respectively. Cover crops were terminated with glyphosate on 4 April 2014 and 9 April 2015 two to three weeks prior to corn planting.

Poultry litter

Pelletized, composted PL was obtained from MightyGrow Organics Inc. (Fruitdale, Alabama, USA). Based on laboratory analysis, PL average nutrient concentrations were: N = 3.33 g kg^{-1} ; P = 1.80 g kg^{-1}; K = 3.18 g kg^{-1}. Average moisture content was 16.7 g kg^{-1}, with a range from 11 to 22 g kg^{-1}, which was analyzed annually for application rate calculations. Poultry litter was broadcast applied on 30 October 2013 and 21 November 2014. Cover crop treatment plots without PL were amended with equivalent amounts of P and K as concentrated super phosphate [$Ca(H_2PO_4)$, 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, Colorado, USA). Dolomitic lime was applied at a rate of 2240 kg ha^{-1} as needed each fall to individual plots with pH values less than 6.0. Prior to the initiation of this research, the field had been managed with conventional tillage.

Sampling Methods

Cover crops and weeds

Immediately prior to termination, four 0.25 m^2 samples of cover crop and annual weed aboveground biomass were harvested from each plot. Samples were sorted by cover crop species or general weed species, weighed, dried in a 65°C oven for 24 h and 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 until C/N analysis. Samples were weighed into microtins and stored in a desiccator until analysis with a Carlo Erba C/N 1500 dry combustion analyzer (Carlo Erba, Milan, Italy). After samples were harvested, weighed, and a subsample analyzed, the remaining residues were returned to the experimental plots.

Soil

Soil nutrient and chemical properties were monitored across two corn crop seasons. Initial soil samples were a composite of eight 1-cm diameter soil probe samples for a 0- to 15-cm depth per plot prior to planting of cover crops and litter application in the fall of 2013. Soil samples were analyzed for extractable nutrients using the Mississippi Soil Test method (Raspberry and Lancaster, 1977). Initial average soil test results were: pH = 6.25; pH ranged from 5.27 to 7.32; P = 127.5 mg kg^{-1} (very high), K = 127.3 mg kg^{-1} (high), Mg = 70.6 mg kg^{-1} (high), Ca = 2050.5 mg kg^{-1} and approximate CEC = 10.9 $cmol_c$ kg^{-1}. Soil samples were collected to determine extractable NH_4^+ and NO_3^- following corn planting and two and four weeks following corn planting. Soil samples were collected using a composite of eight 1-cm diameter soil cores per plot at depths of 0- to 15-cm, 15- to 30-cm, and 30- to 60-cm on 24 April, 8 May, and 22 May in both 2014 and 2015. Soil sampling dates in 2014 corresponded to 20 d, 34 d, and 48 d after cover crop termination, and 0 d, 14 d, and 28 d after fertilizer application. In 2015 soil sampling dates correspond to 15 d, 29 d, and 43 d after cover crop termination, and 0 d, 14 d, and 28 d after fertilizer application. Soil samples were placed on ice in the field, and then stored at 4°C until extracted. Defrosted, crushed and mixed soil was extracted for NO_3^- and NH_4^+ using 1 M KCl (Rice et al., 1984) and NO_3^- and NH_4^+ were analyzed colorimetrically using an OI Analytical FS 3100 analyzer (College Station, Texas, USA). Extractable soil N (total NO_3^- + NH_4^+ per depth) results were averaged across the 3 depths. Excess extractable soil N by treatments was calculated by subtracting average extractable soil N of the 0 N control plots for each of the 3 sampling dates.

Statistical methods

An ANOVA was used to determine treatment effects. Response variables included cover crop species and winter annual weed biomass, N content, and C:N of residues. Coefficients of variation were calculated for cover crop biomass data to examine variability between years. The distribution of all data was examined visually and normality was evaluated using the Shapiro-Wilk test. Data that failed to meet the assumptions of normality were log-transformed. Treatment means and standard errors are reported after back-transformation. Analysis of cover crop and weed biomass and N content and available soil N were conducted with PROC GLM in SAS (SAS Inst. 2003). Fisher's Protected Least Significant Difference (LSD) was used for mean separation among treatments for cover crop and weed N contents and biomass and extractable soil N data. All tests were performed at a significance level of $\alpha = 0.05$.

Ecosystem services trade-offs

Ecosystem services were compared by examining the effects of cover crop treatments (Rye, Legume, LegumeRye and WF) with and without PL on specific response variables. Spider plots were created to visually display the data of ecosystem services for these management schemes. Spider plots have been promoted for use as decision-making tools for farmers, policy-makers, researchers, and extension agents (Gareau et al., 2010). Using normalized response variables in spider plots, biomass production (above-ground cover crop biomass), residue N content (N content of above-ground cover crop residues), extractable soil N (total soil NO_3^- and NH_4^+ averaged for 0- to 60-cm depth), weed suppression (relative above-ground weed biomass), and effects on corn yield effect (from Chapter 2). Response variables were normalized using the following equation:

$$X_{new} = \frac{X - X_{min}}{(X_{max} - X_{min})}$$

where X_{new} is the normalized value for the treatment response variable (X), X_{max} and X_{min} represents the maximum and minimum values of the treatment response variable, respectively (Schipanski et al., 2015). This equation was used for all response variables except for weed biomass. Since weed suppression was quantified using the weed biomass, normalization for weed suppression was performed using the above equation modified by using the maximum rather than minimum weed biomass in relation to each treatment response variable.

3.4 Results

Cover crop biomass and N content

A significant overall effect of PL on cover crop biomass yield was apparent in both years of the study ($p < 0.0001$ in 2014 and $p \leq 0.043$ in 2015). Poultry litter increased cover crop species yield in monocultures and biculture treatments, but the effect varied (Fig. 1; Table 1). In 2014, Legume/Rye PL produced the greatest total biomass, which was 59% greater than the Legume/Rye ($p = 0.009$). In 2015, Legume/Rye PL was not the most productive treatment and was only 6% greater than Legume/Rye without PL ($p = 0.157$). There was an increase in Rye biomass due to PL in 2014 (51%, $p = 0.007$), but the difference was not significant in 2015

(30%, $p = 0.056$). Increases in Legume biomass due to PL were not significant either year ($p = 0.097$ in 2014 and $p = 0.220$ in 2015).

The effects of cover crop and PL on N content of residue were significant in 2014 and 2015 ($p \leq 0.004$, Fig. 1). Cover crop N content was greatest for Legume PL residues at 36% and 42% greater than Legume without PL in 2014 and 2015, respectively ($p = 0.041$ and $p = 0.002$; Fig. 2; Table 2). Poultry litter did not consistently increase Rye N content in monoculture ($p = 0.011$ in 2014 and $p = 0.369$ in 2015). The Legume/Rye biculture N content was greater with PL in 2014 ($p = 0.009$) but not in 2015 ($p = 0.157$).

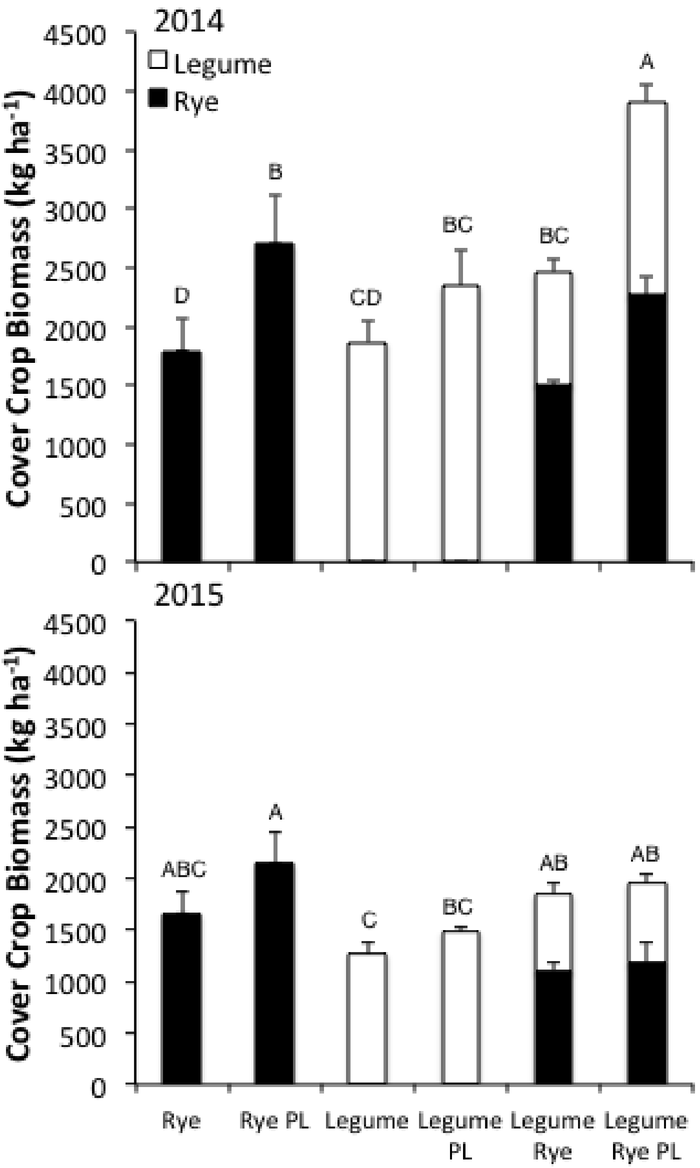

Fig. 1. – Total cover crop biomass of cover crop × poultry litter treatments in 2014 and 2015. Letters represent LSD mean separation of total treatment biomass.

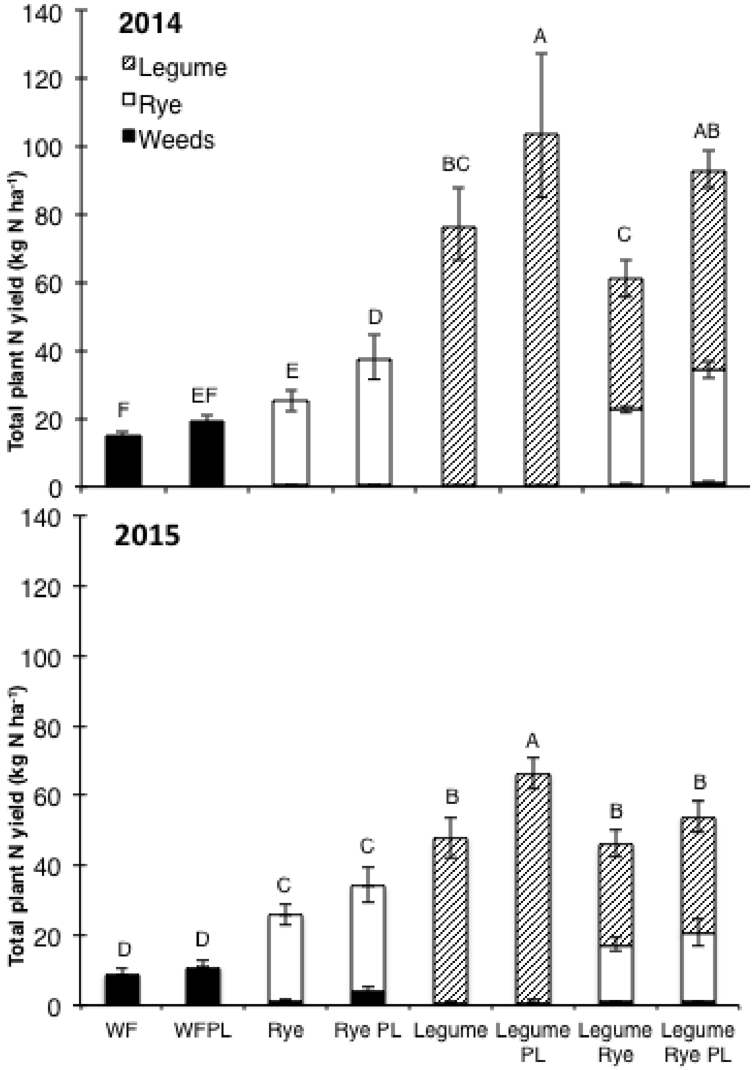

Fig. 2. Cover crop N yield cover crop × poultry litter treatments in 2014 and 2015. Letters represent LSD mean separation of treatment totals.

Table 1. ANOVA Table for total cover crop biomass yield of cover crop × poultry litter treatments in 2014 and 2015.

	2014 ($kg\ ha^{-1}$)	**2015 ($kg\ ha^{-1}$)**
L	1925.8	1320.1
LPL	2449.2	1636.6
R	1807.4	1695.0
RPL	2724.7	2208.6
LR	2476.4	1884.7
LRPL	3941.8	2000.7
ANOVA	**P>F**	
CC	0.0003	0.0246
PL	<0.0001	0.0433
CC*PL	0.1109	0.5385
CONTRASTS	**P>F**	
L v. LPL	0.0974	0.2203
R v. RPL	0.0073	0.0556
LR v. LRPL	0.0002	0.6459
L v. LR	0.0826	0.0375
LPL v. LRPL	0.0001	0.1619

CC = cover crop and PL = poultry litter. Treatments are Legume (L), LegumePL (LPL), Rye (R), Rye PL (RPL), LegumeRye (LR), LegumeRye PL (LRPL), Winter Fallow (WF), and Winter Fallow PL (WFPL).

Table 2. ANOVA Table for total cover crop N yield of cover crop × poultry litter treatments in 2014 and 2015

	2014 (kg N ha^{-1})	**2015 (kg N ha^{-1})**
L	77.69	49.70
LPL	106.66	70.51
R	25.29	25.61
RPL	37.90	32.46
LR	60.92	47.21
LRPL	92.89	56.06
WF	15.25	9.19
WFPL	19.12	11.39
ANOVA	**P>F**	
CC	<0.0001	<0.0001
PL	0.0001	0.0042
CC*PL	0.7667	0.1919
CONTRASTS	**P>F**	
L v. LPL	0.0410	0.0024
R v. RPL	0.0112	0.2686
LR v. LRPL	0.0086	0.1570
WF v. WFPL	0.1349	0.7186
L v. LR	0.1097	0.6837
LPL v. LRPL	0.3533	0.0259

CC = cover crop and PL = poultry litter. Treatments are Legume (L), LegumePL (LPL), Rye (R), Rye PL (RPL), LegumeRye (LR), LegumeRye PL (LRPL), Winter Fallow (WF), and Winter Fallow PL (WFPL).

C:N ratios

The C:N of legumes was more variable with PL and in biculture, while rye C:N was static across the different treatments (Table 4). Rye C:N was unaffected by PL application or when grown with legume in biculture and was consistently around 30:1 among treatments ($p > 0.1$). On the other hand, legume residue had the lowest C:N in the legume residues of Legume PL treatments both years. In 2014, the C:N of legume residue in the Legume/Rye PL treatment was significantly greater than the C:N of legume residue in Legume PL ($p < 0.05$). In contrast in 2015, legume C:N without PL was greater than the C:N with PL, and differences between legume C:N in Legume/Rye were not significant.

Weed C:N also differed among treatments both years (CC $p < 0.001$, PL $p = 0.004$ in 2014 and NS 2015, CC*PL $p = 0.013$ in 2014 and NS 2015). Weed species were analyzed together and dominated by henbit (*Lamium amplexicaule* L.), chickweed (*Stellaria media* (L.) Vill.), and annual bluegrass (*Poa annua* L.). Weeds within the Legume PL treatment plots had the lowest C:N among all treatments, equal to 18.1 in 2014 and 14.4 in 2015. Conversely, the greatest C:N of weed residues was in the Rye and Rye PL treatments, which were as high as 32.0 in 2014 and as low as 22.2 in 2015.

Table 3. C:N ratio for each cover crop species and weeds for cover crop × poultry litter treatments in 2014 and 2015.

		2014			**2015**	
	Rye	**Legume**	**Weeds**	**Rye**	**Legume**	**Weeds**
L	-	11.02	19.77	-	11.86	17.66
LPL	-	9.75	18.10	-	10.15	14.45
R	31.59	-	32.13	30.64	-	22.46
RPL	32.10	-	22.63	32.28	-	23.08
LR	30.98	11.35	23.98	31.01	11.27	19.00
LRPL	30.75	12.64	22.74	26.32	10.48	18.17
WF	-	-	25.96	-	-	19.39
WFPL	-	-	24.92	-	-	22.09
ANOVA			**P>F**			
CC	0.3163	0.0201	<0.0001	0.1411	0.7933	0.0009
PL	0.8807	0.9793	0.0041	0.4017	0.0304	0.8959
CC*PL	0.7010	0.0512	0.0131	0.1006	0.3643	0.1987
CONTRASTS			**P>F**			
L v. LPL	-	0.1521	0.3808	-	0.0345	0.1092
R v. RPL	0.7060	-	0.0002	0.5189	-	0.6970
LR v. LRPL	0.8679	0.1436	0.5150	0.0876	0.0760	0.6694
WF v. WFPL	-	-	0.9840	-	-	0.1746
L v. LR	-	0.6915	0.0354	-	0.4088	0.1092
LPL v. LRPL	-	0.006	0.0217	-	0.6394	0.0659
LPL v. WFPL			0.0016	-	-	0.0007

CC = cover crop and PL = poultry litter. Treatments are Legume (L), LegumePL (LPL), Rye (R), Rye PL (RPL), LegumeRye (LR), LegumeRye PL (LRPL), Winter Fallow (WF), and Winter Fallow PL (WFPL).

Extractable Soil N

Average extractable soil N from fertilized and cover crop treatments did not differ at the first sampling date. Following N fertilization at 0 days after planting (DAP), there were significant treatment differences at 14 and 28 DAP both years. All fertilizer N rates showed an increase in extractable soil N at 14 DAP and a decrease in response to increased in corn N recovery by 28 DAP (Fig. 2). Extractable soil N levels in 2015 suggest there was no residual N after the winter fallow period in 2014.

In 2014, the cover crop × PL treatments differed after the first sampling date, which was 20 days cover crop post-termination in 2014 and 15 days post-termination in 2015. LegumePL produced consistently greater extractable soil N than other treatments for all samplings and years. At the second sampling, LegumePL treatments had greater soil N than all other treatments ($p < 0.009$) except Legume ($p = 0.608$). Six weeks following cover crop termination (third sampling), LegumePL treatments had greater extractable soil N than LegumeRye PL ($p = 0.020$), but not significantly different from Legume ($p = 0.710$). In 2015, LegumePL again produced the greatest amount of extractable soil N and by the third sampling date, more than two times the extractable soil N of the nearest treatment (LegumeRyePL, $p = 0.004$). The extractable soil N from Rye and RyePL treatments never differed either year, and were negative or negligible compared to the 0 N control. The LegumeRye and LegumeRye PL treatments also never differed, but by the third sampling in 2015, produced similar soil N as Legume without PL.

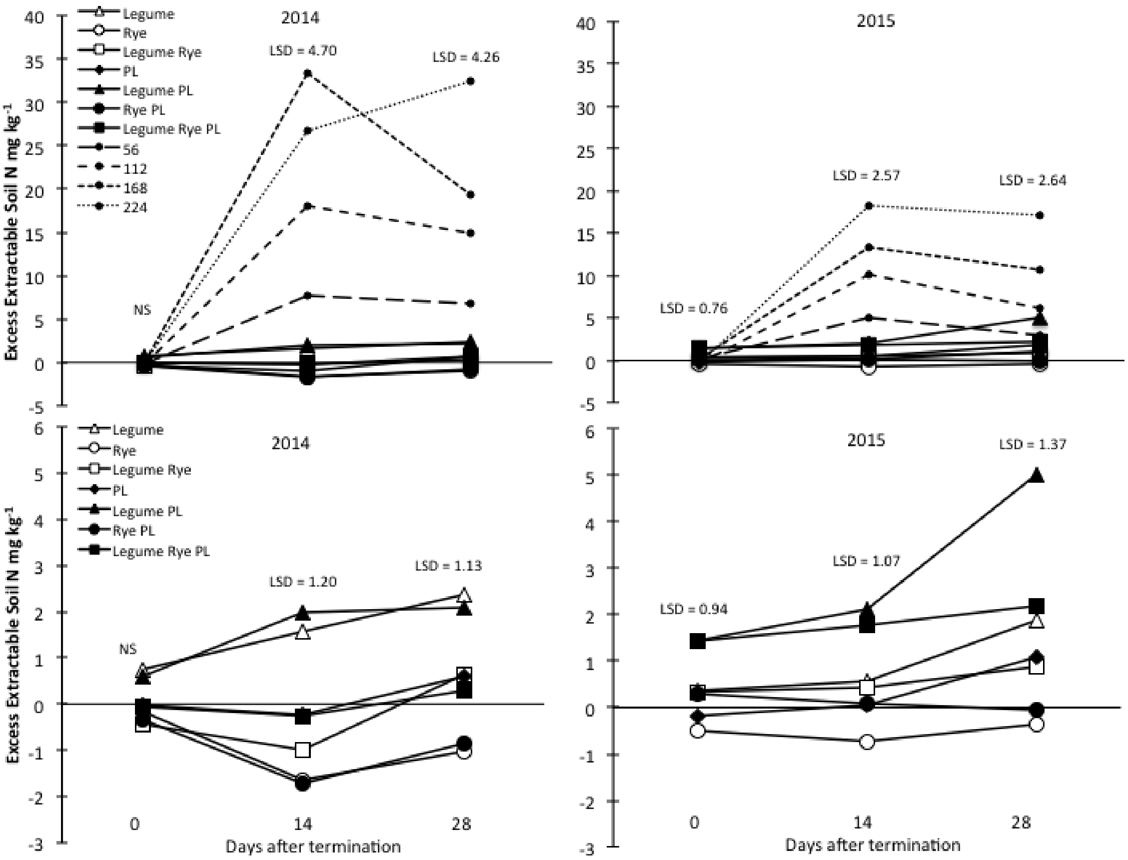

Figure 3 – Excess extractable soil mineral N averaged across 0 to 60 cm depths in 2014 and 2015. Upper panels include all fertilizer N and cover crop/poultry litter treatments. Lower panels include only cover crop and poultry litter treatments. LSD values are reported for the sampling dates that had significant treatment effects (significant p values all ≤ 0.03). NS represents non-significant treatment effects.

Table 4. ANOVA of excess extractable soil N due to treatment (minus native soil N from 0 N control) averaged across 0 to 60 cm depths for all fertilizer N and cover crop × poultry litter treatments in 2014 and 2015.

	2014 (days post-termination)			**2015 (days post-termination)**		
	0	**14**	**28**	**0**	**14**	**28**
L	-0.024	1.187	1.783	0.273	0.430	1.424
LPL	0.444	1.484	1.579	1.083	1.588	3.764
R	-0.123	-1.229	-0.761	-0.376	-0.534	-0.271
RPL	-0.256	-1.296	-0.630	0.217	0.065	-0.038
LR	-0.334	-0.744	0.485	0.254	0.340	0.665
LRPL	-0.036	-0.192	0.204	1.075	1.347	1.647
WFPL	-0.029	-0.175	0.456	-0.135	0.055	0.824
56 N	-0.126	5.779	5.139	0.121	3.805	2.237
112 N	-0.203	13.580	11.233	-0.286	7.573	4.610
168 N	-0.036	25.088	14.482	0.083	10.003	8.023
224 N	-0.056	19.980	24.304	-0.260	13.679	12.936
ANOVA			**P>F**			
TRT	0.0988	<0.0001	<0.0001	0.0034	<0.0001	<0.0001
CONTRASTS			**P>F**			
L v. LPL	0.6834	0.8980	0.9228	0.0377	0.3528	0.0799
R v. RPL	0.6510	0.9370	0.9505	0.1212	0.6283	0.8581
LR v. LRPL	0.3107	0.8118	0.8934	0.0352	0.4181	0.4530
L v. LR	0.0042	0.4073	0.5385	0.9588	0.9417	0.5611
LPL v. LRPL	0.1084	0.4714	0.5146	0.9835	0.8455	0.1114
LPL v. WFPL	0.1130	0.4758	0.5942	0.0027	0.2211	0.0301
LPL v. 56 N	0.0588	0.0714	0.0981	0.0149	0.0045	0.2463
LPL v. 112 N	0.0333	<0.0001	<0.0001	0.0009	<0.0001	0.5178

Weed suppression

Weed suppression was effectively accomplished with Rye and Legume/Rye cover crops compared to the no cover treatments (WF and WFPL) both years (Fig. 4). Weed biomass in fallow plots was greater in 2014 than in 2015 ($p < 0.0001$), while cover cropped plots had greater weed biomass in 2015 than in 2014 ($p = 0.001$). The primary weed species were henbit, chickweed, and annual bluegrass. In 2014, Legume PL reduced weed biomass least effectively, but still achieved 95% suppression compared to WF. Also in 2014, Legume/Rye suppressed weeds most effectively among the treatments (99%) but did not differ from Rye, Rye PL or

Legume/Rye PL treatments. In 2015, Legume PL weed biomass did not differ from the weed biomass of the WFPL treatment ($p = 0.11$). However, all other treatments that included rye had at least 90% less weed biomass than the no cover treatments and 74% less than Legume PL in 2015 ($p < 0.05$). Under WF conditions, PL increased weed biomass by 20% in 2014 and 28% in 2015, but the difference was not significant either year ($p = 0.77$ and $p = 0.58$). Poultry litter did not significantly increase weed biomass within cover crop monocultures or biculture.

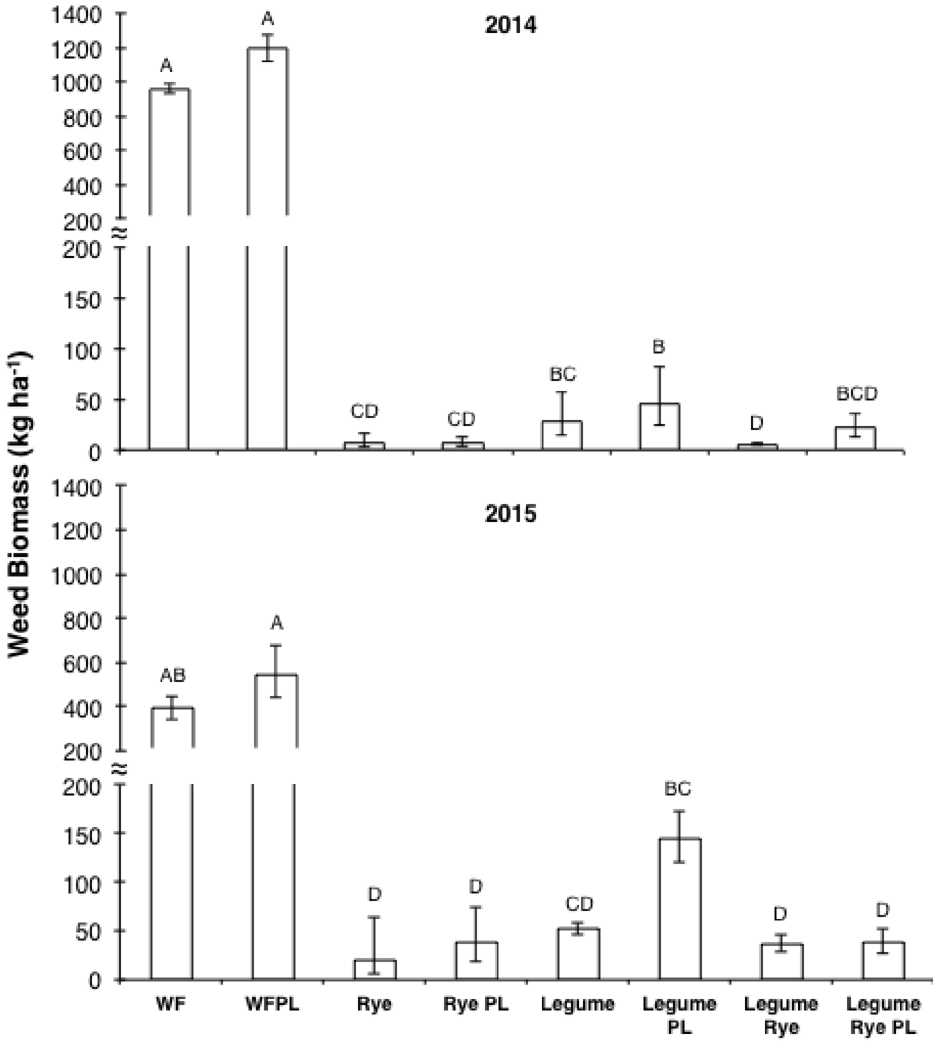

Figure 4. Weed above-ground biomass (kg ha^{-1}) harvested immediately prior to cover crop termination of cover crop × poultry litter treatments. The means and standard error are reported as back-transformed values and letter separate means using LSD.

Table 5. ANOVA of weed above-ground biomass (kg ha^{-1}) harvested immediately prior to cover crop termination of cover crop × poultry litter treatments.

	2014 (kg ha^{-1})	**2015** (kg ha^{-1})
L	28.64	51.67
LPL	44.68	143.65
R	5.95	19.71
RPL	6.88	34.42
LR	5.52	36.66
LRPL	21.57	37.15
WF	961.26	392.39
WFPL	1193.80	548.66
ANOVA	**P>F**	
CC	<0.0001	<0.0001
PL	0.1559	0.1130
CC*PL	0.6255	0.6702
CONTRASTS	**P>F**	
L v. LPL	0.5428	0.1001
R v. RPL	0.8562	0.3335
LR v. LRPL	0.0723	0.9823
WF v. WFPL	0.7661	0.5781
L v. LR	0.0329	0.5690
LPL v. LRPL	0.3228	0.0336

3.5 Discussion

Functionally diverse cover crop species, such as grasses and legumes, and management practices, such as fall applied PL, can be designed to simultaneously deliver multiple ecosystem services (Storkey et al., 2015). This field study quantified the tradeoffs and benefits of grass, legume, and biculture cover crops coupled with fall-applied PL in conservation-tilled corn. What emerges from the results is not a simple recommendation, but a suite of data that can be applied to design complex, high-functioning agroecosystems that target specific ecosystem services. The visualization of these data in spider plots can aid in species selection based on service prioritization (Fig. 5). Of the ecosystem services plotted, cover crop productivity and N content, as well as C:N, were related to soil mineral N and crop productivity. On the other hand, weed suppression was more directly related to cover crop biomass productivity. These results corroborate a study on 8 species mixtures that concluded the relationship between cover crop biomass and ecosystem services was dependent upon the specific service (Finney et al., 2015). Furthermore, our results suggest the addition of fall-applied PL to cover crop, particularly a legume, enhances N related ecosystem services such as post-termination soil N. Targeting specific ecosystem services may be achieved through species selection and management practices.

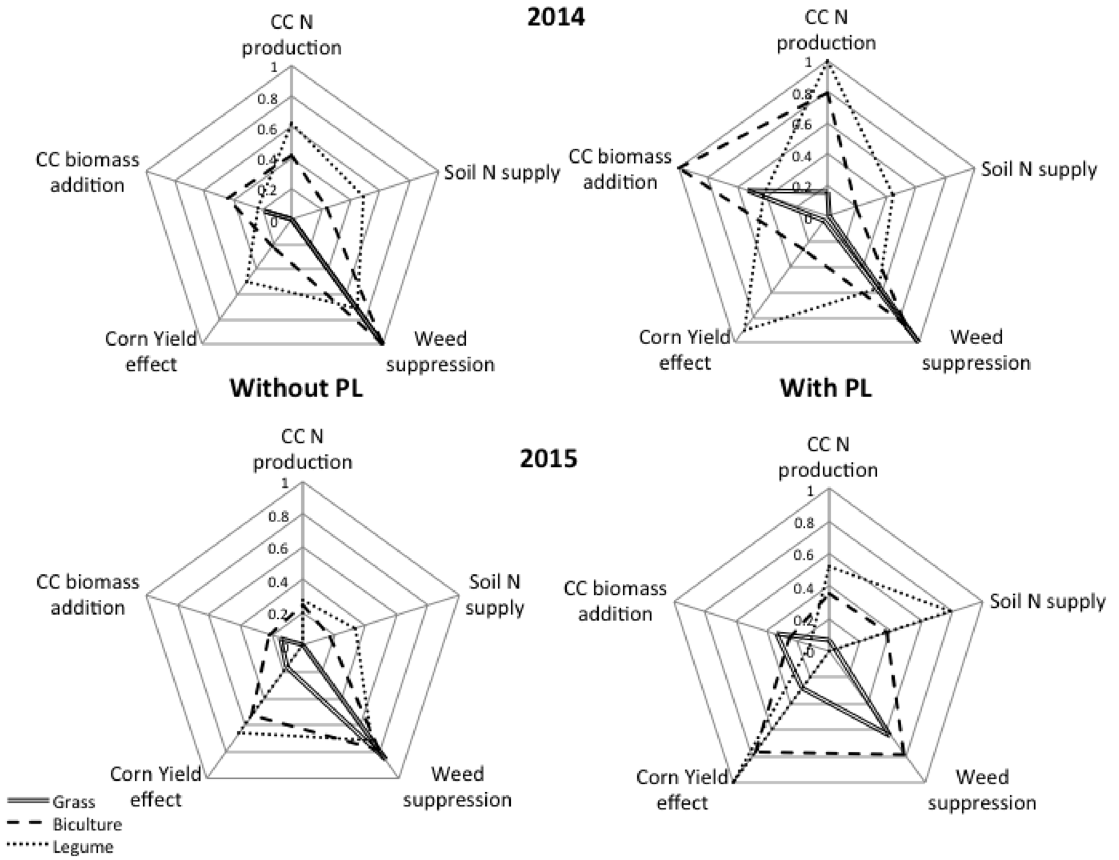

Figure 5 - Normalized mean values of 2014 and 2015 cover crop (CC) biomass production, cover crop N production, early season soil N supply, fertilizer utilization, weed suppression, and corn yield effect of grass, legume and biculture cover crop treatments on the left and grassPL, legumePL and bicuturePL on the right . Normalized values are calculated relative to the range of the values.

Cover crop biomass productivity and variation in productivity between years increased with fall application of PL, even with relatively low nutrient additions (approximately 60 kg N, 30 kg P, and 45 kg K ha^{-1}). Differences and variability were likely due to differences in cumulative precipitation, and disease pressure (*Phytophthora* sp.) on legume in 2015 (Supplementary Fig. 1). The coefficient of variation of the yield data suggest greater variability in monocultures (25.6 Legume and 25.1 Rye) than in mixtures (17.9), greater variability with PL application in both mono- and bicultures (30.5 LegumePL, 29.6 RyePL, 37.1 Legume/RyePL), and approximately equal variability in monocultures of the legume and grass (25.6 Legume and 25.1 Rye). While these results may be more robust in a longterm study, they support the well-established hypothesis that functionally diverse species, as in a legume-grass biculture cover crop, stabilize biomass productivity, possibly by maximizing different resource niches (Silvertown, 2004). On the other hand, variability in cover crop biomass was greater with PL, which may be due weather variations that subsequently led to PL nutrient losses from volatilization or leaching (Adeli et al., 2006).

While N content and C:N differences in legumes suggest a greater effect of PL on the legume than rye cover crops, the mechanism remains unclear. Fall-applied PL was recovered by Legume and though not significant, Legume biomass was greater with PL. Any additional effect of poultry litter on biological N_2 fixation in the legume treatment may be minor but is a mechanistic consideration. A previous study did not find a strong inverse relationship between soil fertility and biological N fixation of either perennial or annual legumes (Schipanski and Drinkwater, 2012). However, in a greenhouse experiment, cowpea root nodule to shoot biomass ratio was greater in monocultures and low-fertility soils than in mixtures with higher fertility soils (Wortman and Dawson, 2015). Further analysis to assess differences in biological N_2 fixation due to soil fertility and interspecific nutrient competition may illuminate the mechanisms affecting legume C:N and N content.

Based on biomass comparisons of monocultures and bicultures, all bicultures yielded more than the component monocultures. The effect of PL on relative yield of biculture compared to monoculture was inconsistent, contrary to our original hypothesis. Greater variability in yields of PL treatments, similar to the variability in cover crop biomass, may be due to nutrient losses from volatilization or leaching (Adeli et al., 2006). Weather variability and potential decreases in legume productivity in bicultures in 2015 may also explain the lower biculture yield differences that year (Supplemental Table 1). Though there was some variability in the mixture yield between years, generally Rye productivity increased and legume productivity decreased in bicultures. This result is further supported by the research synthesis in Chapter 4.

Lower residue C:N of LegumePL suggests an effect of PL on legume residue quality, but no consistent interspecific effect of biculture on the C:N of the legume component. The consistent and relatively fixed C:N of Rye residues among treatments may lend evidence to support the general popularity of Rye in cover crop systems based on the stability of Rye biomass production and residue quality (2015 Cover Crop Survey). Understanding differences in residue quality and quantity of individual species in mixtures and with PL, will aid in maximizing ecosystem services, including crop productivity.

Generally, cover crop and PL treatment effects on extractable soil N were greater with the addition of PL and legume, with LegumePL providing consistently greater levels of soil N compared to other treatments, albeit low levels relative to fertilized plots. Minimal extractable soil N resulting from the biculture with the legume proportion of 33% in 2014 and the extractable soil N comparable to fertilizer rate of 56 kg N ha^{-1} in 2015 with a legume proportion of 50% in 2015, support prior recommendations of at least 40% legume in biculture to increase availability of N from residues (Kou and Sainju, 1998). The greater concentration of extractable soil N resulting from the mixture in the second year may reflect the increase in legume proportion and also a general increase in soil organic matter and “soil natural capital” (Brady et al., 2015). Biculture soil N varied between years, but did not differ significantly from the extractable soil N of legume monoculture by year two.

Weed suppression was successful with cover crop treatments compared to WF, and PL did not significantly increase weed biomass. Residue productivity is often correlated with weed suppression (Finney et al., 2015), and cover crop mixtures tend to overyield compared to monocultures (Chapter 4). However, in a recent study there was no difference in weed suppression due to a five species cover crop mixture and the most productive monoculture species (Smith et al., 2014). Additionally, Hayden and others (2014) found an inverse relationship between weed suppression and legume proportion when comparing bicultures at 17% seed rate increments. This study illustrates that grass-legume bicultures at seeding rates of 67:33 and 50:50 were as effective as the Rye monoculture for weed suppression.

3.6 Conclusion

These results aid in the selection of cover crop/PL combinations according to preferred ecosystem services, while considering complex interspecific and management interactions. Legume N yield is increased and C:N ratio is decreased by PL application. Additionally, a legume cover crop in combination with PL resulted in greater soil mineral N concentrations, but slightly more weed pressure. Poultry litter increased Rye biomass, but not residue N content. Rye retains soil N, is most effective at suppressing weeds, but may decrease corn productivity without additional fertilizer N inputs. Bicultures overyield compared to monocultures, are not consistently affected by PL, result in similar soil N availability compared to a legume monoculture following multiple years of practice, and suppress weeds as effectively as Rye.

Prioritizing multiple provisioning, supporting, and regulating ecosystem services using cover crops in complex agroecosystems will ultimately create more sustainable production that enhances agroecosystem function. A consolidation of field data that includes multiple cover crop species in monoculture and mixtures and more ecosystem services would be useful in engineering diverse systems that meet management and environmental priorities. Production systems designed to provide multiple ecosystem services meet the goals of conservation agriculture and will ultimately lead to higher-functioning, more sustainable agroecosystems.

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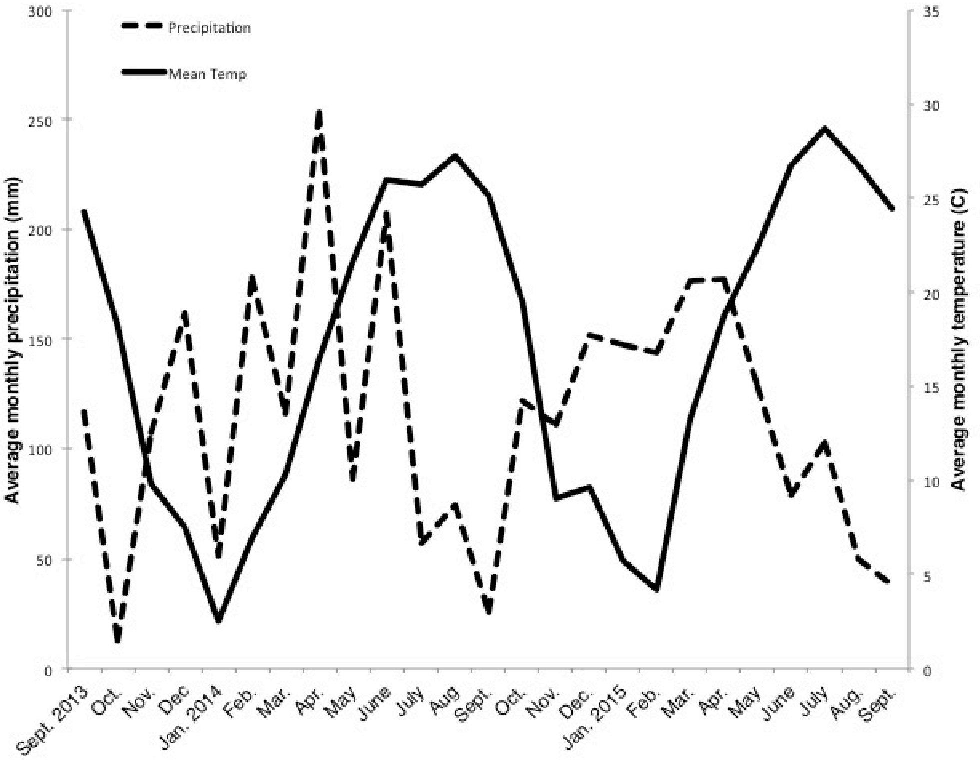

Chapter 3 Supplemental Figure 1. Line graph represents the average monthly precipitation (mm, dashed line) and temperature (°C, solid line) values for the duration of the experiment (September 2012 to September 2015).

CHAPTER 4 - Cover crop functional groups differ in productivity when grown in mixtures

4.1 Abstract

In natural systems, there is a positive relationship between species diversity and biomass productivity. Diversity effects on cover crop production remain unresolved. This study examined the effect of bicultures and multiple species mixtures on productivity of cover crop residue, functional group residue, and cash crop yield using synthetic review. Relevant studies were summarized to assess cover crop biomass of species functional groups in mixtures and monocultures and the subsequent effect on cash crop yield. Data were summarized from 31 studies. Land Equivalent Ratio (LER) was used to determine that mixtures of cover crops overyield by 14.2%, with 84% of observations overyielding. Grasses overyielded in mixtures by 60% compared to grass yield in monocultures with 78% of observations reporting that grasses overyield. Conversely, legumes yielded 10% less when in mixtures compared with legume monocultures in 62% of the observations. Finally, mixed cover crops increased cash crop yields by 13.5% overall, compared to a predicted outcome from monoculture component species, but results varied by cropping system. Unfertilized corn had 5% greater yield following mixes than fertilized corn, which was still 10% more than the predicted yield. While the sample size for fertilized horticultural crops was small, these crops were generally unaffected by mixed cover crop but creamer potato and processing tomato had 29% and 17% greater yields following mixes than predicted, respectively. Similar to grassland ecosystems, diverse cover crop mixtures overyield compared to monocultures, while grass, brassica, and legume cover crops and cash crop yields differ by species and management.

4.2 Introduction

In grassland ecosystems, diverse plant communities of at least five species or two functional groups typically maximize resource-use efficiency and productivity (Tilman et al., 1997). Niche partitioning, functional group complementarity (Hooper et al., 2005) and an examination of intercropping (Vandermeer, 1989) suggest that mixed cover cropping systems could have facilitative effects on cover crop biomass, residue composition, and consequently, nutrient cycling in agroecosystems. The relationship between diversity and productivity has been used as a theoretical framework to investigate crop rotations and intercropping, and has increasingly been applied to predict the impacts of cover crop mixtures in agroecosystems (Wortman et al., 2015). Research in cover crop mixtures is increasing but there has yet to be a synthetic analysis examining diversity-productivity relationships in a variety of mixed species of cover cropping systems.

The USDA promotes 58 plant species as cover crops, which are categorized into various functional groups based on phenology, growth cycle, plant architecture, relative water usage, and seasonality (Liebig and Johnson, 2015). These functional groups can have different effects on nutrient cycling, soil conservation, weed suppression, and other ecosystem services (Cherr et al., 2006, Dabney et al., 2001, Bàrberi and Mazzoncini, 2001, Finney et al., 2015). Grasses and legumes are the most common functional groups in cover cropping systems, especially in winter. Typically grass species are grown to conserve soil, retain residual nutrients, add organic matter through residues, and suppress weeds (Dabney et al., 2001). Unlike grass species, legumes add more N through residues with lower C:N but are neither as productive nor as suppressive of weeds (Clark et al., 1994). Grasses and legumes are often combined in biculture and mixed cover cropping systems to obtain benefits from both species (Ranells and Wagger, 1997). Individual studies on the competitive or facilitative relationship between cover crop species or functional group have been inconclusive.

To understand the competitive, facilitative or synergistic effects between cover crops grown in mixtures compared to monocultures, land equivalent ratio (LER) can be calculated. LER represents the amount of land required to produce equal biomass yield from mixed species compared to monoculture crops (Mead and Willey, 1980; Vandermeer, 1989). The calculation has recently been applied to compare mixtures to monoculture cover crops. An LER greater than 1 indicates that more land area would be required to grow the same yield of cover crops as monocultures compared to mixtures. An LER less than 1 suggests monocultures produce more biomass than mixtures, requiring less land. Individual studies on bicultures and mixtures that include LER have suggested mixtures tend to produce greater biomass (Creamer et al., 1997; Brainard et al. 2012; Wortman et al., 2012; Halde et al., 2014). However, a conflicting study found mixes do not overyield compared to monoculture components in a water-limited environment (Nielsen et al., 2015).

Overyielding may not enhance all ecosystem services however (Smith et al., 2014). Increased cover crop residue, especially high C:N residues, may negatively impact inorganic N availability in mixed cover crop systems (Finney et al., 2015). Other studies have also found variable mixture effects on residue quality and biological N_2 fixation. Wortman and Dawson (2015) examined the root nodulation and biological N fixation of cowpea (*Vigna unguiculata* L. Walp.) and found the lowest rates of these processes in cover crop blends with four species (Wortman and Dawson, 2015). Mixing complementary species can affect both the residue quantity and quality, which consequently affects associated ecosystem services and ultimately cash crop yield.

Resolving the effect of mixed cover crops on cash crop yield is essential to production system management. Legumes generally increase crop yield, due to the addition of biologically fixed N and greater mineralization of residues (Frye et al., 1988). The effect of grasses on crop yield is often neutral (Miguez and Bollero, 2005, Tonitto et al., 2006) or negative due to immobilization of N, especially under low fertility conditions (Raimbault et al., 1989). While the effect of mixtures on crop yield has not been extensively studied, corn yield has been shown to increase with increasing legume percentage in mixtures under conditions with no additional fertilizer (Clark et al. 1994, Tosti et al. 2012)

This study examined the effect of bicultures and multiple species mixtures on total cover crop residue productivity, individual species or functional group productivity, and cash crop productivity using synthetic review and meta-analysis. Specifically, we expected total cover crop biomass to increase and Land Equivalent Ratio (LER) to be greater than one, suggesting mixtures overyield compared to monocultures. We also examined the productivity of individual functional groups (e.g., grasses, legumes, brassicas) grown in mixtures and monocultures, expecting yield differences by species or functional group. For studies that also included measures of variance, we performed a formal meta-analysis, using Hedge’s d to calculate effect sizes comparing yield of mixtures to monocultures (Hedges and Olkin, 1985). Finally, the effect of mixtures on cash crop yield was examined by synthesizing yield differences following monoculture and mixed cover crops in different production systems. The results of these analyses have implications for the management and design of mixed cover cropping systems to maximize functional group production to generate heterogeneous residues, facilitate cash crop production, and prioritize other ecosystem services.

4.3 Methods

Included studies were selected through an exhaustive search of the primary literature using the Agricola database and the search string “cover crop” AND “mix*”, which returned 213 references. All 213 abstracts were reviewed for relevance. Ninety-eight full manuscripts were screened for cover crop yields of individual species in monoculture and mixtures. Papers were selected which included data on cover crop species biomass yields both in mixes and monocultures and seeding rates for both mixes and monocultures. Also included were those papers that reported calculated values for LER, with or without reported biomass data. The reference list of each selected paper was also searched for other relevant papers. Additionally, we reviewed the work of the authors that were already included in the synthesis. Papers were excluded from the synthesis if they did not include data on both monoculture and mixed species of cover crops in the same experiment. Thirty-one papers were ultimately selected for the synthesis. Data extracted included monoculture cover crop biomass yield, either combined species or separated species biomass yield in mixtures, seeding rates and cash crop yield following monoculture and mixed species cover crops. Descriptive data of the cover cropping season, species in the mixture, study location, soil type, and experimental years were also summarized.

LER

Land Equivalent Ratio (LER) was calculated using the reported values of monoculture and mixed cover crop species biomass using different calculations defined below. A Calculated LER (“CLER”) was used when separated biomass of individual species grown in mixtures was reported, which would allow for the calculation of relative yield by species.

$$CLER = RY_{species\ i} + \ RY_{species\ j} + \cdots + RY_{species\ k}$$

$$RY_{species\ i} = \frac{Yield_{species\ i\ \ mix}}{Yield_{species\ i\ \ mono}}$$

where $RY_{species\ i}$ is the Relative Yield of species i, and Relative Yield is defined as the yield of species i in the mixture ($Yield_{species\ i\ mix}$) divided by the yield of species i in the monoculture ($Yield_{species\ 1\ mono}$). Calculated LER is then the sum of the relative yield of each component species in the mixture.

Using a different approach, we were also able to utilize papers that reported the combined biomass of the mixtures, rather than the individual species components by calculating a “Theoretical LER” or “TLER”. TLER was calculated using the following equation:

$$TLER = \frac{Yield_{mix}}{(Yield_{species\ i\ mono} \ \times \ SP_{species\ i}) + (Yield_{species\ j\ in\ mono} \ \times \ SP_{species\ j}\ )}$$

where $Yield_{mix}$ is the total yield of the mixture, $Yield_{species\ i\ mono}$ is the yield of species i in the monoculture, and $SP_{species\ i}$ is the seed proportion of the seed rates of species i in the mixture relative to the seed rate of species i in the monoculture. The *SP* was defined as the weight of seed of one species in the mix relative to the weight of seed of that species in the monoculture. For example, if a grass was planted at a rate of 25 kg ha^{-1} when grown with legume in biculture and at a rate of 60 kg ha^{-1} in monoculture, then the *SP* would equal 0.42. The SP value for each individual species observation was never greater than 1.

The various permutations of LER calculations were used to utilize the variety of reported data on mixed cover crop yields. There were also 3 papers that reported LER values. Total LER is the average of calculated individual observations of CLER, TLER, and reported LER values. All LER calculations greater than 1 suggest mixed cover crops overyield compared to the monoculture components. An LER value less than 1 indicates that mixed cover crops yield less compared to the monoculture components.

Mixture Effect

To examine the effect of mixture on yield of different cover crop functional groups or species, a *Mixture Effect* was calculated using the following equation:

$$Mixture\ Effect_{species\ i} = \frac{Yield_{species\ i\ mix}}{Yield_{species\ i\ mono} \times SP_{species\ i\ mix}}$$

where the *Mixture Effect* is defined for individual species or functional group based on the yield of the species in mixture ($Yield_{species\ i\ mix}$), divided by the yield of the same species in monoculture ($Yield_{species\ i\ mono.}$), corrected by the Seed Proportion ($SP_{species\ i}$). *SP* was defined as the weight of seed of one species in the mix relative to the weight of seed of that species in the monoculture as in the above calculation for TLER. This calculation of *Mixture Effect* allows for a more direct comparison of the differences between functional group or species yield in mixtures and monocultures while taking into account the planting density. In other studies this has been indirectly addressed using a partial LER calculation or pLER (Smith et al. 2014; Wortman et al. 2012).

Finally, a formal meta-analysis was conducted using Hedge's d as the metric for those papers that reported variance or from which variance could be calculated from the data provided. Hedge's d was calculated to compare the effect of cultivation in mixture on individual cover crop functional groups. Hedge's d is the yield of cover crop species in the mixture to the yield of the cover crop species in monoculture. Hedge's d was calculated for a grass species or a legume species with MetaWin software using the following equation:

$$Hedge's\ d = \frac{XE - XC}{S} \times J$$

where *XE* is the mean of the species biomass in the mixture, *XC* is the mean of the species biomass in the monoculture, *S* is the pooled standard deviation and *J* is a correction factor based on the degrees of freedom (Rosenberg et al., 1997). For those papers that did not explicitly publish the values for means and variance, we used the software program "Data Thief" to extract those values from figures (Tummers, 2006).

Crop Mixture Effect

Crop mixture data was explored by calculating a standardized effect of mixed cover crops on cash crop yield. All available observations on crop yield involved a grass and legume biculture. The *Mixture Yield Effect* was a comparison of yield of cash crop following mixed cover crops and a proportional cash crop yield from monocultures using the following equation:

$$\frac{Crop\ Yield_{mix}}{\left(Crop\ Yield\ g\ \times\ SP_g\right) + \left(Crop\ Yield\ l\ \times\ SP_l\right)}$$

where $Crop\ Yield_{mix}$ is the cash crop yield produced following mixed cover crop, *Crop Yield g* is the cash crop yield following grass, SP_g is the proportion of grass seed in the mixture by weight, *Crop Yield l* is the cash crop yield following legume, and SP_l is the proportion of legume seed in the mixture by weight. We also explored differences in mixture yield effect on crop species including corn, broccoli, strawberries, tomato, soybean, cotton, and sorghum. Mixed cover crop effects on corn yield were based on several studies, while the sample size is small for other individual crops. The effects of mixed cover crops in fertilized and unfertilized systems were also analyzed separately when relevant data were provided to isolate the effect of mixtures with and without additional fertilizer. Values greater than 1 suggest the mixture produced more crop than the predicted outcome based on the relative proportions of monocultures. A value less than 1 means the predicted yield from the component monocultures was greater than the yield produced by the mixture. It does not necessarily mean that yield following cover crop mixtures was greater than the yield following an individual cover crop species, such as legume monoculture.

4.4 Results

Of the 31 studies analyzed, the dominant cover crop season was winter (84%) with only 5 studies that focused on summer cover crops. Three studies included experiments in both winter and summer or other seasonal periods such as late spring. The most common mixture of cover crops was a biculture (84% of studies). Mixtures of 3 to 8 species made up the remaining 16% of the studies.

Of the 31 studies analyzed, 87% included rye, the most frequently planted cover crop species in the US (SARE/CTIC/ASTA Joint 2014-2015 Cover Crop Report). Other dominant grass species, in decreasing order, were oat (*Avena sativa*), barley (*Hordeum vulgare*), wheat (*Triticum* spp.), and ryegrass (*Lolium multiflorum*). The second most common cover crop and the most commonly grown legume was vetch; *Vicia* spp. were included in 81% of studies in the analysis. Vetch species include primarily hairy or woolly pod vetch in the USA (65% of studies), purple (*V. benghalensis*) in Florida and France (6%), and common vetch (*V. sativa*) in Italy and Argentina (6%). Other legumes in mixtures were clovers (*Trifolium* spp.), including crimson (*T. incarnatum*), white (*T. repens*), and squarrosum (*T. squarrosum*), winter and field pea (*Pisum sativum* and *P. arvense*). Other cover crop studies included mustard species (canola; *Brassica napus* and turnip rape; *Brassica rapa*), sorghum sudangrass (*Sorghum bicolor* x *sudanese*), triticale (*Triticale hexaploide*), buckwheat (*Fagopyrum esculentum*), millet (*Setaria* spp. and

Echinochloa spp.), cowpea, velvetbean (*Mucuna pruriens*), and sunn hemp (*Crotalaria juncea*), but were represented collectively in only about 30% of the studies.

Cover crop mixture studies were predominantly located in the United States (70%), with most of those (83%) along the east coast from Florida to New Hampshire. One study was located in Nebraska and two were in western Washington. Eight studies were conducted outside the United States including two in Canada, three in Italy, and one each in France, Germany, Argentina, and Australia (Figure 1).

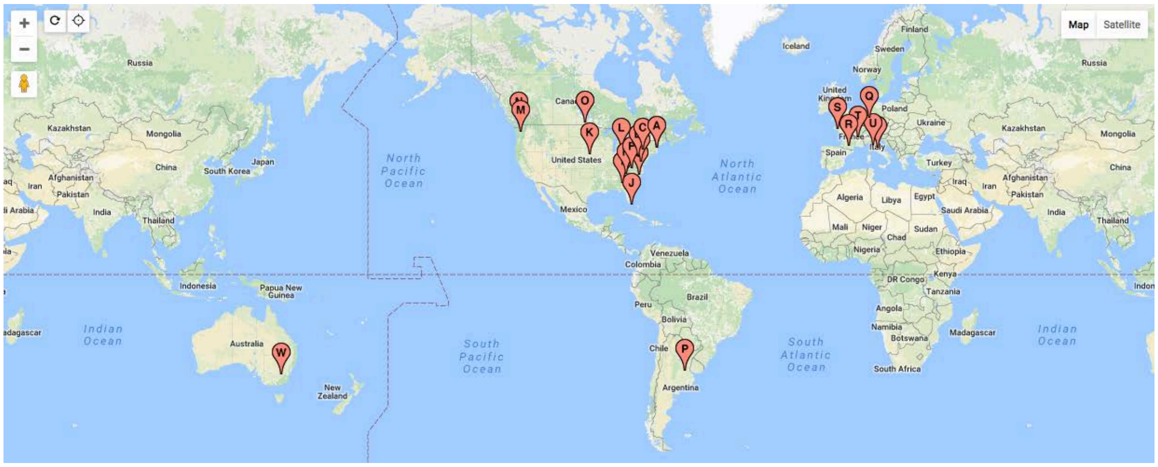

Figure 1. Locations of cover crop mixture study sites included in the analysis.

All studies were conducted on experimental sites with soil orders that included alfisols, inceptisols, ultisols, mollisols and entisols, in decreasing order. Experimental trials included in the analysis were conducted as early as 1964 up to 2013, which was published in July 2016. The frequency of trials was generally steady from the mid-1990s to the present (Figure 2).

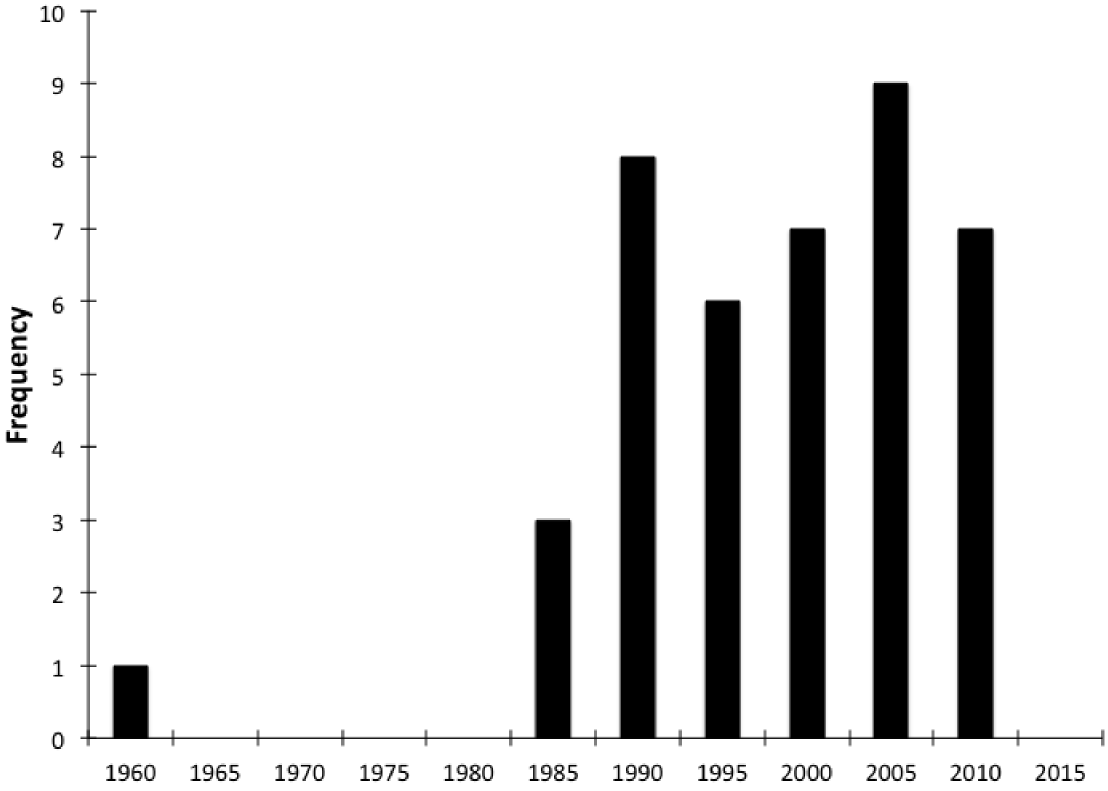

Figure 2. Histogram of study experimental year for cover crop mixture studies included in synthesis summed over 5 year intervals.

By combining all permutations of the LER calculations, which included 214 observations and 29 studies, the average of the Total LER values suggests mixtures overyield by 14% (LER = 1.142, 95% CI [1.094, 1.190], Figure 3). Studies that included biomass measurements but reported total mixed biomass, rather than separated individual species' biomass, were used to calculate TLER. Those included 15 studies and 101 observations. The calculations of TLER suggest mixtures overyield by 9% compared to their monoculture components (TLER = 1.087, 95% CI [0.995, 1.180]). Ten studies and 73 observations reported separated biomass of component species and were used to generate CLER. Again the yield comparison suggests overyielding of mixtures by 18%, a greater increase than the TLER calculations (CLER = 1.185, 95% CI [1.135, 1.234]). There were also 3 studies and 24 observations that reported LER values rather than the original biomass data. The LER average value of these, which was added to the Total LER, was 1.260, again reporting an overyielding effect (95% CI [1.196, 1.323], Figure 3).

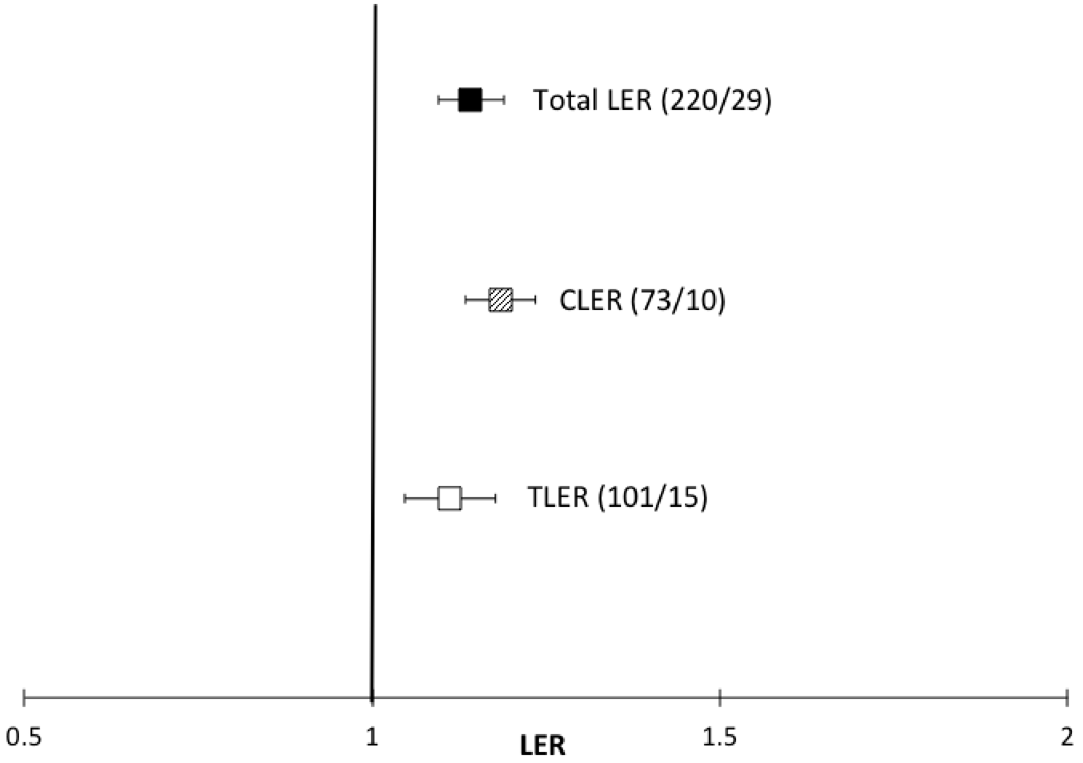

Figure 3. Average and 95% Confidence Interval of calculated (CLER), theoretical (TLER), and Total Land Equivalence Ratio values for mixed species cover crops. Numbers in parentheses indicate the number of observations and the number of studies included in the calculation (#Obs / #studies).

Based on the CLER calculations that included individual species biomass in mixtures and monocultures, in 84% of observations mixtures produce more biomass (overyield) than expected compared to the monocultures of component grasses or legumes. In 59% of those overyielding observations, the overyielding was transgressive. In other words, the total mixture biomass was greater than the most productive monoculture component. When the mixture did not transgressively overyield, grass monocultures produced greater biomass than the total mixture biomass in 27% of the observations, while legumes produced more yield than the mixture in only 13% of observations. The small sample size for brassicas and other functional groups make transgressive overyielding in these mixtures difficult to assess.

Examining the mixture effect on individual component cover crop species indicates there are differences between species or functional groups. Grass yields in mixtures were greater than in monocultures in 81% of the 10 studies and 74 observations synthesized (Figure 4). When grasses were grown in mixtures, yield was nearly 60% greater than yield in monoculture (Mixture effect on grass = 1.591, 95% CI [1.4190, 1.762]). Average brassica yield increased when grown in mixtures by 32% (1.3196, 95% CI [1.124, 1.515]), but results are perhaps limited by small sample size. In contrast, legumes tended to yield less in mixtures than in monocultures in 63% of the studies surveyed. Yield of legumes was reduced by approximately 10% when grown in mixtures compared to what would be expected from the relative seeding rates in mixtures and monocultures (0.908, 95% CI [0.779, 1.037]; Figure 4).

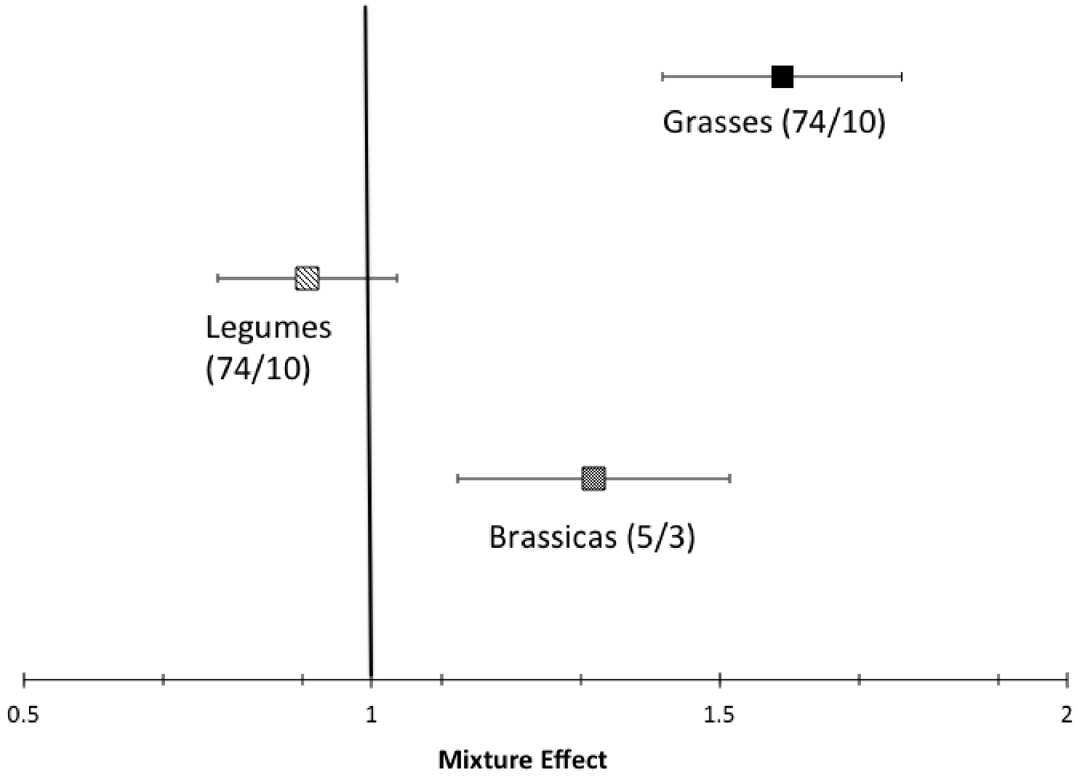

Figure 4. Mixture effects on functional groups of grasses, legumes, and brassicas. Bars represent the 95% confidence interval of the means. Numbers in parentheses are observations and studies included in the calculation (#Observations / #studies).

There were four studies that included separated species' biomass and variance that could be used for formal meta-analysis. These studies included 29 observations on rye, barley, sorghum sudangrass, Japanese millet, vetch, pea and soybean. Because these calculations are measuring effect size of mixtures compared to controls, an effect size greater than 0 suggests an increase in yield and an effect size less than 0 suggests a decrease in yield for the functional group. For grasses, the mixture effect size (Hedge's d) was 1.28 [95% CI 0.9935, 1.5758]. For legumes, the mixture effect size (Hedge's d) was -0.36 ([95% CI -0.6230, -0.1005], Figure 5). These values show a similar trend to the mixture effect calculations, which support the hypothesis that grass biomass increases, while legume biomass decreases when grown in mixtures.

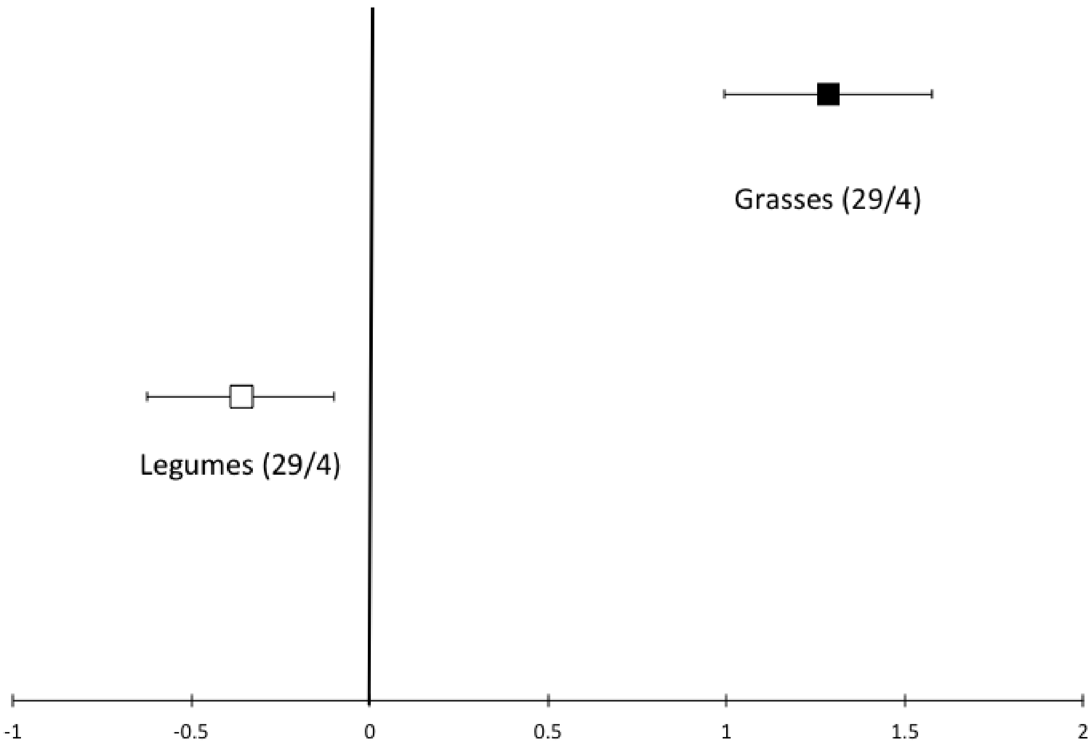

Figure 5. Hedge's d – Effect size of functional group (grasses and legumes) yield in mixtures and monocultures calculated using 4 studies and 29 observations that included means of yield in monoculture and mixture and measures of variance for each. Bar represent the 95% confidence interval. Numbers in parentheses are observations and studies included in the calculation (#Observations / #studies).

Finally, using a synthesis of 11 studies and 51 observations, cover crop mixture effects on cash crop yield were examined. The cash crop was grain corn in 64% of the studies. Studies also included cotton, sorghum, soybean, tomato, potato, broccoli, and strawberry. Across all crops and experiments, there was an average increase in crop yield due to the calculated mixture yield effect of 13.5% (1.135 [95% CI 1.089, 1.181], Figure 5). In 77% of all observations, the mixture yield effect was positive, or greater than 1. In unfertilized corn experiments, mixed cover crops increased corn yield by 15.5% (1.155 [95% CI 1.093, 1.217]) compared to the predicted yield of the component monocultures. When fertilizer was applied in addition to mixed cover crops, the average effect was only 10% but not significantly different (1.101 [95% CI 1.029, 1.173]).

Cover crop mixture effects were also diminished in other non-corn crop species that were grown with fertilizer, resulting in an increase in yield of 9.3% (1.093 [95% CI 1.022, 1.174]). However, based on the average responses of fertilized broccoli, fertilized cotton, and unfertilized soybean to mixed cover crops, the mixture yield effects were negative, or yield was reduced following mixed cover crop compared to the predicted yield from component monocultures. Fertilized strawberry yield was unaffected by mixed cover crop. In contrast, yields of processing tomato and creamer potato increased with mixed cover crops compared to the expected value of the yields from monoculture cover crop treatments (Mixture yield effect = 1.172 and 1.286, respectively). While these results suggest interesting differences in the effect of mixed cover crops among cash crop species, the data are limited and sample size is small for individual species.

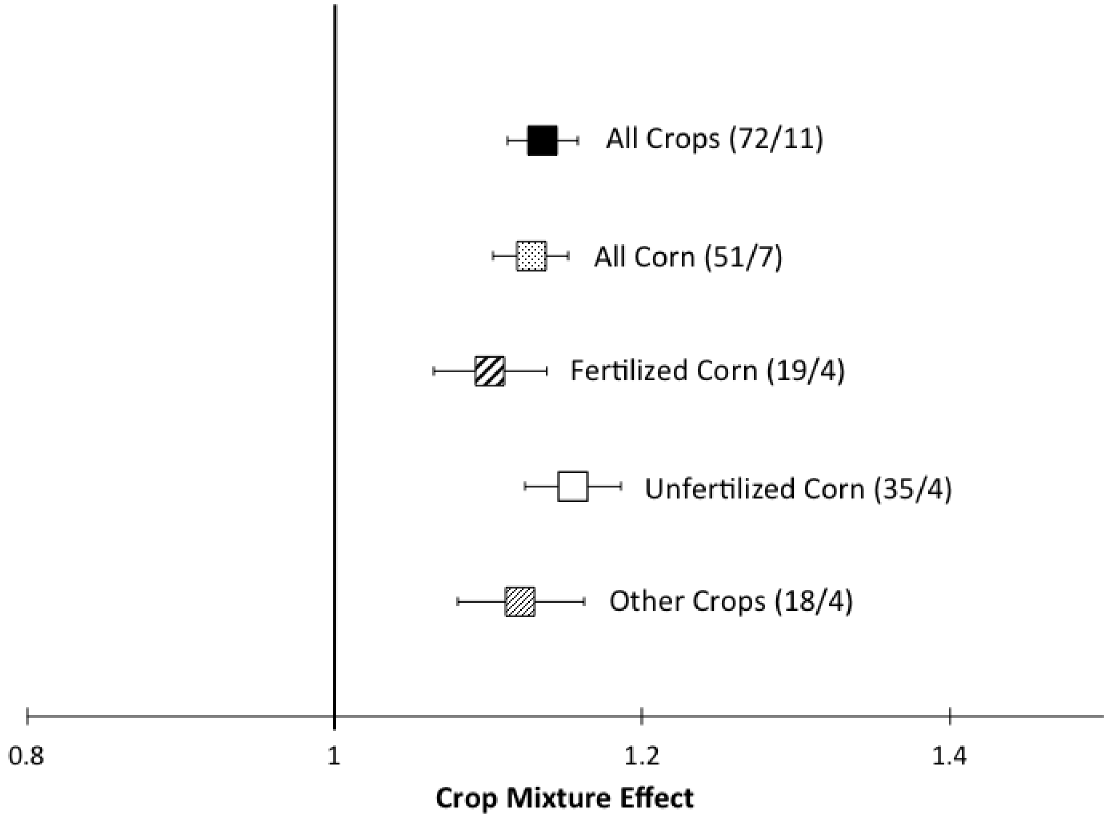

Figure 6. Mean crop mixture effect on yield of all crops, fertilized and unfertilized corn, and all other vegetable crops following a grass legume cover crop. Bars represent the standard error of the means. Numbers in parentheses are observations and studies included in the calculation (#Observations/#studies).

4.5 Discussion

Overyielding

Based on multiple approaches of calculating LER, there was a consensus among these studies that mixed cover crops of complimentary functional groups yield more biomass than the component monocultures by 14%. Comparing the LER of cover crop mixes and monocultures may introduce some variability when standardizing effects of different monoculture yields (Oyejola and Mead 1982). About half of the studies included in the synthesis (52%) use an additive approach to combining species in mixtures, resulting in mixtures being overseeded. The remaining studies use a replacement design that changed seed rates of each component species to maintain similar total plant density (Jolliffe, 2000). Ultimately, including both seed rate and yield to compare mixed and monoculture cover crops summarizes the mixture effect on individual species and functional group yield taking into account plant density. Regardless of the design of seed rates or calculation method, the LER > 1 suggests biculture and mixed species of cover crops yield more than the component monocultures, which we found to be the case in 67% of all observations. The method of calculating LER affected the variability within the results, but overall the conclusion from all methods (CLER, TLER and RLER) is that mixtures overyield from 9 to 20%.

Transgressive Overyielding

Transgressive overyielding is an alternative interpretation of yield comparisons in diverse natural ecosystems (Cardinale et al. 2007; Schmid et al. 2008) and in cover crop systems (Finney et al. 2015; Smith et al. 2014). Transgressive overyielding occurs when mixed cover crops produce more biomass than the single most productive species in the mixture. This study suggests that transgressive overyielding of mixtures occurs in about 60% of observations. When transgressive overyielding does not occur, grasses tend to be the functional group that yields more than the mixture because of high biomass productivity and competition for resources (Ranells and Wagger, 1997; Karpenstein-Machan and Stuelpnagel, 2000). Alternatively, another possible mechanism for grass monocultures yielding more than grass in mixtures may be related to greater reduction of grass seed rate in mixtures. Regardless, the majority of studies and this survey support mixtures overyielding, even when compared to the greatest yielding monocultures. As mixed cover crop systems are developed, an understanding of the complex interactions of functional groups can inform seeding rates and single or multiple species selection, especially when residue management is a concern.

Mixture Effect on Cover Crop

Results suggest facilitative and competitive relationships between grasses and legumes when grown in mixtures. While the mechanisms affecting cover crop yields in mixtures versus monocultures could not be analyzed in this synthesis, several individual studies have suggested grasses benefit from the transfer of fixed atmospheric N by a legume (Fujita et al. 1992), particularly in perennial forage systems. There may also be complementarity from the architecture of grass species, allowing for upward twining and increased growth of some legumes (Karpenstein-Machan and Stuelpnagel, 2000). However, our results suggest that mixtures and bicultures actually reduce the biomass of legumes when relative seed rates are considered. The reduction of legume biomass in mixtures across the synthesized studies may be a due to reduced legume emergence or reduced winter survival, depending on weather conditions and species (Creamer et al., 1997; Hayden et al. 2014). If disease or other pressures exist, the mixture effect of cover crop bicultures on the legume proportion may shift toward the positive (Snapp et al. 2004), as grass may facilitate legume production under more stressful conditions by reducing soil contact and moisture effects.

Crop Mixture Effects

Mixed cover crops that include legumes increased cash crop yield over all crop types and fertility conditions, compared to the predicted effect of component monocultures. In unfertilized corn, the effect of mixed cover crops on corn yield is likely correlated with residue quality, which would add N in residues and increase nutrient availability. Legume monocultures have lower

C:N ratio and mineralize more N than mixes with lower legume proportions or monoculture grasses (Ranells and Wagger 1996). The synthesis of corn yield results suggests legume monoculture produces the greatest yield, followed by the mixture, while the high C:N grasses may decrease yield with and without additional fertilization. In a recent meta-analysis that compared corn yield following legume, grass, and legume-grass bicultures to no cover controls, Miguez and Bollero (2005) conclude yield increased 37% with legume cover crop and 21% with grass-legume biculture. In the synthesis of fertilized corn studies presented here, we found a 10% increase in fertilized corn yield due to the mixed cover crop, which may be due to a rotation effect of cover crops and other changes in soil quality (Snapp et al. 2004), rather than the direct effect of mixtures on soil available N. The effect of mixed cover crops on yield of other, fertilized, crops showed species yield differences and a general decrease in benefits, compared to unfertilized crops. Generally, solanaceous crops had the greater mixture yield advantage than strawberry, broccoli or cotton, albeit sample size was small. The reduced benefit of mixed cover crops on fertilized, high-value cash crop yield is likely due to high fertilizer inputs.

4.6 Conclusions

Using Land Equivalent Ratios and cover crop and crop yield effects from cover crop mixtures, we can conclude that mixed cover crop systems tend to overyield by 10 to 20%, supporting the diversity-productivity hypothesis in cover cropping systems. In the majority of studies, mixture yield is greater than the most productive monoculture in the mixture, also known as transgressive overyielding, but variations in seeding rate effect the comparison. In cover crop systems, mixtures and bicultures increase the biomass of grasses and brassica cover crops, while legume biomass tended decrease. Cash crop yield is also variably affected by the application of mixed grass-legume cover crops, while the greatest increase in cash crop yield is produced following a monoculture legume cover crop. Overall mixed grass-legumes benefited all crops by increasing yield 13.5% on average, with variable effects on corn, potato, tomato, broccoli, strawberry and other crops under fertilized or unfertilized conditions. As interest in designing cover crop biculture and mixtures to provide specific ecosystem services continues, this information can be applied to maximize productivity. Understanding the complex interactions between mixed functional groups of cover crops and the effects on biomass quantity and quality can aid in species selection and relative seeding rates. Applying this information to management practices that prioritize other ecosystems services associated with cover crop biomass productivity, such as weed suppression, will also help farmers and researchers design efficient and sustainable mixed cover crop systems. These complex cover crop systems can be developed to increase other ecosystem services and agroecosystems functioning overall as research on individual cover crop species and mixtures continues.

Author	Year	Journal	# cover crop species	Cover crop species	Cash crop species
Abdul-Baki et al.	1997	Hort. Sci. 32:836	2	Millet & soybean	Broccoli
Benincasa et al.	2010	J. Sustain. Ag. 34:705	2	Field bean, vetch clover, rapeseed, barley, ryegrass	Corn
Brainard et al.	2011	Weed Tech. 25:473	2	Sorghum sudan, millet soybean, cowpea	None
Carrera et al.	2005	Amer. J. Potato Research 82:471	2	Rye, rape, crimson clover	Creamer potato
Clark et al.	1994	Agron. J. 86:1065	2	Rye & hairy vetch	Corn
Clark et al.	1997	Agron. J. 89:427	2	Rye & hairy vetch	None
Finney et al.	2015	Agron. J. 108:39	8	Mixed from 12 sps. grass, brassicas, legumes	None
Garland et al.	2011	Hort. Sci. 46:985	2	Sorghum sudan, millet velvetbean, soybean	Strawberry
Halde et al.	2014	Agron. J. 106:1193	4	Barley, pea, vetch	None
Hayden et al.	2014	Agron. J. 106:904	2	Rye & hairy vetch	None
Hodgdon et al.	2016	Agron. J. 108:1624	2	Rye & hairy vetch	None
Karpenstein-Machan and Stuelpnagel	2000	PlantSoil 218:215	2	Rye, winter pea, crimson clover	None
Kuo and Jellum	2002	Agron. J. 94:501	2	Rye, ryegrass, vetch	Corn
Mariotti et al.	2009	Grass Forage Sci. 64, 401	2	Barley, white lupine	None
Moschler et al.	1967	Agron. J. 59:547	2	Ryegrass, oat, crimson	None
Odhiambo and Bomke	2001	Agron. J. 93:299	2	Wheat, rye, ryegrass clover	None
Poffenbarger et al.	2015	Agron. J. 107:2069	2	Rye & hairy vetch	None
Ranells and Wagger	1996	Agron. J. 88:777	2	Rye & crimson	None
Restovich et al.	2012	Field Crops Research 128:62	2	Oats & common vetch	Corn & soybean
Sainju et al.	2005	Agron. J. 97:1403	2	Rye & hairy vetch	Cotton & sorghum
Smith et al.	2014	PLos1 e97351	5	Buckwheat, rye, vetch, pea, SXS, mustards	None
Sullivan et al.	1991	Amer. J. Alt. Ag. 6:106	2	Rye & hairy vetch	None
Teasdale and Abdul-Baki	1998	Hort. Sci. 33: 1163	3	Rye, crimson, vetch	None
Tosti et al.	2012	Euro. J. Agron. 43:136	2	Barley & hairy vetch	Corn & tomato
Tribouilllois et al.	2016	Plant Soil 401:347	2	10 sps grass, legume, brassica, tansy	None
Vaughan and Evanylo	1998	Agron. J. 90:536	2	Rye & hairy vetch	Corn
Vaughan et al.	2000	Commun. Soil Sci. Plant Anal. 31:1017	2	Rye, wheat, hairy vetch	Corn
Wang et al.	2015	JSustainableAg. 36:423	2	Mixes of summer and winter legume and grass	None
Wayman et al.	2014	Renew. Ag. Food. Syst. 30:450	2	Barley& hairy vetch	None
Wortman et al.	2012	Agron. J. 104:699	8	Vetch, mustards, pea, clover, radish, rape	None
Zhou et al.	2012	Applied Soil Ecol. 53:49	2	Rye & hairy vetch	None

Table 1. List of cover crop mixture studies used in the analysis, the number and species in mix and cash crop species.

Author	Experimental Years	Location	LER	Mixture Effect	Hedge's D
Abdul-Baki et al.	1995	MD, VA	CLER	Y	N
Benincasa et al.	2002-2003	Italy	TLER	N	N
Brainard et al.	2005-2006	NY	CLER	Y	Y
Carrera et al.	2000	MD, VA	TLER	N	N
Clark et al.	1989	MD	TLER	N	N
Clark et al.	1990-1991	MD	TLER	N	N
Finney et al.	2011-2012	PA	CLER	N	N
Garland et al.	2007-2008	NC	CLER	Y	N
Halde et al.	2010-2012	Manitoba	CLER	Y	N
Hauggaard-Nielsen et al.	1998 -2000	Denmark	None	N	Y
Hayden et al.	2010-2011	MI	RLER	N	Y
Hodgdon et al.	2011-2012	NH	TLER	N	N
Karpenstein-Machan and Stuelpnagel	1990-1994	Germany	RLER, CLER	Y	N
Kuo and Jellum	1994-1998	WA	TLER	N	N
Mariotti et al.	2003-2004	Italy	RLER	N	N
Moschler et al.	1964	VA	TLER	N	N
Odhiambo and Bomke	1994-1995	BC	TLER	N	N
Poffenbarger et al.	2011-2012	MD	CLER	Y	N
Ranells and Wagger	1993-1994	NC	CLER	Y	N
Restovich et al.	2005-2010	Argentina	TLER	N	N
Sainju et al.	2000-2002	GA	TLER	N	N
Smith et al.	2011-2012	NH	RLER	N	N
Sullivan et al.	1988-1989	VA	TLER	N	N
Teasdale and Abdul-Baki	1995-1997	MD	TLER	N	N
Tosti et al.	2006-2007	Italy	CLER	Y	Y
Tribouilllois et al.	2012	France	None	N	N
Vaughan and Evanylo	1992-1993	VA	CLER	Y	N
Vaughan et al.	1995	NC	LER	Y	N
Wang et al.	2007-2008	FL	CLER	N	N
Wayman et al.	2012-2013	WA	TLER	N	N
Wortman et al.	2010-2011	NE	RLER	N	N
Zhou et al.	2009	Australia	TLER	N	N

Table 2. Included studies by first author, experimental year, location and calculations.

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CHAPTER 5 Summary

Environmental concerns and a growing global population necessitate increased agricultural production with reduced environmental impacts. Cover crops, originally implemented to reduce erosion, are currently being managed to provide a range of ecosystem services. Cover crops can mitigate nutrient losses and increase soil organic matter and nutrients. Complex cover crop species mixes may not be necessary if agroecosystems can be designed to exploit functional differences and target specific ecosystem services.

Cover crops supplement inorganic N and conserve nutrients from fall-applied poultry litter. Coupling C and N in organic inputs may tighten nutrient cycling and create a “slow-release fertilizer” (McSwiney et al. 2010). When legume cover crops and poultry litter are coupled there is the potential to replace about 100 kg N ha^{-1}, based on the FNEQ values, which increased over the three-year study. Legume-containing biculture also provided substantial fertilizer credit to corn by the end of the study. In contrast, Rye and RyePL resulted in zero or negative FNEQ in the 3 years of the study, and reduced corn yield slightly below the winter fallow control treatment.

In choosing cover crop species and poultry litter combinations, there are tradeoffs of ecosystem services. Bicultures overyield compared to monocultures, are not consistently affected by poultry litter, result in similar N availability compared to legume, and suppress weeds as effectively as rye. Nitrogen yield and C:N of legume is improved by poultry litter application, and legume residues result in greater soil mineral N concentrations, but more weed pressure. Poultry litter increased rye biomass, but not N content, and rye retains soil N, is most effective at suppressing weeds, but may decrease corn productivity without additional fertilizer input.

Land Equivalent Ratios synthesized from over 200 observations corroborate results from several individual studies that show biculture and mixed species overyield compared to monoculture component species. Our results suggest a range of increase of 10 to 20% in cover crop yield. Sixty percent of the time, the overyielding is transgressive and the most productive monoculture does not yield as much as the mix. Individual functional groups yield variably in mixes; grasses and brassica cover crop yield increase by 30 to 60%, while legume biomass may decrease by 10%. Cash crop yield is also variably affected by the application of mixed cover crops. Overall mixed cover crop benefited all crop yield by 8 to 18%, with variable effects on corn, potato, tomato, broccoli, strawberry and other crops under fertilized or unfertilized conditions. As interest in biculture and mixed cover crops continues, this information can aid in cover and cash crop species selection to maximize productivity.

The use of cover crops as a tool to manage for ecosystem services fits within the goals of sustainable, conservation, and organic agriculture production. Coupling cover crops with poultry litter in fall is one method of waste management that may reduce nutrient losses. Further exploration of the effects and diversity of mixtures on individual cover crop species residue quality and quantity, with and without fall-applied poultry litter, and in different seasons, soil types, and climatic conditions will allow for site-specific agroecosystems design that prioritizes particular ecosystem services.

Appendix A. Additional Figures and Tables

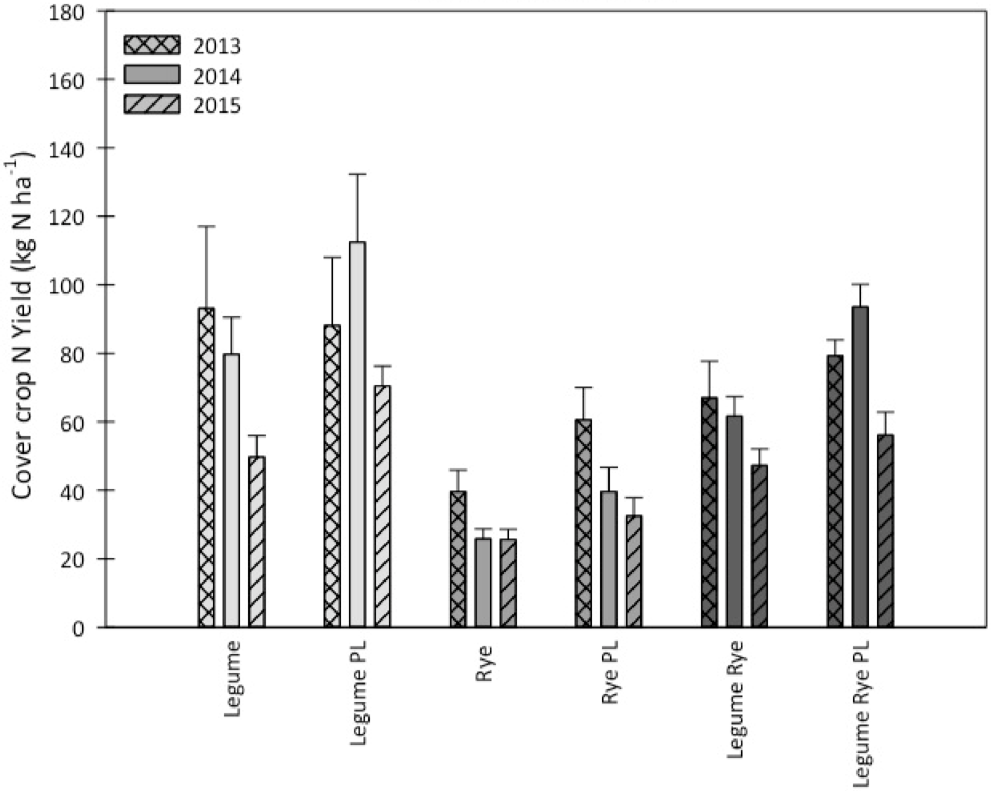

FIGURE 1 – TOTAL COVER CROP N YIELD AT FINAL HARVEST Average final total N yield of each of the six cover crop treatments (kg N ha^{-1}), including combined species treatments with standard error. Years are distinguished by bar textures for 2013 (cross hatch), 2014 (none) and 2015 (diagonal lines).

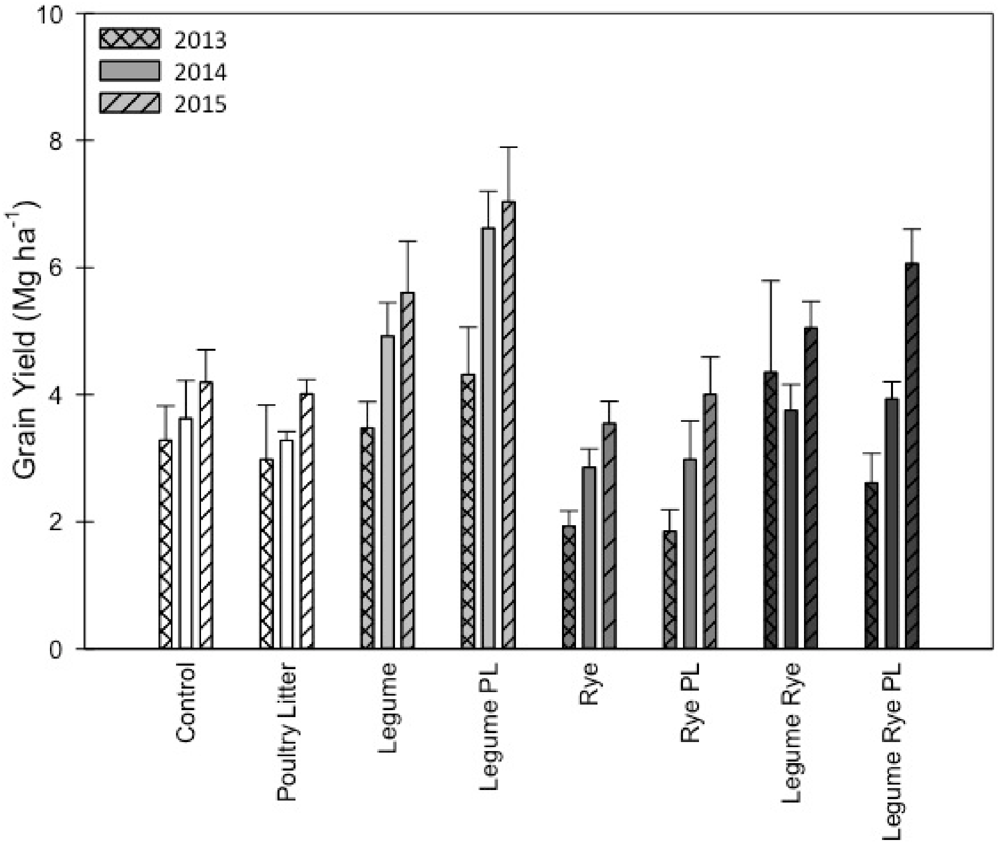

FIGURE 2- GRAIN YIELD. Average grain yield at 15.5% moisture and standard errors for all treatments including the cover crop and poultry litter combinations, the no cover/ no poultry litter control, and the no cover poultry litter control. Years are distinguished by bar textures for 2013 (cross hatch), 2014 (none) and 2015 (diagonal lines).

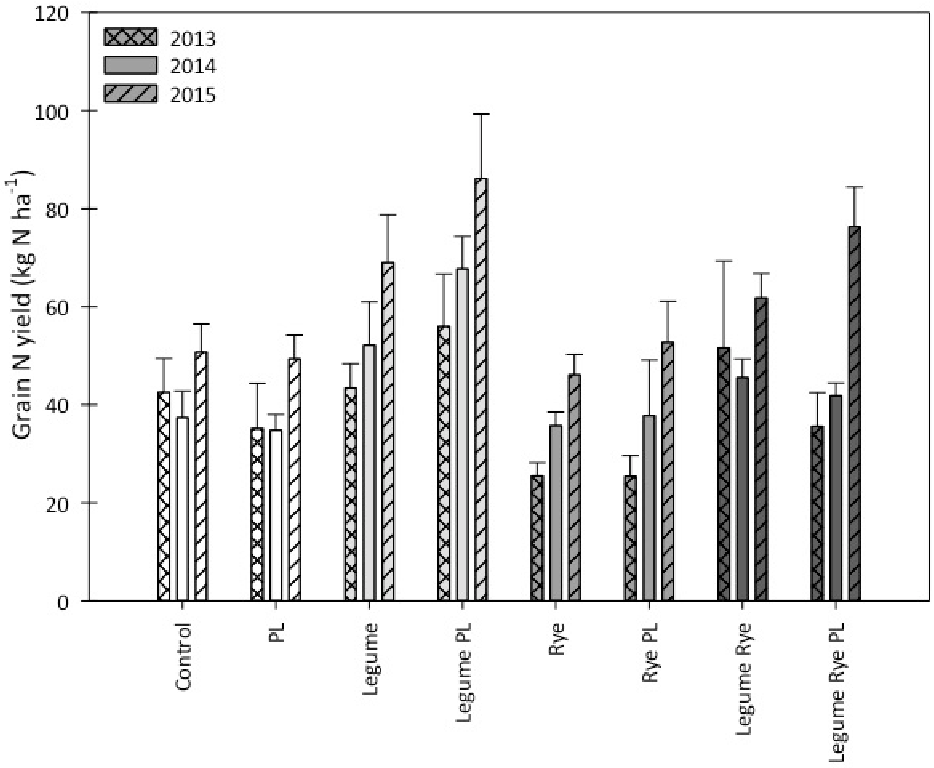

FIGURE 3- GRAIN N CONTENT Average grain N yield at 15.5% moisture and standard errors for all treatments including the cover crop and poultry litter combinations, the no cover/ no poultry litter control, and the no cover poultry litter control. Years are distinguished by bar textures for 2013 (cross hatch), 2014 (none) and 2015 (diagonal lines).

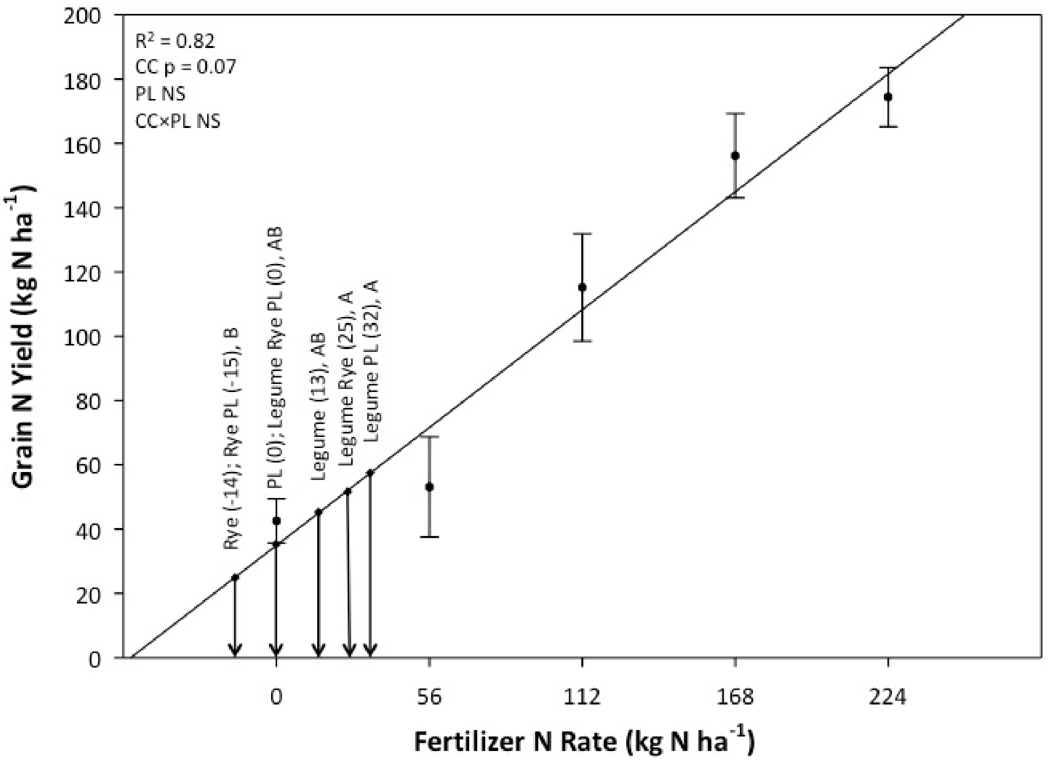

FIGURE 4- 2013 FERTILIZER EQUIVALENCE BASED ON GRAIN N YIELD
FEQ for each treatment is labeled with the calculated average value in parentheses and the following letter representative of LSD mean separation, based on grain N yield. R^2 of regression is shown and the p-value of treatment effects based on grain N is given. CC represents cover crop effect, PL poultry litter and CC*PL is the cover crop and poultry litter interaction. Significance is indicated by NS for not significant or p value.

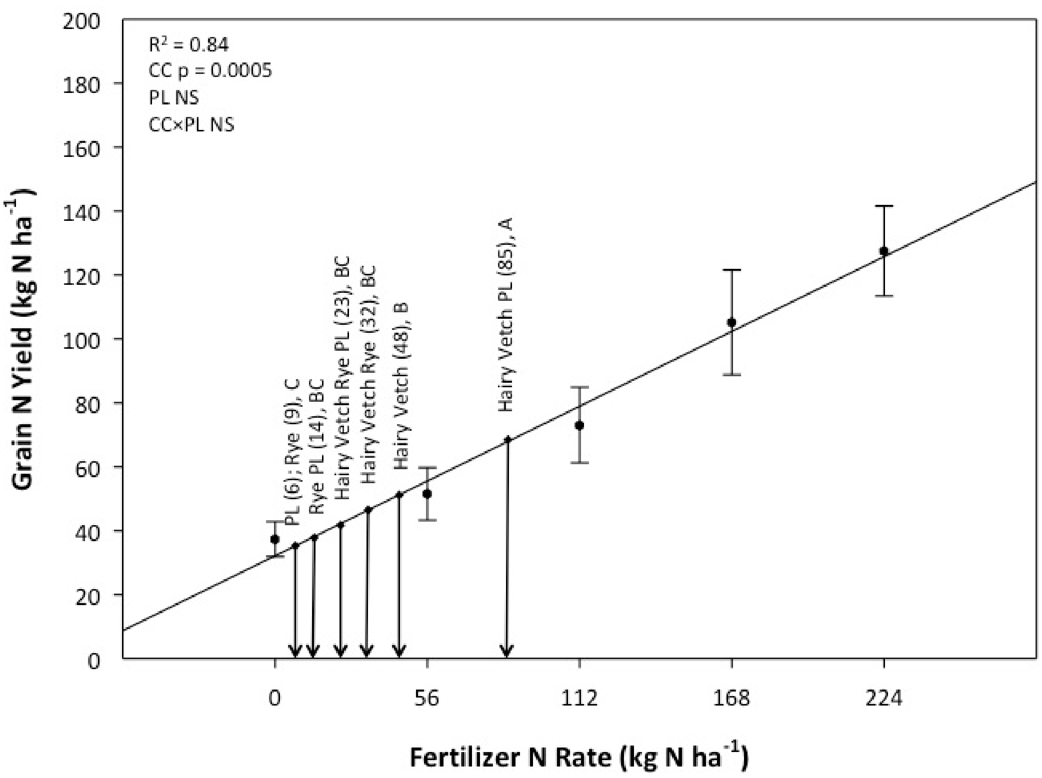

FIGURE 5 – 2014 FERTILIZER EQUIVALENTS BASED ON GRAIN N YIELD
FEQ for each treatment is labeled with the calculated average value in parentheses and the following letter representative of LSD mean separation, based on grain N yield. R^2 of regression is shown and the p-value of treatment effects based on grain N is given. If treatment factor is not listed the effect was not significant. CC represents cover crop effect, PL poultry litter and CC*PL is the cover crop and poultry litter interaction. Significance is indicated by NS for not significant or p value.

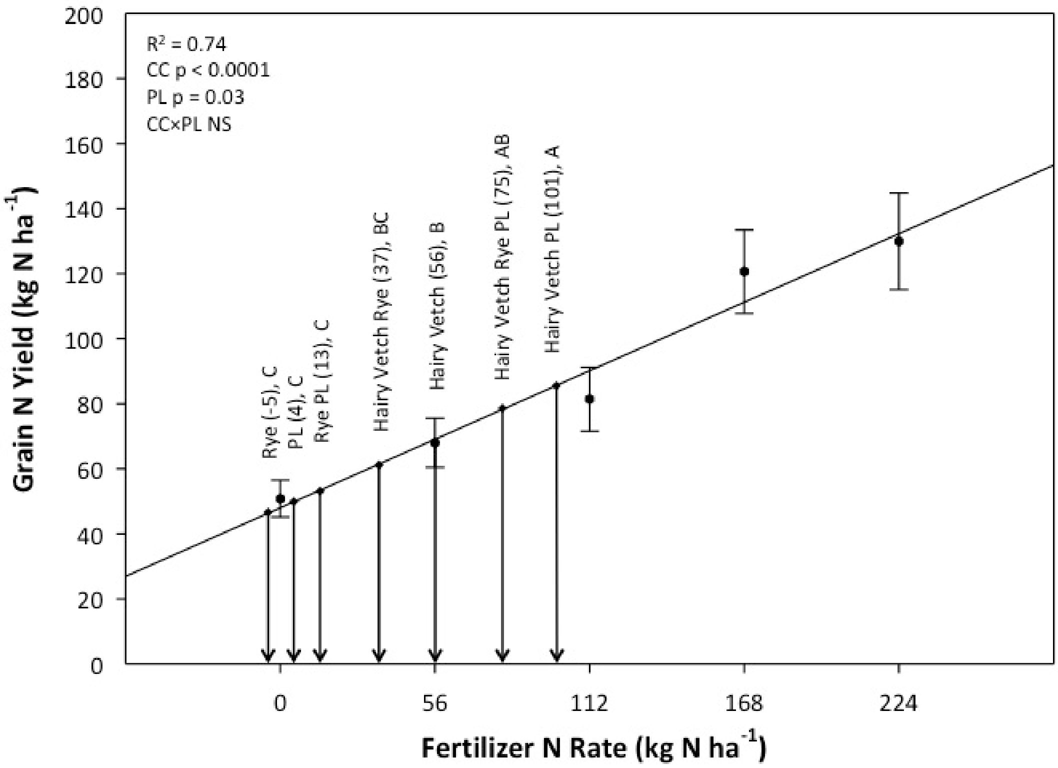

FIGURE 6- 2015 FERTILIZER EQUIVALENTS BASED ON GRAIN N YIELD
FEQ for each treatment is labeled with the calculated average value in parentheses and the following letter representative of LSD mean separation, based on grain N yield. R^2 of regression is shown and the p-value of treatment effects based on grain N is given. If treatment factor is not listed the effect was not significant. CC represents cover crop effect, PL poultry litter and CC*PL is the cover crop and poultry litter interaction. Significance is indicated by NS for not significant or p value.

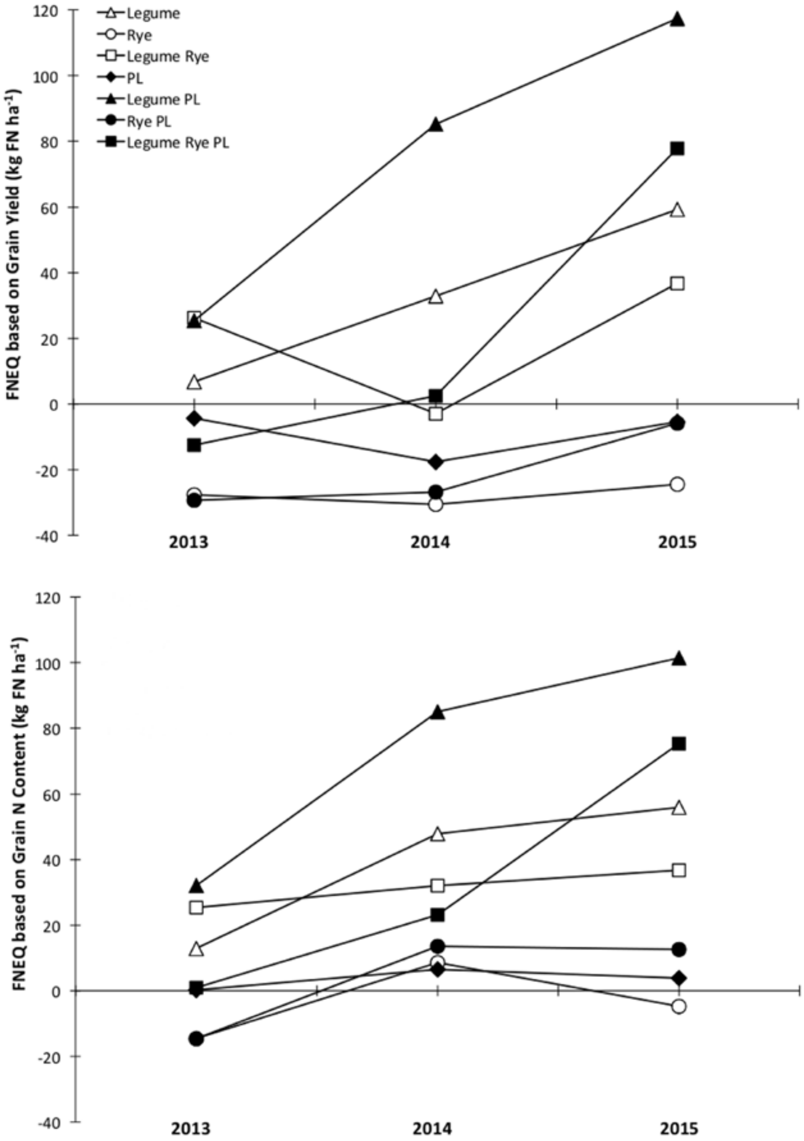

FIGURE 7- FNEQ VALUES
Average fertilizer N equivalence based on grain yield and grain N content calculations for cover crop × poultry litter treatments over the 3 year study period

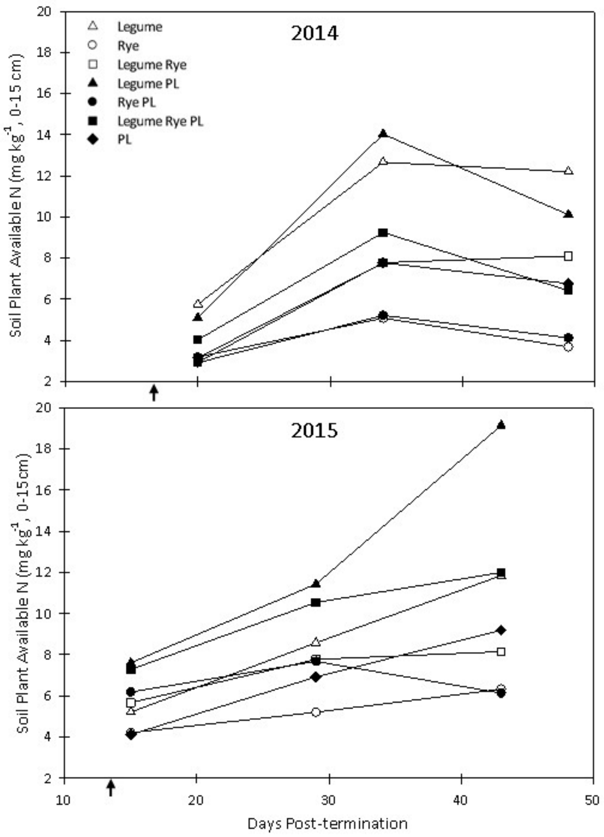

FIGURE 8 -SOIL MINERAL N (NO3- and NH4+ upper 15cm)
Combined nitrate and ammonium in upper 15 cm of soil profile on three sampling days after cover crop termination. Arrow represents time of corn planting.

TABLE 1 – Corn N content

The average grain N, stover N and total plant N for each treatment are given for the 1-meter harvest at black layer. Upper case letter indicate significant differences among treatment means.

Treatment	**2014**			**2015**		
	1M Grain N (kg N ha^{-1})	**1M Stover N (kg N ha^{-1})**	**1M Total N (kg N ha^{-1})**	**1M Grain N (kg N ha^{-1})**	**1M Stover N (kg N ha^{-1})**	**1M Total N (kg N ha^{-1})**
Legume	45.3 AB	26.2 B	71.4 B	70.3 B	36.6 BC	106.9 B
Rye	30.2 BC	17.3 C	47.5 D	41.6 D	22.0 E	63.6 D
Legume Rye	37.8 BC	24.4 BC	62.3 BCD	64.3 BC	32.9 BCD	97.2 BC
Legume PL	60.0 A	35.4 A	95.4 A	40.9 A	45.1 A	138.4 A
Rye PL	31.0 BC	22.0 BC	53.0 BCD	93.3 CD	29.1 CDE	79.5 CD
Legume Rye PL	40.4 BC	27.8 B	68.2 BC	50.4 AB	39.1 AB	117.5 AB
PL	26.0 C	23.8 BC	49.8 CD	78.4 D	28.1 DE	69.0 D

TABLE 2 The average grain N, stover N and total plant N for each N rate treatment (Ammonium Nitrate) are given for the 1-meter harvest at black layer. Upper case letter indicate significant differences among treatment means using LSD.

	2014			2015		
Treatment	**1M Grain N (kg N ha^{-1})**	**1M Stover N (kg N ha^{-1})**	**1M Total N (kg N ha^{-1})**	**1M Grain N (kg N ha^{-1})**	**1M Stover N (kg N ha^{-1})**	**1M Total N (kg N ha^{-1})**
0 N	30.0 (6.3) C	24.3 (2.0) D	54.2 (8.2) C	56.8 (5.7) C	30.8 (1.3) C	87.7 (6.6) C
56 N	41.8 (4.7) BC	36.4 (4.3) C	78.2 (6.0) C	66.2 (3.2) C	33.8 (1.9) BC	100.0 (4.8) C
112 N	67.8 (10.9) B	45.6 (3.9) BC	113.4 (14.4) B	84.5 (6.9) BC	41.3 (3.0) B	125.8 (9.8) BC
168 N	102.7 (15.1) A	54.8 (5.3) AB	157.5 (17.4) A	115.8 (13.9) AB	51.7 (5.1) A	167.5 (17.3) AB
224 N	120.9 (15.2) A	61.0 (5.8) A	181.9 (18.2) A	125.5 (18.1) A	54.3 (6.4) A	179.8 (24.0) A

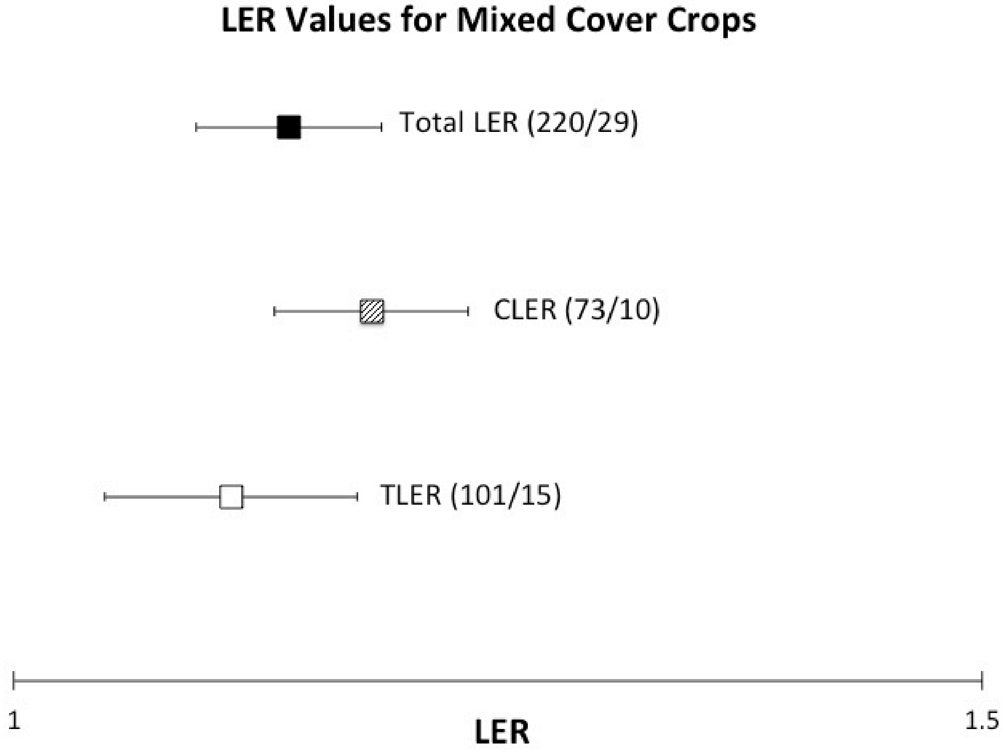

FIGURE 9. Land Equivalence Ratio (LER) of multiple species cover crops including Total LER, CLER, TLER. Bars represent the 95% confidence interval of the means. Numbers in parentheses are observations and studies included in the calculation (#Observations / #studies).

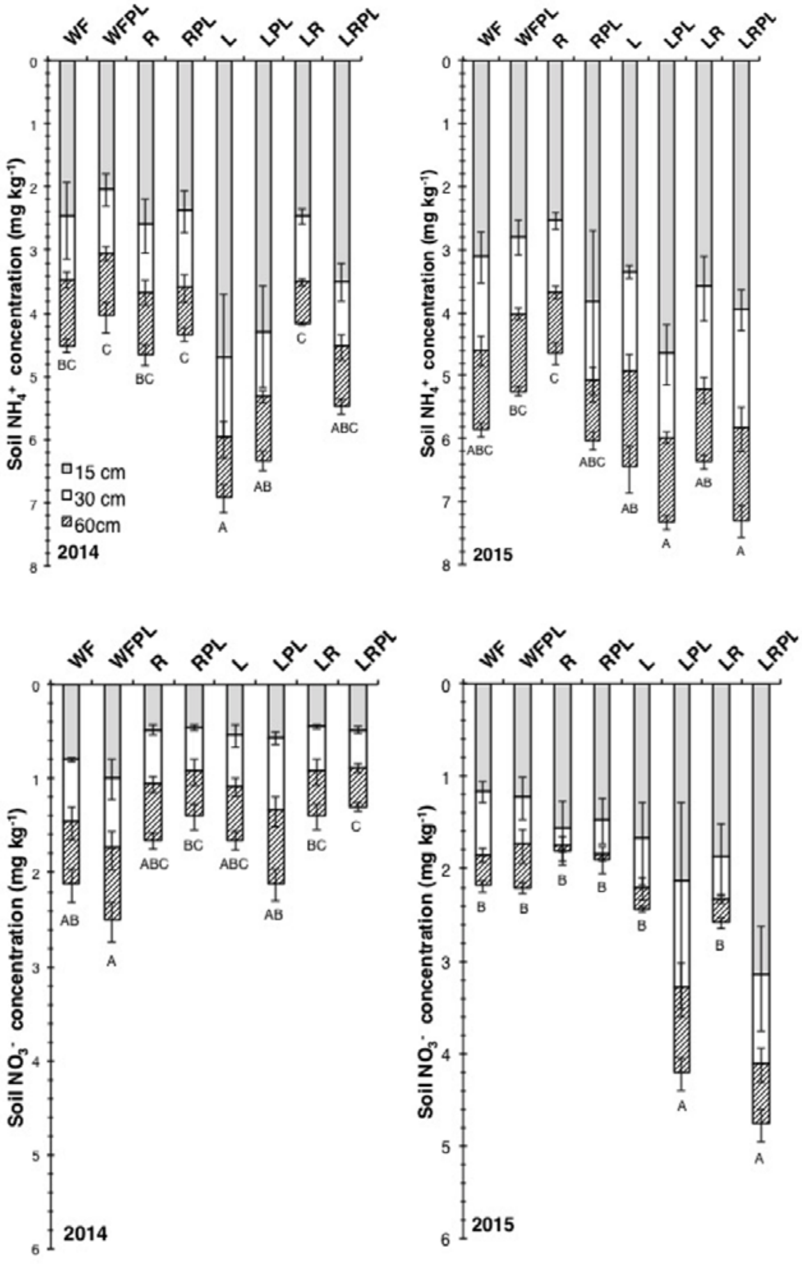

FIGURE 10. Soil ammonium and nitrate concentrations from 0 – 15 cm, 15 – 30 cm, and 30 – 60 cm, two weeks after cover crop termination in 2014 and 2015 cover crop × poultry litter treatments. Treatments are represented by WF = winter fallow, WFPL = winter fallow with fall-applied poultry litter, R = Rye, RPL = Rye poultry litter, L = Legume, LPL = Legume poultry litter, LR = Legume Rye, LRPL = Legume Rye poultry litter. Bars represent the back-transformed mean for 0 to 15 cm depth- light grey, 15 to 30 cm- white bars, and 30 to 60 cm – diagonal lined grey. Error bars represent the back-transformed standard error interval for each depth. Letters represent mean LSD mean separation of total soil ammonium and nitrate from 0 to 60 cm depth within each year.

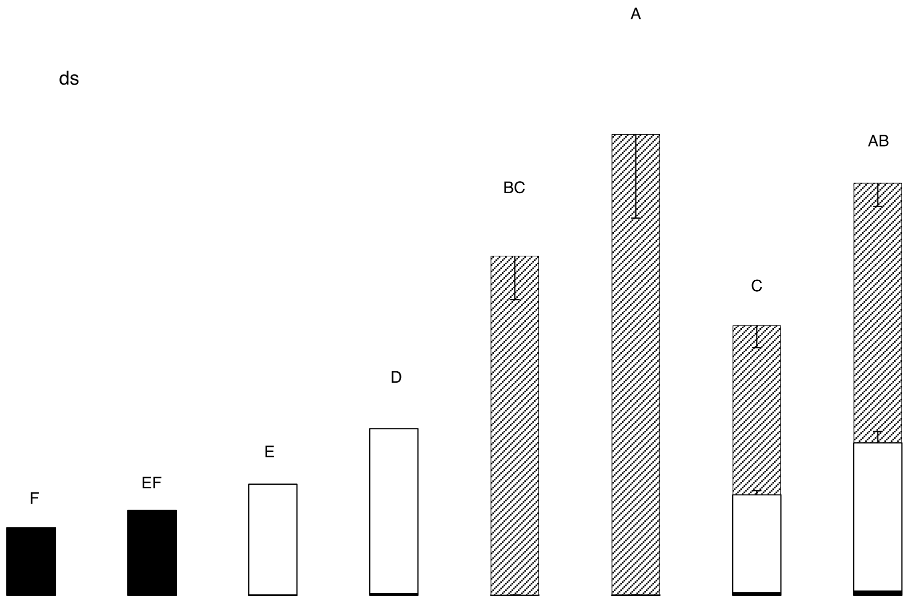

FIGURE 11. Total plant yield of cover crop × poultry litter treatments for 2014. Rye N content is represented by white bars, Legume N content is grey and Weed N content is black. Error bars represent the back-transformed standard error interval for each plant species component. Letters represent mean LSD mean separation of total treatment N content.

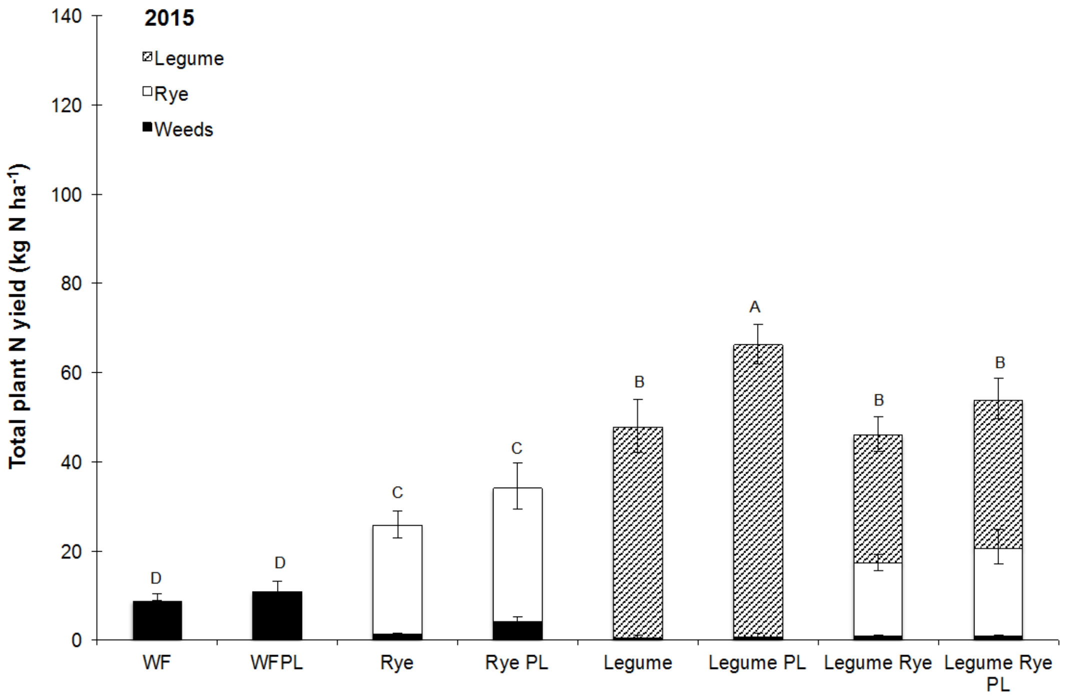

FIGURE 12 Total plant yield of cover crop × poultry litter treatments for 2015. Rye N content is represented by white bars, Legume N content is grey and Weed N content is black. Error bars represent the back-transformed standard error interval for each plant species component. Letters represent mean LSD mean separation of total treatment N content.

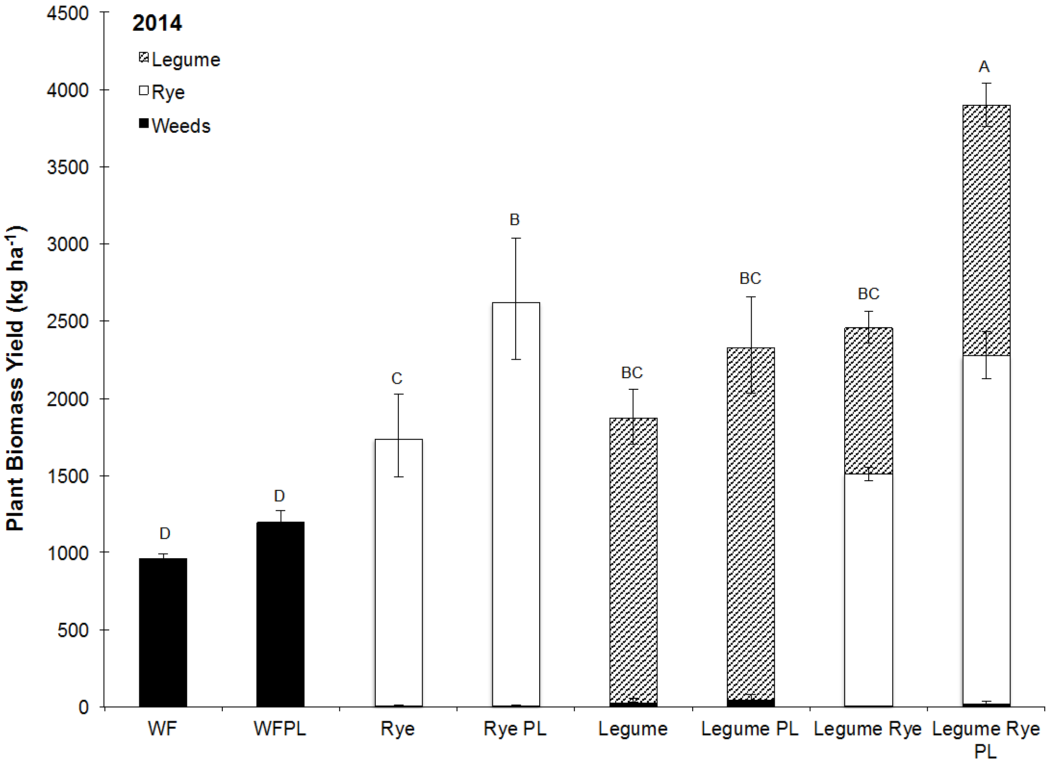

FIGURE 13 Total plant biomass yield of cover crop × poultry litter treatments for 2014. Rye N content is represented by white bars, Legume N content is grey and Weed N content is black. Error bars represent the back-transformed standard error interval for each plant species component. Letters represent mean LSD mean separation of total treatment biomass.

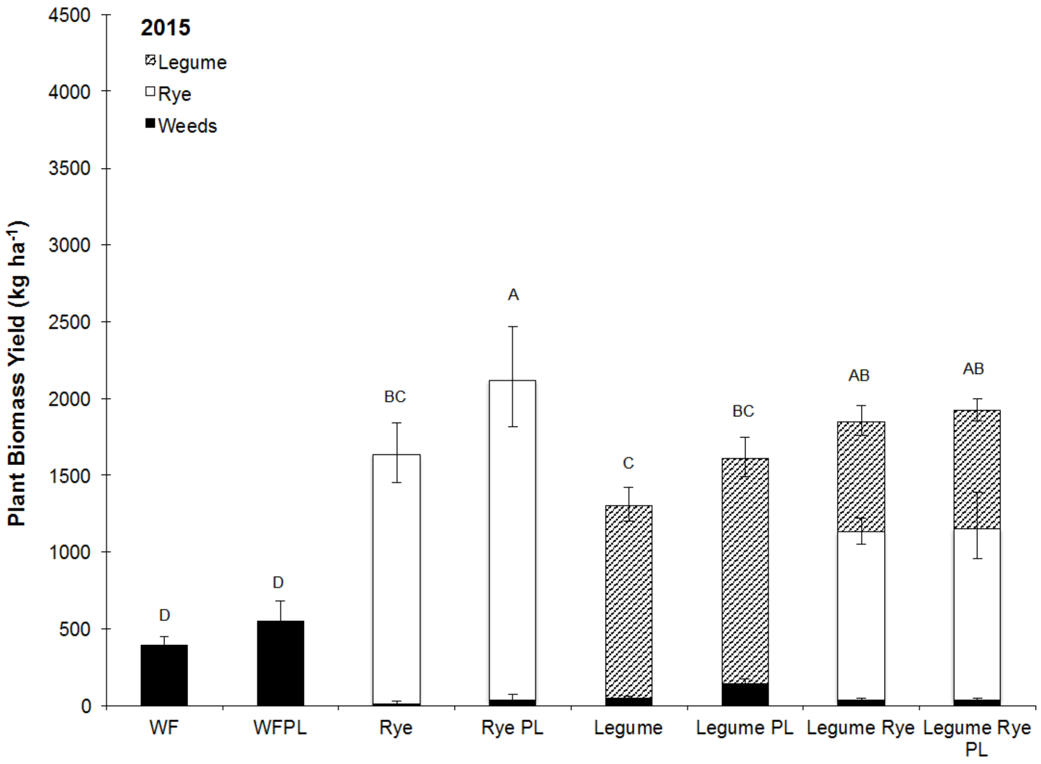

FIGURE 14. Total plant biomass yield of cover crop × poultry litter treatments for 2014. Rye N content is represented by white bars, Legume N content is grey and Weed N content is black. Error bars represent the back-transformed standard error interval for each plant species component. Letters represent mean LSD mean separation of total treatment biomass.

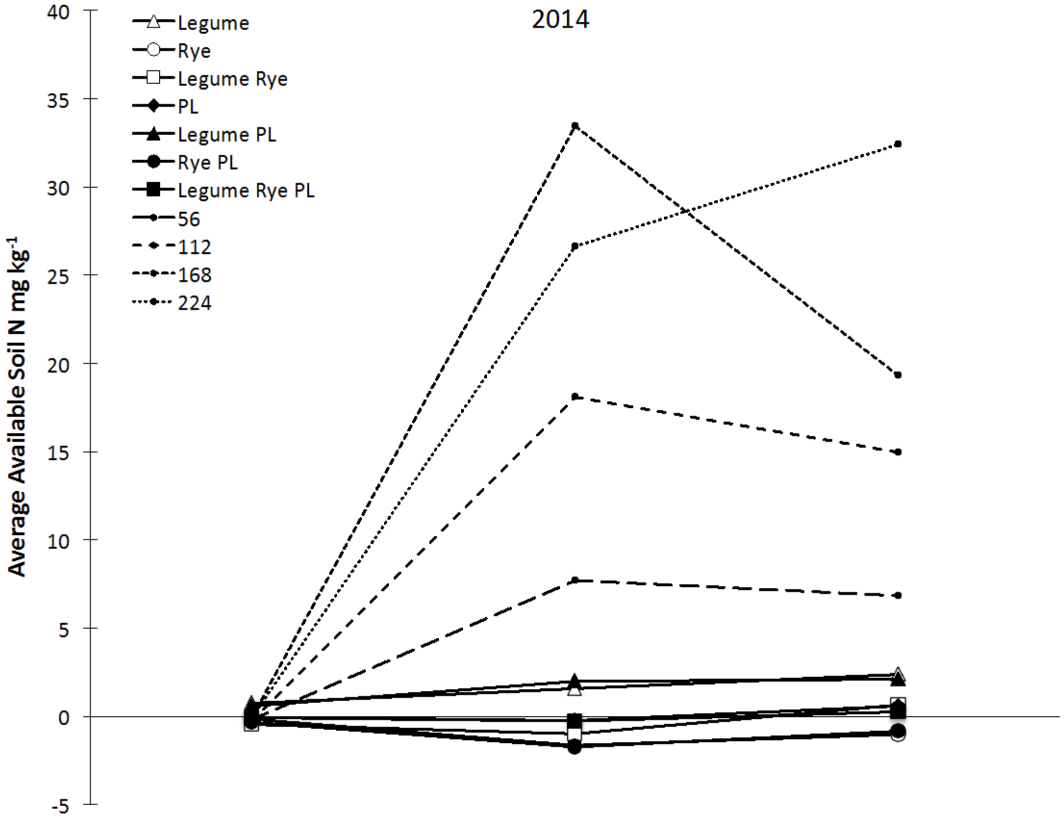

FIGURE 15. Average extractable soil N due to cover crop × poultry litter treatments and fertilizer N for 2014, at 0, 14, and 28 days post-termination.

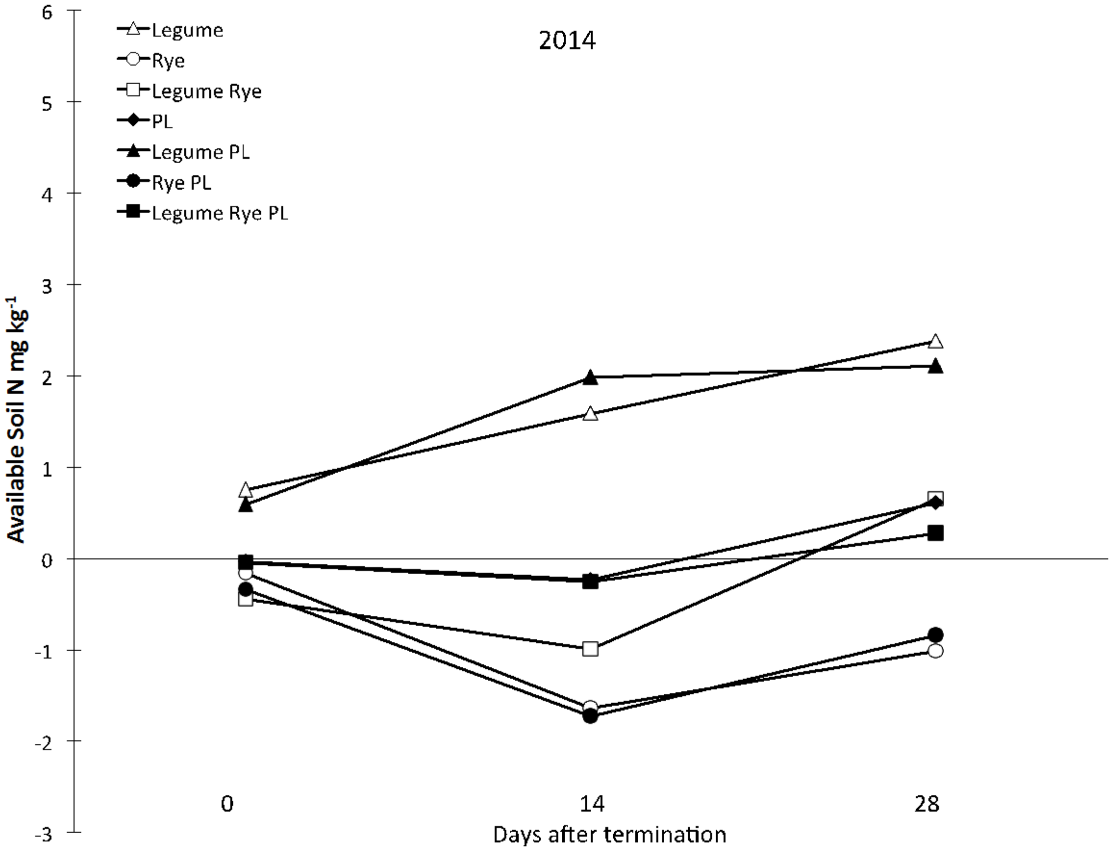

FIGURE 16. Average extractable soil N due to cover crop × poultry litter treatments for 2014, at 0, 14, and 28 days post-termination.

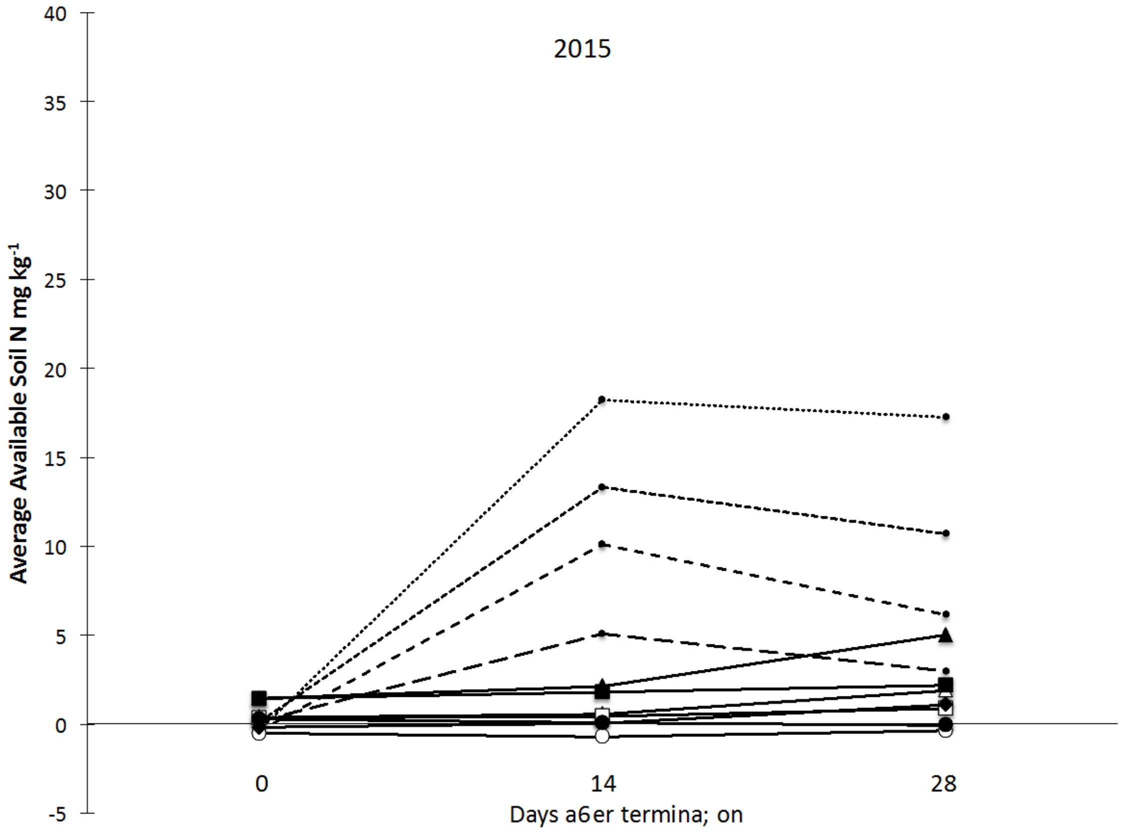

FIGURE 17. Average extractable soil N due to cover crop × poultry litter treatments and fertilizer N for 2015, at 0, 14, and 28 days post-termination.

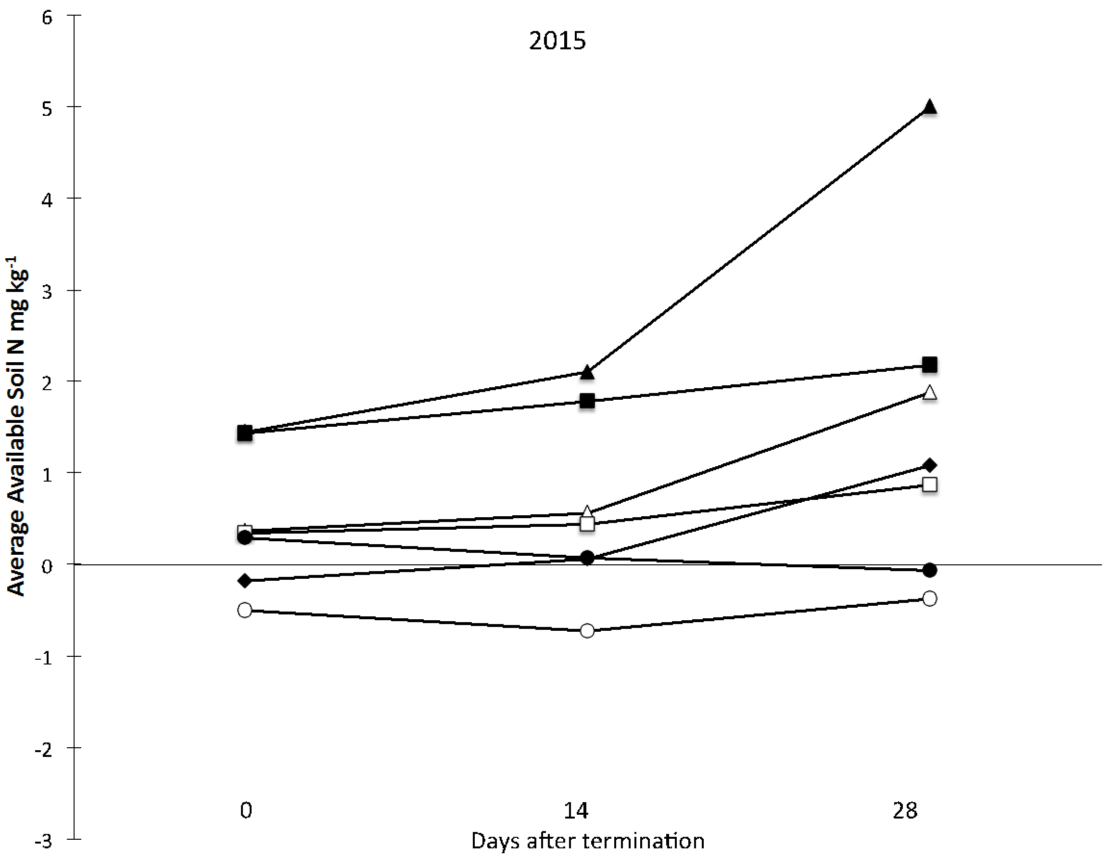

FIGURE 18. Average extractable soil N due to cover crop × poultry litter treatments for 2015, at 0, 14, and 28 days post-termination.

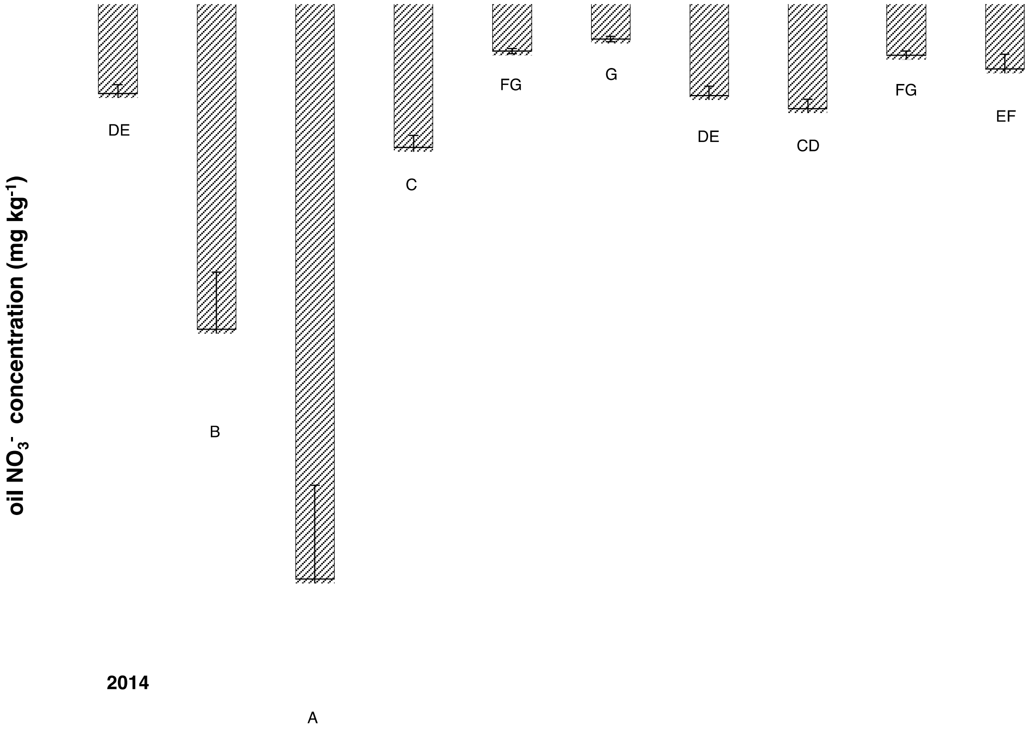

FIGURE 19. Soil nitrate concentration at 15 cm depth for cover crop × poultry litter treatments and fertilizer treatments of 56 and 112 kg N ha^{-1} in 2014.

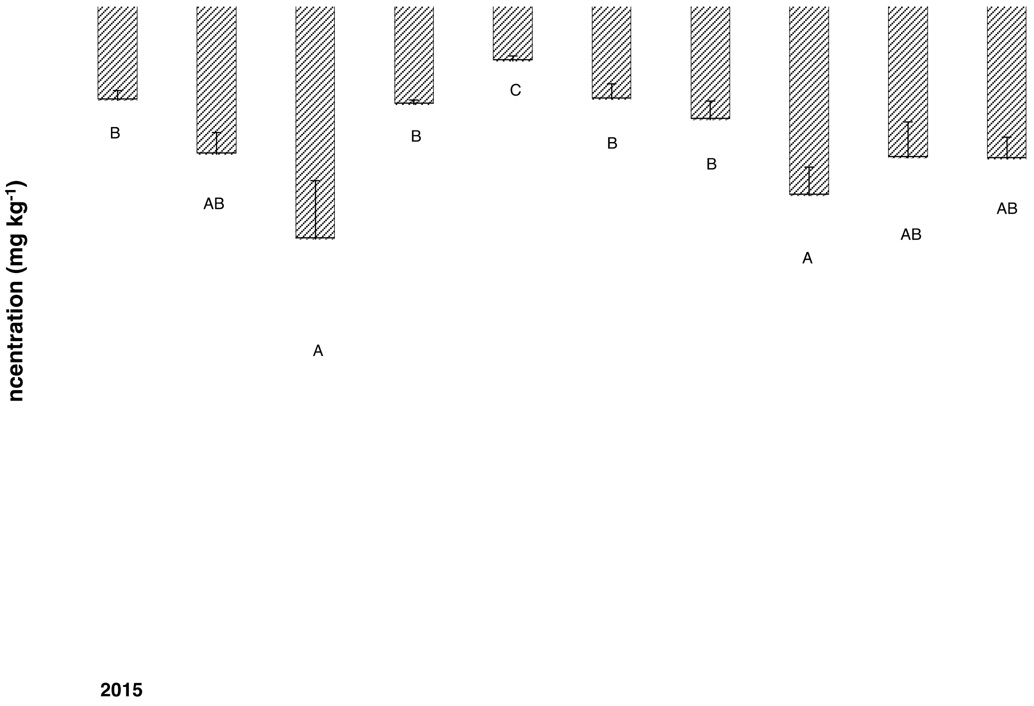

FIGURE 20. Soil nitrate concentration at 15 cm depth for cover crop × poultry litter treatments and fertilizer treatments of 56 and 112 kg N ha^{-1} in 2015.

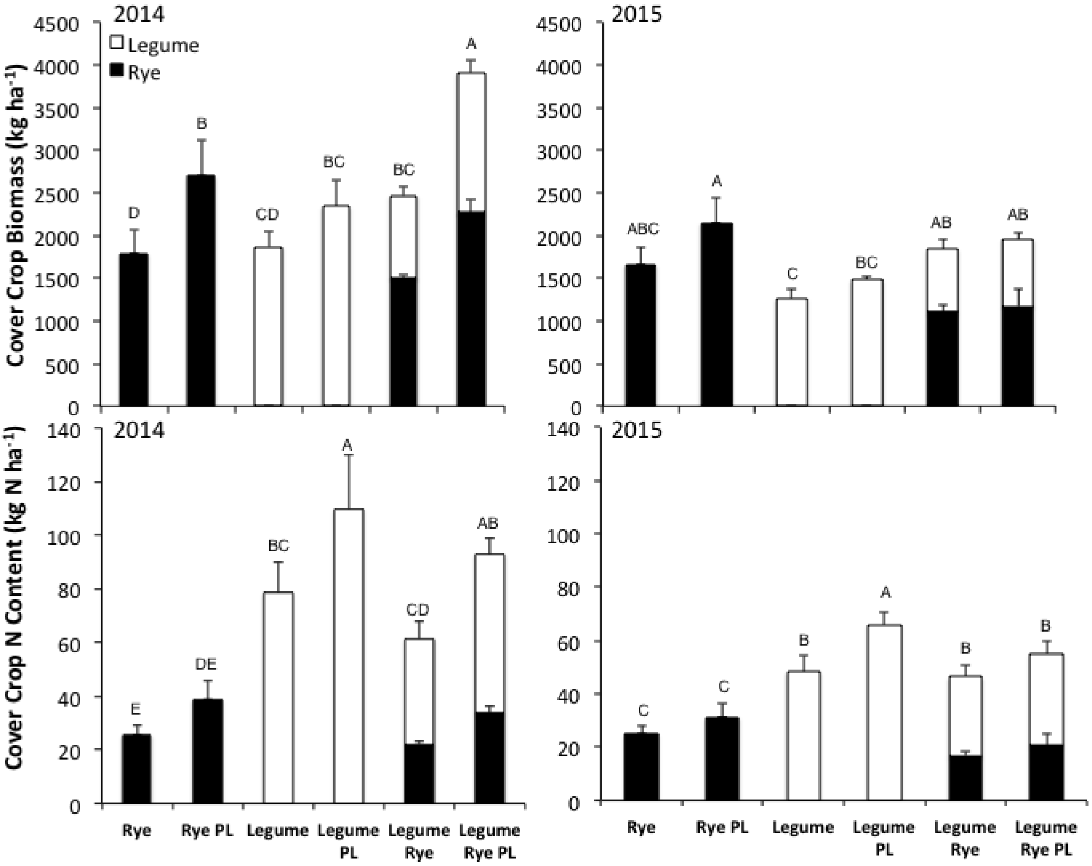

FIGURE 21 - Plant above-ground biomass (kg ha^{-1}) and N content yield (kg N ha^{-1}) of cover crop × poultry litter treatments, separated by species. Bars represent the back-transformed means for rye (black) and legume (white). Error bars represent the back-transformed standard error for each species component. Letters represent LSD mean separation of total treatment biomass and N content within years.

TABLE 3. Cover crop and weed residue C:N, and Land equivalent ratio (LER) and Nitrogen LER (NLER) of biculture cover crop treatments, and mixture effects of cover crop × poultry litter treatments for 2014 and 2015.

Treatment	**Legume**	**Rye**	**Biculture**		**LegumePL**	**RyePL**	**BiculturePL**		**PL**	**Control**
Plant	**Legume**	**Rye**	**Legume**	**Rye**	**Legume**	**Rye**	**Legume**	**Rye**	**Weeds**	**Weeds**
2014										
C:N	11.0 (0.6) AB	31.6 (1.0) NS	11.3 (0.6) AB	31.0 (1.0) NS	9.8 (0.7) B	32.1 (0.8) NS	12.6 (0.1) A	30.8 (1.0) NS	24.9 (0.6)	25 (1)
LER			1.38				1.58			
NLER			1.38				1.46			
Mixture effects			1.03	1.74			1.43	1.72		
2015										
C:N	11.9 (0.6) A	30.6 (0.8) NS	11.3 (0.3) AB	31.0 (1.9) NS	10.1 (0.2) B	32.3 (2.1) NS	10.5 (0.5) AB	26.3 (1.1) NS	22.1 (0.7)	19.4 (0.7)
LER			1.25				1.07			
NLER			1.28				1.19			
Mixture Effects			0.70	1.58			0.64	1.30		

Letters represent LSD mean separation of C:N of component plant tissues within the treatments (i.e. Legume residue C:N in monoculture and Legume residue C:N in biculture with and without PL. No significant difference is represented by NS.

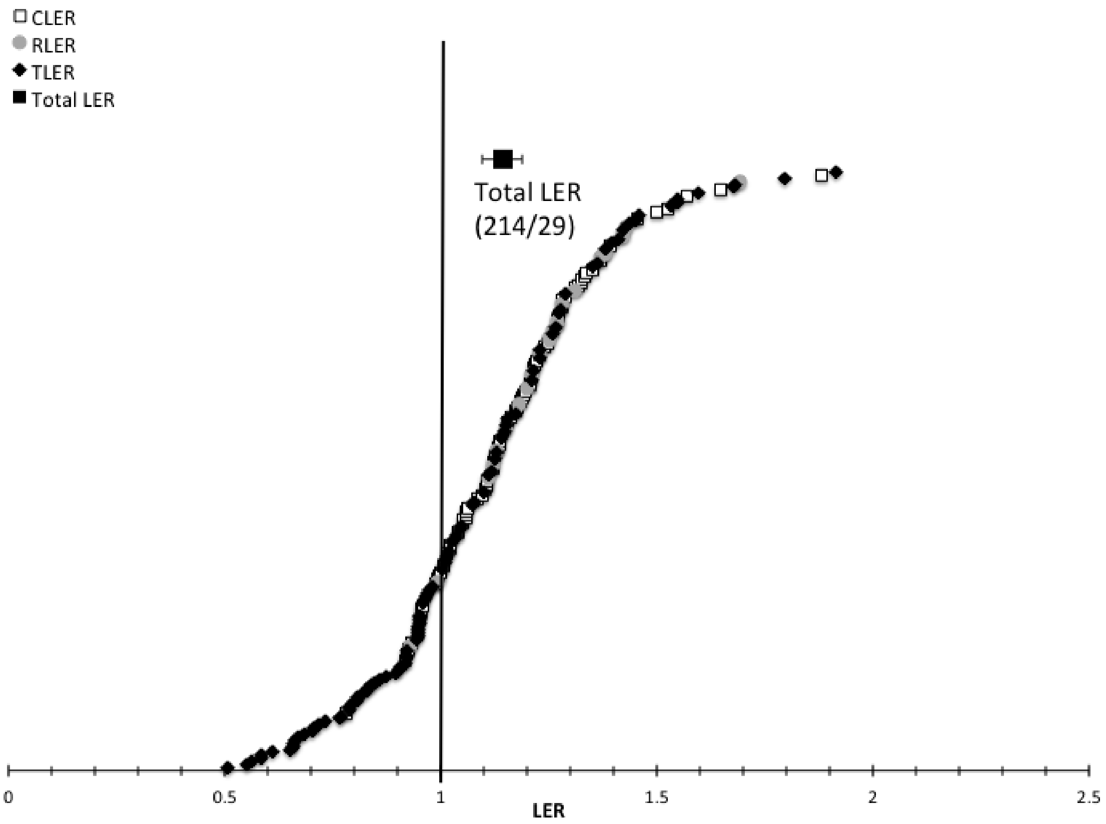

FIGURE 22. Plot of calculated (CLER), theoretical (TLER), and reported (RLER) Land Equivalence Ratio values for mixed species cover crops ordered by rank. Total LER is the average and the 95% confidence interval of all LER calculations.