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Use of legume green manures as nitrogen sources for corn production

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Abstract

Recent volatility in supplies and prices of natural gas and synthetic nitrogen (N) fertilizer suggests a need to develop and refine alternative strategies for supplying N to corn. In this study, conducted in north-eastern Iowa, we examined the use of red clover and alfalfa green manures as means of supplying N to a succeeding corn crop. Red clover intercropped with oat produced significantly more biomass and contained more N than alfalfa intercropped with oat. Tilling green manures in the fall or delaying tillage until the following spring did not have a consistent effect on green manure N content. Without N fertilizer, corn grain yield following oat–red clover and oat–alfalfa was 25–63% greater than following oat grown alone, but at the highest fertilizer rate (202 kg N ha^{-1}), there was no difference in corn yield between oat–legume and oat-alone treatments. These patterns support the premise that legume green manure effects on corn yield were N-related. Red clover green manure had an N fertilizer replacement value for corn of 87–184 kg N ha^{-1}; alfalfa supplied corn with the equivalent of 70–121 kg N ha^{-1}. At a fossil energy cost for N fertilizer of 57 MJ kg^{-1} N, reducing synthetic N fertilizer applications to corn by 70–184 kg N ha^{-1} would represent a fossil fuel savings of 3990–10,488 MJ ha^{-1}, equivalent to the energy content of 104–274 m^3 of natural gas. These types of savings are likely to become increasingly important as fossil energy supplies become scarcer and fertilizer prices rise.

Key words: alfalfa, corn production systems, legume green manure, nitrogen fertilizer replacement value, red clover

Introduction

Nitrogen (N) fertility is a critical component of corn (*Zea mays* L.) production. Within the USA, N is the nutrient supplied to crops in the greatest quantities as synthetic fertilizer and more N is applied to corn than any other crop[1]. In 2005, US farmers applied 4.6×10^6 Mg of synthetic N fertilizer to 96% of the area planted with corn at a mean rate of 155 kg N ha^{-1}.[1]

N fertilizer is not only applied to corn in large quantities, but it is also energy-intensive to synthesize. Consequently, it can comprise a major portion of the fossil-energy budget for corn production. Shapouri et al.[2] reported that N fertilizer requires, on average, 57 MJ of fossil energy (principally natural gas) per kg of N synthesized. Assuming an average fertilizer application rate of 155 kg N ha^{-1} at an energy cost of 57 MJ kg^{-1} N, and an energy content for natural gas of 38.3 MJ m^{-3}, [3] N application to 1 ha of corn would require the equivalent of 231 m^3 of natural gas. For conventional corn production in the Midwestern US, N fertilizer currently consumes 50% of the fossil energy used in the production cycle[2].

Recent volatility in supplies and prices of natural gas and synthetic N fertilizer[4] suggests a need to develop and refine alternative strategies for supplying N to corn that rely more on ecological processes, such as biological N fixation, than the Haber–Bosch industrial process. One such strategy is the use of legume green manures that fix atmospheric N$_2$ and are used as nutrient sources for subsequent crops[5]. Legume green manures suitable for the Midwestern US and southern Canada include alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.), which can be intercropped with small grains grown as cash or feed crops, preceding corn production[6–8].

N availability to a subsequent crop from legume green manures can vary with management options, such as tillage; soil and climate conditions, such as temperature and soil moisture; and tissue quality characteristics, such as

Table 1. Chronology of field operations during 2000–2002.

Field operation	Experiment 1 (2000–2001)	Experiment 2 (2001–2002)
Shallow cultivation	April 6, 2000	April 16, 2001
Oat, red clover and alfalfa planted	April 6, 2000	April 17, 2001
Oat harvested	July 21, 2000	July 30, 2001
Oat straw baled	July 21, 2000	July 31, 2001
Oat-only sub-plots mowed	August 11, September 1, 2000	August 21, September 11, 2001
Moldboard plowing for fall incorporation	November 14, 2000	November 22, 2001
Moldboard plowing for spring incorporation	April 20, 2001	April 16, 2002
Shallow cultivation	April 20, 2001	April 17, 2002
Shallow cultivation	May 12, 2001	May 4, 2002
Corn planted	May 12, 2001	May 4, 2002
Pre-emergence herbicide applied	May 12, 2001	May 4, 2002
Post-emergence herbicide applied	June 9, 2001	June 1, 2002
N fertilizer applied	June 11, 2001	June 7, 2002
Inter-row cultivation	June 11, 2001	June 7, 2002
Corn harvested	October 16, 2001	October 6, 2002

content of C, N, cellulose, lignin and polyphenols[9–12]. In general, tillage induces more rapid mineralization of green manure materials via shredding and mixing with soil and associated micro-organisms; N release from green manures occurs slowly at or below 5°C, increases to a maximum at 30–35°C, and declines at higher temperatures; N release is favored by soil conditions that are neither exceptionally dry nor flooded (-0.1 to -1 MPa moisture tension); and tissues with carbon to nitrogen (C : N) ratios <20 release N more quickly than those with C : N ratios >25[13,14].

N may be lost from incorporated green manures if between tillage and growth of a subsequent crop there is a long interval when soils are warm enough for organic matter mineralization and precipitation is great enough for nitrate leaching to occur[15]. Alternatively, if temperatures after incorporation of green manure are cool enough to inhibit mineralization, as might be the case with late fall plowing in temperate areas, nutrients might be retained until warmer conditions prevail in the spring. In much of the Midwestern US, it is often drier in the fall than it is in the spring, which facilitates plowing. However, fall plowing eliminates the erosion–reduction benefits of cover provided by an overwintering green manure crop.

Given the potential trade-offs and uncertainties associated with using and managing legume green manures, we conducted an experiment in north-eastern Iowa to determine the effects of using red clover or alfalfa as short-duration green manures preceding corn. Our specific objectives were to determine (i) biomass production and N-related characteristics of alfalfa and red clover intercropped with oat (*Avena sativa* L.) and incorporated in the fall or spring preceding corn production; (ii) corn yield responses to alfalfa and red clover green manure; and (iii) the N fertilizer replacement value (FRV) for corn of fall and spring tilled alfalfa and red clover green manures.

Materials and Methods

Site description and experimental design

Field research was conducted at the Iowa State University (ISU) Northeast Research and Demonstration Farm (NERDF) in Nashua, IA (42°57′N, 92°32′W). Predominant soil types are Kenyon loam (fine-loamy, mixed, mesic Typic Hapludolls), Floyd loam (fine-loamy, mixed, mesic Aquic Hapludolls) and Clyde loam (fine-loamy, mixed, mesic Typic Endoaquolls). Environmental data, including ambient air temperature, soil temperature and precipitation, were collected from the Iowa Environmental Mesonet[16] for the ISU-NERDF (Station ID: Nashua-a135879).

The first part of the field study (referred to as Experiment 1) was planted with oat and legumes in spring 2000 and was followed by field corn in 2001. The second part (referred to as Experiment 2) began with oat and legumes in spring 2001 and was followed by field corn in 2002. Oat and barley were grown in 1999 preceding the oat and legume crops of Experiment 1; soybean was grown in 2000 preceding the oat and legume crops of Experiment 2. Experiments 1 and 2 were conducted on separate fields, and each experiment was conducted using a randomized complete block, split-plot design. Whole-plot treatments comprised a factorial of three cropping systems (oat–alfalfa followed by corn, oat–red clover followed by corn and oat alone followed by corn) and two times of tillage (fall or spring plowing preceding corn). The six treatments were replicated four times and allocated randomly to strips within each of the two fields. The whole plots ($61\,\mathrm{m} \times 4.6\,\mathrm{m}$) were split into randomized sub-plots ($14.6\,\mathrm{m} \times 4.6\,\mathrm{m}$) that received a set of N fertilizer rates (0, 67, 134 and 202 kg N ha^{-1}) during the corn phase to determine the N FRV of the legume crops for the subsequent corn crop. Dates for field operations are presented in Table 1.

Green manure phase

Oat and legume species were planted in April for each green-manure phase (Table 1). 'Jerry' oat was planted at a rate of 108 kg ha^{-1}, and 'Red Comet' red clover and 'Pioneer 53H81' alfalfa were planted at 17 kg ha^{-1}. Red clover and alfalfa seeds were treated with *Rhizobium* inoculants; alfalfa seeds were also treated with a fungicide (mefenoxam: (R)-2-[(2,6-dimethylphenyl)-methoxyacetyl-amino]-propionic acid methyl ester). Oat was harvested for grain and the straw was baled in July of both years. Oat plots were mowed 21 and 42 days after oat harvest to control weeds.

Prior to fall and spring incorporation, four 0.25 m^2 quadrats were taken in each green manure × tillage timing treatment replicate to determine biomass of legumes, oat and weeds. Plant material, both living and dead, was cut at a height of about 1 cm above ground level on October 17, 2000 and April 18, 2001 (Experiment 1) and October 17, 2001 and April 12, 2002 (Experiment 2), dried at 60°C for 48 h and then weighed. Biomass samples were processed through a 2.0-mm screen with a Thomas–Wiley mill and ground further with a Cyclone sample mill to pass through a 1.0-mm screen (Model 014, UDY Corporation, Fort Collins, CO, USA). Sub-samples were analyzed for total C and total N concentrations by dry combustion (modified Dumas method)[17] using a LECO CHN-2000 elemental analyzer (LECO Corp., St. Joseph, MI, USA).

Incorporation of oat residue, new oat growth from seeds not recovered by the combine, and legumes was done with a moldboard plowed to a depth of 25 cm in the fall and spring (Table 1). Immediately after spring incorporation, all plots were field cultivated once to break up the soil clods in the spring-incorporated plots and to control spring-emerging volunteer legumes in the fall-incorporated plots.

Corn phase

Prior to corn planting, all plots were field cultivated twice for weed control and seedbed preparation. Corn was planted on May 12, 2001 (Golden Harvest 2390) and on May 4, 2002 (Golden Harvest 8250) at 80,000 seeds per hectare in 0.76 m rows. Pre-emergence herbicides were applied at the time of corn planting for grass weed control. In Experiment 1, acetochlor (2-chloro-2′-methyl-6′-ethyl-*N*-ethoxymethyl-acetanilide) was applied at a rate of 2.7 kg a.i. ha^{-1}; in Year 2, dimethenamid-P ([S]-2-chloro-N-[(1-methyl-2-methoxy) ethyl]-N-[2,4-dimethyl-thien-3-yl]-acetamide) was applied at a rate of 1.1 kg a.i. ha^{-1}. A post-emergence herbicide, dicamba (diglycolamine salt of 3,6-dichloro-o-anisic acid) was used, at rates of 280 g a.i. ha^{-1} in Experiment 1 and 350 g a.i. ha^{-1} in Experiment 2, to control broadleaf weeds.

Soil cores (3.2-cm-diameter) were taken at a depth of 15 cm during May and June in 2001 and 2002, respectively, at three or four randomly selected locations within each non-fertilized sub-plot. Soil samples were placed in sealed plastic bags and stored at 4°C prior to analysis. Field-moist samples were then pushed through an 8-mm-diameter sieve and soil water content was measured gravimetrically for sub-samples (15–20 g) after oven drying overnight at 105°C. Other field-moist 8-mm-sieved sub-samples (10 g) were extracted with 2 M KCl and NO_3-N concentration in the filtrate was determined using flow injection technology (Lachat Instruments, Milwaukee, WI, USA).

Urea fertilizer was applied at rates of 0, 67, 134 or 202 kg N ha^{-1} at the vegetative sixth leaf corn-growth stage (V6)[18] using a drop spreader (Gandy Company, Owatonna, MN, USA) in the appropriate sub-plots on June 11, 2001 and June 7, 2002 (Table 1). The center holes of the spreader were sealed to prevent fertilizer from dropping into the corn whorl and damaging leaves. All plots were row cultivated once the same day to incorporate the fertilizer.

Corn plant density was determined on June 12, 2001 and June 21, 2002 by counting each plant in three central plot rows. Weed density was determined on May 29, 2001 and May 20, 2002 from eight randomly located 0.25 m^2 quadrats placed within the whole-plots. Cornstalk sections were taken on October 13, 2001 and September 27, 2002 from 15 randomly selected plants in three center rows of each sub-plot[19]. The samples were dried and ground and stalk NO_3-N concentration was quantified using flow-injection technology (Lachat Instruments, Milwaukee, WI, USA) on filtrate extracted from 0.250-g sub-samples with 50 ml 2 M KCl, shaken for 30 min. Three central sub-plot rows were harvested with a combine to determine grain yield; yields were adjusted to a moisture content of 155 g kg^{-1}.

Statistical analyses

Data were analyzed using procedures in JMP 7.0.2[20]. Linear mixed-effects model analyses were conducted for plant variables in the green manure and corn phases of Experiments 1 and 2 using time of tillage and crop treatments as main plot factors, and N fertilizer rate treatments applied to corn as the split-plot factor. Random effects were included for replicate blocks and main plots. Fixed effects were included for tillage timing, crop system, fertilizer rate and all possible interactions among these factors. Mean separations among tillage timing × crop system combinations were conducted using linear contrasts. A repeated measures approach was used to examine the effects of time of tillage, preceding crop, sampling date and their interactions on soil NO_3-N concentrations in sub-plots not receiving N fertilizer within the corn phases of Experiments 1 and 2.

Corn grain yield responses to N fertilizer rate were further analyzed using polynomial regressions. Possible associations between corn population density and grain yield were examined by adding corn density as a covariate to models that included block, tillage timing, crop system, fertilizer rate and interactions among the factors. Relationships between soil NO_3-N concentrations, green manure characteristics and corn grain yield in sub-plots not

Table 2. Mean monthly precipitation and air temperature in 2000–2002 and 50-year averages (1951–2000), at the experimental sites.

	Mean monthly rainfall				Mean monthly air temperature			
Month	2000	2001	2002	50-yr avg.	2000	2001	2002	50-yr avg.
	mm				°C			
January	18	27	10	23	−7.7	−8.0	−2.4	−9.3
February	23	33	22	21	−1.1	−9.8	−2.2	−6.0
March	16	20	17	49	5.4	−2.9	−1.7	0.0
April	49	99	97	85	9.1	10.6	8.3	8.2
May	102	127	69	105	16.2	15.3	13.6	15.0
June	143	56	66	115	18.9	20.3	21.7	20.0
July	79	61	135	114	21.3	23.2	23.7	22.1
August	88	64	150	109	21.4	21.3	20.6	21.1
September	56	132	48	83	16.8	15.2	17.9	16.4
October	55	36	51	62	11.7	9.2	6.7	10.3
November	56	32	7	46	−0.8	8.1	0.6	1.4
December	65	7	9	28	−13.8	−2.1	−3.0	−6.1

receiving N fertilizer were examined using correlation analyses.

The significance level for all comparisons and inclusion of terms in regression models was $P \leqslant 0.05$. Preliminary analyses indicated that experiment by treatment interactions occurred for many variables, so all data were analyzed separately by experiment.

Results and Discussion

Environmental conditions

Air temperature. Mean monthly air temperatures during the period of April–October for the green manure and corn phases of the experiment in Experiment 1 (2000–2001) and Experiment 2 (2001–2002) were similar to the 50-year average (Table 2). Air temperatures during the winter months (November–March) of the green manure phase averaged 7°C cooler in Experiment 1 compared to Experiment 2. Corn growing degree-days from planting to harvest were 1412 in Experiment 1 and 1533 in Experiment 2, with a base temperature of 10°C and an upper threshold of 30°C[21]. In both experiments, the growing degree-days accumulated were adequate for corn to achieve physiological maturity.

Soil temperature. Paul and Clark[13] noted that microbial decomposition of organic matter is slow below 5°C and above 40°C, and that the optimum range for nitrification is between 30 and 35°C. In temperate climates, nitrification rates are greatest in the spring through early fall and lowest in the winter. Soil temperatures recorded at a depth of 10 cm indicate that soil cooled to below 5°C by November 8, 2000 in Experiment 1 and by November 20, 2001 in Experiment 2 of the green-manure phase (data not shown). Fall incorporation of green-manure crops occurred within a week after these dates, so very little net N mineralization would have been likely at ambient temperatures until soil warmed the following spring. In the spring, soil temperatures maintained levels above 5°C by the first week in April in both experiments (data not shown). Spring incorporation of green manures occurred by the third week in April when soil temperatures were maintaining levels above 10°C. Assuming other conditions were adequate (moisture, aeration and organic matter composition), these soil temperatures would be warm enough to support net N mineralization for either the fall-incorporated biomass or the newly incorporated spring biomass. During the periods of corn production, air and soil temperature conditions should not have been limiting factors for microbial decomposition of organic matter.

Precipitation. In the green-manure phase of each crop sequence, total precipitation was greater in Experiment 1 (2000) than in Experiment 2 (2001) during the period of May–October (Table 2). In the months of June, July and August, mean monthly precipitation was 103 mm in the green-manure phase of Experiment 1 compared with 60 mm in Experiment 2. In the corn phase of the Experiment 1 crop sequence (2001), total precipitation was less than in Experiment 2 (2002) during the period from May to October, particularly in the months of June, July and August when the mean monthly rainfall was 60 mm in Year 1 compared with 117 mm in Experiment 2. Growing season totals for corn phases in both Experiment 1 (2001) and Experiment 2 (2002) were below the 50-year average (Table 2). However, neither drought nor flooded conditions were present in either year of corn production, suggesting that soil moisture availability was not an important constraint on green-manure decomposition.

Green manure phase

Green manure aboveground biomass. In Experiment 1, mean biomass of spring tilled green manure treatments was 5660 kg DM ha^{-1}, which was significantly greater than the mean for fall tilled green manures (3490 kg ha^{-1})

Table 3. Total aboveground biomass of crops and weeds, biomass N and C concentrations, total N and C contents and C : N for plant materials incorporated before corn production.

	Fall tillage			Spring tillage				Source of variation		
Response	**Oat–red clover**	**Oat–alfalfa**	**Oat alone**	**Oat–red clover**	**Oat–alfalfa**	**Oat alone**	**SE**	**Time of tillage (T)**	**Crop (C)**	**T × C**
								-------------	P>F	-------------
Experiment 1[1]										
Biomass (kg ha^{-1})	5796	3121	1554	6845	5294	4840	735	0.0025	0.0023	0.3401
N concentration (g N kg^{-1})	27	22	18	29	31	11	1	0.2785	<0.0001	<0.0001
N content (kg N ha^{-1})	158	69	28	201	168	53	20	0.0043	<0.0001	0.1944
C concentration (g C kg^{-1})	423	445	407	422	367	359	19	0.0182	0.1572	0.1651
C content (kg C ha^{-1})	2301	1306	594	2884	1870	1716	284	0.0205	0.0016	0.6011
C:N ratio	15	20	22	15	12	34	2	0.7920	<0.0001	0.0019
Experiment 2										
Biomass (kg ha^{-1})	5721	2184	1254	4880	1667	2845	343	0.7848	<0.0001	0.0058
N concentration, (g N kg^{-1})	26	23	14	40	29	13	4	0.0485	0.0002	0.1303
N content (kg N ha^{-1})	149	51	18	192	51	36	14	0.0867	<0.0001	0.3092
C concentration (g C kg^{-1})	429	436	407	381	378	325	9	<0.0001	0.0005	0.1887
C content (kg C ha^{-1})	2436	952	510	1833	630	918	125	0.1110	<0.0001	0.0030
C:N ratio	17	19	30	10	15	26	2	0.0055	<0.0001	0.6810

[1] Data for the fall tillage treatment are for samples collected on October 17, 2000 (Experiment 1) and October 17, 2001 (Experiment 2); data for the spring tillage treatment are for samples collected on April 18, 2001 (Experiment 1) and April 12, 2002 (Experiment 2).

(Table 3). Most of the biomass in the spring tillage oat-alone treatment was comprised of volunteer oat, whereas most of the biomass in the spring tillage oat–red clover and oat–alfalfa treatments was comprised of legume material, some of which was from new growth and some of which was residue of winter-killed fall growth (Fig. 1). Abundant precipitation in Experiment 1 (Table 2) may have increased soil moisture and contributed to increased late fall and early spring growth.

In Experiment 2, biomass of the spring tilled oat-alone treatment was significantly greater than the fall tilled oat-alone treatment, whereas tillage timing had no significant effect on biomass production in the oat–red clover and oat–alfalfa treatments (Table 3). We do not know why there was a reduced amount of legume biomass in the spring of Experiment 2 compared with Experiment 1. In particular, we did not monitor snow and ice cover, which might have affected winter kill, though soil temperatures did not differ greatly during the winter periods of Experiments 1 and 2 (data not shown).

In both experiments, the oat–red clover treatment produced significantly more biomass than the oat–alfalfa and the oat-alone treatments for both the fall and spring tillage regimes (Table 3). This was an expected result; in Wisconsin, when grown with a companion crop such as oat, red clover yield during the seeding year is generally 6–10 Mg ha^{-1},[22] whereas alfalfa yield is 2–3 Mg ha^{-1}.[23]

Botanical composition of aboveground biomass. Aboveground biomass from the oat–red clover treatment consistently contained greater than 80% legumes, with a range of 83–94% (Fig. 1). Spring tilled biomass from the oat–red clover treatment tended to be slightly lower in legumes and higher in oat stubble and regrowth than the fall-tilled biomass; weeds were a very minor component. Winter kill or slower regrowth in the spring of red clover or volunteer oat growth in late fall or early spring may have been contributing factors to the lower legume percentage and increased oat percentage in the spring biomass.

The legume composition of the oat–alfalfa treatment biomass was similar in the fall for both experiments: 55% in Experiment 1 and 56% in Experiment 2 (Fig. 1). The percentage of legume material with spring tillage was 40–50% greater than with fall tillage for the oat–alfalfa treatment and similar to the legume concentration in the spring tilled oat–red clover treatment (Fig. 1).

After oat harvest, weeds were controlled in the oat-alone treatment by mowing twice after harvest (Table 1). Weed regrowth after mowing, however, was present in the fall biomass samples. Weeds were a major component in the fall tilled oat-alone treatment for Experiments 1 and 2, but not in the spring tilled oat-alone treatments (Fig. 1).

Weeds were minimal to non-existent in all the spring tillage treatments for both experiments (Fig. 1), presumably because the majority of weeds were annual grasses and had yet to germinate when biomass samples were taken.

Aboveground biomass N content. Green manure biomass N content was calculated from the product of aboveground biomass and N concentration. In both years, green manure N content was closely correlated with N concentration (Experiment 1: $r = 0.8022$, $p < 0.0001$; Experiment 2: $r = 0.8934$, $p < 0.0001$) (Table 3).

In Experiment 1, spring tilled treatments contained significantly more N than fall tilled treatments (141 versus

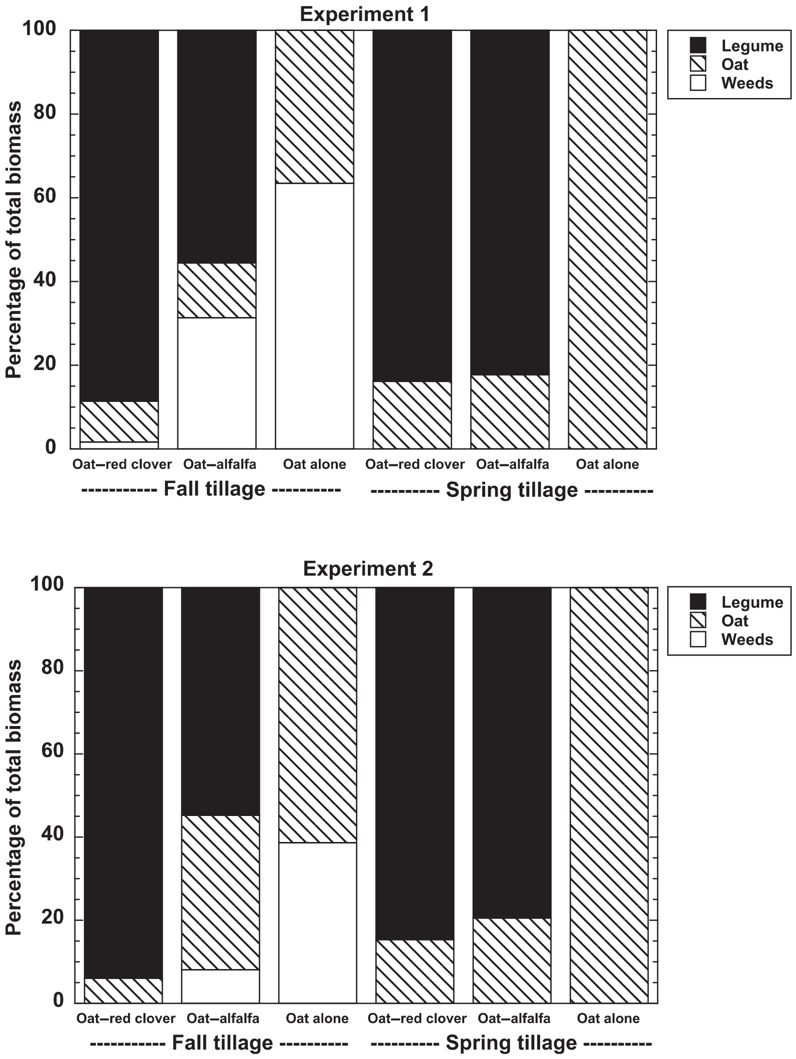

Figure 1. Botanical composition of green manure biomass.

85 kg N ha^{-1}; Table 3). Averaged over tillage regimes in Experiment 1, the oat–red clover treatment contained significantly more N than the oat–alfalfa treatment (179 versus 118 kg N ha^{-1}), and both of the oat–legume treatments contained significantly more N than the oat-alone treatment (40 kg N ha^{-1}) (Table 3).

In Experiment 2, tillage timing had no significant effect on green manure N content (Table 3), but crop effects were evident. Averaged across tillage regimes in Experiment 2, oat–red clover contained significantly more N (170 kg N ha^{-1}) than oat–alfalfa (51 kg N ha^{-1}) and oat-alone (27 kg N ha^{-1}), which did not differ statistically (Table 3).

Aboveground biomass C:N ratio. Soil environmental conditions and the composition of organic substrates are key factors affecting microbial decomposition of organic matter. The C:N ratio of organic materials is an important factor influencing net mineralization or immobilization of N[13,14]. In both years, C:N was strongly negatively correlated with green manure N concentration (Experiment 1: $r = -0.9410$, $P < 0.0001$; Experiment 2: $r = -0.9188$, $P < 0.0001$), but was not related to green manure C concentration (Experiment 1: $r = -0.0867$, $P = 0.7085$; Experiment 2: $r = -0.1114$, $P = 0.6043$) (Table 3).

In Experiment 1, green manure C:N was affected by an interaction between tillage timing and crop identity (Table 3). More specifically, C:N did not differ among crop types for the fall tillage regime, but was significantly greater in the oat-alone treatment than the oat–legume treatments for the spring tillage regime (Table 3).

In Experiment 2, fall-tilled treatments had significantly higher C:N values than did spring-tilled treatments (22 versus 17; Table 3). Averaged over tillage regimes, the oat-alone treatment had a significantly higher C:N value than did the oat–alfalfa treatment, which was in turn significantly higher than the oat–red clover treatment (28 versus 17 versus 13; Table 3).

With a C:N value close to or greater than 25 : 1 in the oat-alone treatments, there may have been immobilization of N and residual soil inorganic N may have been depleted[24]. In contrast, the lower C:N values for the oat-legume treatments favored more rapid microbial decomposition if other conditions were adequate.

Table 4. Densities of corn and weeds in corn as affected by time of tillage and preceding crop.

Time of tillage and preceding crop	Corn[1]		Weeds[2]	
	Experiment 1	**Experiment 2**	**Experiment 1**	**Experiment 2**
	------------no. ha^{-1}------------		------------no. m^{-2}------------	
Fall tillage				
Oat–red clover	75,260	73,945	4 (1.8)	7 (2.4)
Oat–alfalfa	72,940	71,555	40 (5.9)	25 (4.9)
Oat	74,640	75,025	14 (3.7)	27 (5.1)
Spring tillage				
Oat–red clover	74,330	62,815	5 (2.2)	10 (2.9)
Oat–alfalfa	71,550	63,300	32 (5.6)	18 (4.1)
Oat	73,560	72,320	17 (3.9)	32 (5.4)
SE	1560	1000	(0.6)	(0.3)
Source of variation	---------------------------------*P>F*---------------------------------			
Time of tillage (T)	0.1852	< 0.0001	0.8087	0.9944
Preceding crop (C)	0.0574	0.0023	< 0.0001	< 0.0001
T × C	0.9725	0.0472	0.8731	0.1240

[1] Corn densities were measured on June 12, 2001 (Experiment 1) and June 21, 2002 (Experiment 2).

[2] Weed densities were measured on May 29, 2001 (Experiment 1) and May 30, 2002 (Experiment 2) and were square root-transformed before analysis of variance. Means and standard errors of transformed data are shown in parentheses.

Corn phase

Corn density. No significant differences in corn density were observed among treatments in Experiment 1, for which mean corn population density was 73,713 plants ha^{-1} (Table 4). In Experiment 2, corn density in both the spring tillage oat–legume treatments was significantly lower than in the oat-alone treatment, and lower than in each of the fall tillage treatments (Table 4). Mean corn density for the two oat-legume treatments tilled in the spring was 63,058 plants ha^{-1}, whereas for the other crop × tillage timing treatments of Experiment 2, mean corn density was 73,211 plants ha^{-1} (Table 4). Although we did not closely inspect corn seed to determine the cause of the lower corn density, reduction of corn density in spring-tilled oat–legume plots may have been related to damage by seed corn maggot (*Delia platura* (Meigen)). Hammond[25] reported that ovipositing female seedcorn maggots are attracted to freshly incorporated green plant matter and that damage to soybean seeds was greater following incorporation of a legume (alfalfa) than non-legumes (weeds and rye). Thus, spring incorporation of legumes in the present study may have favored this insect pest.

Weed density in corn. Weed density in corn was determined in late May after all plots had received a pre-emergence herbicide application at planting, but before post-emergence herbicide application (Table 1). Dominant weed species were yellow foxtail (*Setaria pumila* (Poir.) Roemer & J.A. Schultes), giant foxtail (*Setaria faberi* Herrm.), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), common waterhemp (*Amaranthus rudis* Sauer), velvetleaf (*Abutilon theophrasti* Medik) and common lambsquarters (*Chenopodium album* L.).

For both experiments and both tillage timing treatments regimes, corn following oat–red clover had fewer weeds than did corn following oat–alfalfa and oat-alone (Table 4). This accords with the observation that in the fall, the percentage of total biomass comprised of weeds was less in the oat–red clover treatment than in the oat–alfalfa and oat-alone treatments (Fig. 1). Suppression of weed biomass production in the oat–red clover treatment was probably accompanied by suppression of weed seed production[26], resulting in fewer weeds in the subsequent corn crop. Mutch et al.[27] reported similar weed-suppressive effects for red clover. Weed density in corn was not significantly affected by tillage timing in either experiment (Table 4).

Soil nitrate dynamics. Repeated measures analyses indicated that soil NO_3-N concentration in corn was affected by significant interactions between sampling date and time of tillage and crop system factors. In general, NO_3-N concentrations were significantly higher following oat–red clover and oat–alfalfa than oat alone, and were affected inconsistently by the time of tillage (Table 5). The oat–red clover and oat–alfalfa treatments tended to show higher NO_3-N concentrations in May and early June before declining in late June, whereas NO_3-N concentrations in the oat-alone treatment did not vary significantly among sampling dates (Table 5).

Corn N uptake and growth are generally rapid in mid to late June in north-eastern Iowa and soil N availability during this period can strongly influence corn yield. Morris et al.[28] reported that for corn following alfalfa, late spring soil NO_3-N concentrations at or above 14 μg g^{-1} in the top 30 cm of soil indicated that corn yield would be unlikely to respond to supplemental N fertilizer. Data from the present experiment represent NO_3-N concentrations in the top 15 cm of soil, so direct comparisons with Morris et al.'s

Table 5. Surface soil (0–15 cm) NO_3-N concentration at selected sampling dates in plots not receiving N fertilizer during the corn phase of Experiments 1 and 2.

	Fall tillage			Spring tillage				Source of variation			Correlation with corn grain yield, Mg ha^{-1}
Sampling date	Oat–red clover	Oat–alfalfa	Oat alone	Oat–red clover	Oat–alfalfa	Oat alone	SE	Time of tillage (T)	Crop (C)	T × C	r
	------------------- μg NO_3-N g^{-1} soil ------------------							---------P>F---------			
Experiment 1[1]											
May 14, 2001	10.63 a	8.64 a	6.48 a	11.77 a	11.54 a	7.89 a	1.42	0.1201	0.0170	0.8003	0.58**
May 29, 2001	15.62 b	9.59 a	4.32 a	9.21 a	10.44 a	6.49 a	1.80	0.4415	0.0007	0.0410	0.43*
June 12, 2001	10.04 a	14.62 a	8.48 a	11.39 a	13.58 a	7.90 a	1.84	0.9543	0.0193	0.7919	0.51*
June 26, 2001	7.42 b	7.00 b	3.73 a	10.71 a	5.66 b	4.76 a	1.52	0.4330	0.0207	0.3383	0.72***
Experiment 2											
May 9, 2002	13.69 a	11.96 a	5.25 a	12.61 b	10.34 b	6.64 a	1.40	0.7021	<0.0001	0.5206	0.72***
May 23, 2002	12.19 b	13.02 a	4.83 a	23.91 a	14.78 a	7.08 a	2.37	0.0082	<0.0001	0.0662	0.49*
June 7, 2002	12.00 b	13.89 a	8.01 a	10.67 b	16.65 a	7.11 a	1.79	0.9023	0.0003	0.4563	0.67***
June 21, 2002	7.33 c	8.07 b	5.32 a	16.57 a	10.30 b	4.24 b	1.35	0.0026	<0.0001	0.0011	0.45*

[1] For each experiment, means within columns not followed by the same letter are significantly different at $P \leqslant 0.05$.

* $P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

study are not possible. Nonetheless, it is notable that soil NO_3-N concentrations were above Morris et al.'s 14 μg g^{-1} threshold in only four of the eight time of tillage × legume green manure × experiment combinations we observed.

Corn stalk nitrate concentration. Binford et al.[29] examined the relationship between corn grain yield and stalk NO_3-N concentration at plant maturity and determined that plants with <700 mg NO_3-N kg^{-1} were N-deficient, those in the range of 700–2000 mg NO_3-N kg^{-1} had sufficient amounts of N to reach their yield potential, and those with >2000 mg NO_3-N kg^{-1} had received excessive amounts of N. In both Experiments 1 and 2 of the present study, fall tilled and spring tilled oat-alone treatments required the highest fertilizer rate used in the experiment (202 kg N ha^{-1}) to reach stalk concentrations above 700 mg NO_3-N kg^{-1} (Table 6). In contrast, corn stalk analyses indicated optimum levels were reached in Experiment 1 at the 134 kg N ha^{-1} fertilizer rate for fall tilled and spring tilled oat–red clover and oat–alfalfa treatments; in Experiment 2, optimum levels were attained for the oat–red clover and oat–alfalfa treatments at 67 kg N ha^{-1} with fall tillage, and at 67 and 134 kg N ha^{-1}, respectively, with spring tillage (Table 6). These results indicate that treatments with legume green manure reached optimum corn stalk NO_3-N levels with less fertilizer than the oat-alone treatment.

Results of contrast analyses indicated that in Experiment 1, averaged over fertilizer and tillage treatments, both legume treatments produced higher stalk NO_3-N concentrations than the oat-alone treatment ($P < 0.0001$), but did not differ from each other ($P = 0.4405$). In Experiment 2, the red clover treatment produced higher stalk NO_3-N concentrations than the alfalfa treatment ($P = 0.0224$), and both legume treatments produced higher levels than the oat-alone treatment ($P < 0.0001$). Time of tillage did not significantly affect stalk NO_3-N concentrations in either experiment (Table 6).

Corn yield. Corn yield in Experiment 1 was significantly affected by an interaction between preceding crop and N fertilizer rate, but there were no main or interaction effects of tillage timing (Table 7). In contrast, corn yield in Experiment 2 was affected by a three-way interaction of preceding crop × tillage timing × N fertilizer rate (Table 7). In general, corn yields following red clover and alfalfa were similar, but greater than corn yields following oat-alone at lower fertilizer rates.

Regression analyses were used to further investigate the impacts of different preceding crops and times of tillage on corn yield response to N fertilizer. Mean, linear and quadratic models were fit for corn yield responses to varying N fertilizer rates for each preceding crop in Experiment 1, when there were no main or interactive effects of tillage timing, and each tillage timing × preceding crop combination in Experiment 2, when tillage timing effects were significant (Table 7). To make parsimonious prediction equations, we rejected model terms that were not significant (i.e., $P > 0.05$). In the case of corn following red clover in both the fall and spring tillage treatments of Experiment 2, there were no significant linear or quadratic trends, so we used mean responses across fertilizer rates. We also fit segmented regressions using quadratic with plateau models, as recommended by Cerrato and Blackmer[30], but found that simpler, non-segmented polynomial models fit the data better.

In both Experiments 1 and 2, corn responded considerably more strongly to N fertilizer in the oat-alone treatment than in the oat–red clover and oat–alfalfa treatments. This is evident in the lower intercept values and larger linear coefficients in the prediction equations for the oat-alone treatment as compared with the legume treatments

Table 6. Cornstalk NO_3-N concentration at maturity as affected by time of tillage, preceding crop and N fertilizer rate.

		Experiment 1[1]				Experiment 2			
		Fertilizer rate (kg N ha^{-1})				Fertilizer rate (kg N ha^{-1})			
Time of tillage	Preceding crop	0	67	134	202	0	67	134	202
		mg NO_3-N kg^{-1}							
Fall	Oat–red clover	11 (2.0)	548 (6.1)	1002 (6.8)	2991 (8.0)	322 (5.4)	1956 (7.5)	3537 (8.2)	4550 (8.4)
Fall	Oat–alfalfa	14 (2.6)	177 (5.0)	1226 (7.1)	2502 (7.8)	437 (2.7)	1182 (6.4)	2330 (7.7)	3042 (8.0)
Fall	Oat alone	7 (1.0)	9 (1.7)	85 (4.1)	921 (6.7)	25 (1.9)	41 (2.2)	318 (5.4)	1040 (6.8)
Spring	Oat–red clover	91 (3.7)	91 (3.7)	1301 (7.1)	2603 (7.9)	108 (4.4)	701 (6.5)	2372 (7.7)	3202 (8.0)
Spring	Oat–alfalfa	13 (2.5)	141 (4.8)	1134 (6.9)	2300 (7.7)	39 (3.6)	575 (6.0)	1831 (7.4)	2976 (8.0)
Spring	Oat alone	5 (0.6)	15 (2.6)	33 (3.2)	700 (6.5)	50 (3.6)	36 (3.6)	137 (4.5)	816 (6.6)
SE		(1.2)				(1.4)			
Source of variation		*P*>*F*							
Time of tillage (T)		0.4834				0.7824			
Preceding crop (C)		<0.0001				<0.0001			
T × C		0.9796				0.2017			
Fertilizer rate (F)		<0.0001				<0.0001			
F × T		0.4836				0.2562			
F × C		0.7815				0.2927			
F × T × C		0.8343				0.2425			

[1] Samples were collected on October 13, 2001 (Year 1) and September 27, 2002 (Year 2). Data were ln-transformed before analysis of variance. Means and standard errors of transformed data are shown in parentheses.

Table 7. Corn yield (at 155 g H_2O kg^{-1}) as affected by time of tillage, preceding crop and N fertilizer rate.

		Experiment 1				Experiment 2			
		Fertilizer rate, kg N ha^{-1}				Fertilizer rate, kg N ha^{-1}			
Time of tillage	Preceding crop	0	67	134	202	0	67	134	202
		Mg ha^{-1}							
Fall	Oat–red clover	9.97	12.38	11.80	12.72	12.80	13.90	13.34	13.03
Fall	Oat–alfalfa	10.29	12.11	11.75	12.43	11.86	12.84	13.08	13.17
Fall	Oat alone	7.89	10.87	11.63	12.09	9.52	11.53	12.59	13.33
Spring	Oat–red clover	11.03	11.61	12.03	12.52	11.42	11.70	12.58	12.41
Spring	Oat–alfalfa	10.34	12.08	12.83	12.23	11.47	12.00	11.75	12.36
Spring	Oat alone	7.86	10.44	11.01	12.09	7.03	10.58	11.90	12.07
SE		0.28				0.34			
Source of variation		*P*>*F*							
Time of tillage (T)		0.9234				<0.0001			
Preceding crop (C)		<0.0001				<0.0001			
T × C		0.2086				0.4492			
Fertilizer rate (F)		<0.0001				<0.0001			
F × T		0.0867				0.3524			
F × C		<0.0001				<0.0001			
F × T × C		0.0712				0.0204			

(Table 8). The three-way interaction detected in Experiment 2 was the result of a stronger response to N fertilizer in the oat-alone treatment for spring tillage than for the fall tillage (Table 8).

Analysis of covariance indicated that corn population density had no significant association with corn yield in Experiment 1 ($P = 0.9843$), but did have a significant association with yield in Experiment 2 ($P = 0.0014$). More specifically, reductions in corn density that occurred with the use of legume green manures and spring tillage in Experiment 2 (Table 4) were significantly associated with lower corn yield.

In both experiments, corn yield in the 0 kg N ha^{-1} fertilizer treatment was significantly positively correlated with green manure aboveground biomass (Experiment 1: $r = 0.55$, $P = 0.0051$; Experiment 2: $r = 0.43$, $P = 0.0377$),

Table 8. Prediction equations for corn yield response (Mg ha^{-1}) to N fertilizer rate (kg N ha^{-1}) for time of tillage × preceding crop combinations in the two experiments, and estimated fertilizer N equivalence of red clover and alfalfa. Standard errors are shown in parentheses.

Experiment	Time of tillage	Preceding crop	Intercept	Linear coefficient	Quadratic coefficient	N fertilizer equivalent (kg N ha^{-1})
1	Fall, spring	Oat–red clover	10.8140 (0.2303)	0.0094 (0.0018)		87
1	Fall, spring	Oat–alfalfa	10.3843 (0.1861)	0.0287 (0.0044)	−0.000096 (0.000021)	70
1	Fall, spring	Oat alone	7.9857 (0.2136)	0.0422 (0.0051)	−0.000111 (0.000024)	
2	Fall	Oat–red clover	13.2694 (0.1999)			184
2	Fall	Oat–alfalfa	12.1081 (0.2735)	0.0062 (0.0022)		121
2	Fall	Oat alone	9.8723 (0.4219)	0.0185 (0.0034)		
2	Spring	Oat–red clover	12.0252 (0.2758)			131
2	Spring	Oat–alfalfa	11.5368 (0.2100)	0.0036 (0.0017)		104
2	Spring	Oat alone	7.0817 (0.5142)	0.0622 (0.0123)	−0.000187 (0.000058)	

but negatively correlated with green manure C:N ratio (Experiment 1: $r = -0.75$, $P < 0.0001$; Experiment 2: $r = -0.59$, $P < 0.0024$). Corn yield in the 0 kg N ha^{-1} fertilizer treatment was also strongly and positively correlated with the green manure N concentration (Experiment 1: $r = 0.76$, $P < 0.0001$; Experiment 2: $r = 0.50$, $P = 0.0125$), green manure N content (Experiment 1: $r = 0.74$, $P < 0.0001$; Experiment 2: $r = 0.51$, $P = 0.0101$), and soil NO_3-N concentrations (Table 5). Taken together, these results indicate that legume green manures that produced the greatest amount of N-rich biomass contributed most strongly to higher corn yields under N limited conditions.

Nitrogen FRV of legume green manures. Legume green manures may have positive impacts on the yield of succeeding crops due to both N-related and non-N-related effects. As described by Pierce and Rice[31], a *non-N rotation effect* is the difference in yield of a target crop at a high fertilizer rate following legume or non-legume, whereas a *total rotation effect* is the yield difference observed following legume and non-legume crops when no fertilizer is applied. The difference between the *total rotation effect* and the *non-N effect* is the *N-rotation effect*.

When we compared the difference in corn yield between the oat–red clover and oat–alfalfa treatments versus the oat-alone treatment for each experiment and for each time of tillage, the yield difference at the 0 kg N ha^{-1} fertilizer rate was significant in each case, whereas the yield difference at the 202 kg N ha^{-1} fertilizer rate was not (Table 7). This indicated that the observed responses to legumes were N rather than non-N effects. Consequently, we used the regression equations for corn responses to N fertilizer (Table 8) to calculate N FRVs following a procedure described by Hesterman et al.[32]. Using the prediction equations for the oat-alone treatments, we calculated N FRVs as the amount of fertilizer needed to give corn yields equivalent to the *y*-intercepts in the prediction equations for the legume treatments.

FRVs for red clover ranged from 87 to 184 kg N ha^{-1}, whereas values for alfalfa ranged from 70 to 121 kg N ha^{-1} (Table 8). We found no consistent patterns for tillage timing effects, but in each experiment, FRVs for red clover were higher than for alfalfa. Values calculated in the present study are similar to those reported by other researchers studying corn yield responses to short-duration legume green manures in temperate areas[6,7,33].

Economic and energetic considerations. The cost of synthetic N fertilizer rose more than 200% during the period of 1991–2006 and closely paralleled increases in the cost of natural gas[34], a key component of its manufacture. If it is assumed that over the longer term, supplies of natural gas will shrink and its cost will rise, then reducing reliance on synthetic N sources is likely to be a useful strategy for limiting costs of corn production. At a fossil energy cost of 57 MJ kg^{-1} N, reducing synthetic N fertilizer applications to corn by the FRVs calculated for legumes used in the present study—70–184 kg N ha^{-1}—would represent a fossil fuel savings of 3990–10,488 MJ ha^{-1}, equivalent to the energy content of 104–274 m^3 of natural gas[2,3].

Two factors are likely to favor the use of red clover over alfalfa as a short-duration green manure: its generally greater N FRV (Table 7) and its lower seed cost. An informal survey of seed suppliers in Iowa in 2011 indicated that red clover seed cost \$3.75–6.10 kg^{-1}, depending on variety, whereas alfalfa seed cost \$7.10–9.50 kg^{-1}. Thus, for the seeding rate used in this study for both species (17 kg ha^{-1}), red clover would be a more economical choice for supplying N to corn. Assuming that (i) red clover seed would cost \$4.50 kg^{-1} and allow a reduction of fertilizer application to corn of 120 kg N ha^{-1}; (ii) alfalfa seed would cost \$8.00 kg^{-1} and allow a reduction in fertilizer application to corn of 90 kg N ha^{-1}; (iii) oat yield would be unaffected by the presence of legume intercrops[35]; and (iv) both red clover and alfalfa would be planted at 17 kg ha^{-1}; then the cost of N fertilizer at which planting legumes with oat would be economically competitive with planting oat alone would be \$0.64 kg^{-1} of N for red clover and \$1.51 kg^{-1} of N for alfalfa. As a point of reference, during the period of 2001–2010, N fertilizer costs in Iowa varied from \$0.44 to \$1.50 kg^{-1} of N, with a mean cost of \$0.73 kg^{-1} of N[36].

The economics of a crop rotation system depend on yields, prices obtained for crop products, government subsidies, and input, land and labor costs. Relatively few studies have evaluated the economic performance of substituting or adding small grains and forage legumes to the corn–soybean system that dominates the US Midwest. Using data from two long-term cropping systems trials in Wisconsin, Chavas et al.[37] found that without government subsidy payments and organic premiums, a corn–soybean system receiving conventional chemical inputs was more profitable than a corn–soybean–wheat/red clover system under organic management; with subsidies and organic premiums, the more diverse system was more profitable. Cruse et al.[38] used 6 years of data from a cropping systems trial in Iowa to compare the economic performance of a conventionally managed corn–soybean system with that of a corn–soybean–small grain/red clover system that received reduced amounts of synthetic N fertilizer and herbicides, but some manure. The small grain crop was initially triticale and then oat. With the assumptions that (i) the manure was a waste product that incurred costs only for labor and machinery associated with its application, and (ii) no government subsidies or premiums were received for the crops, returns from the two systems were found to be statistically equivalent, differing by \$9 ha^{-1} in favor of the corn–soybean system. However, fossil energy use, including energy used for producing N fertilizer, was 41% lower in the more diverse system[38], suggesting that increases in fossil fuel costs that were disproportionately greater than potential increases in crop value would make the corn–soybean–small grain/red clover economically advantageous. As noted by Cherr et al.[5], future research efforts concerning the use of green manure legumes should take a systemic approach toward identifying the full set of services that they might provide and the factors affecting their adoption by farmers, including economic and energetic efficiency.

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