Reduced Nitrogen Losses after Conversion of Row Crop Agriculture to Perennial Biofuel Crops

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Current biofuel feedstock crops such as corn lead to large environmental losses of N through nitrate leaching and N_2O emissions; second-generation cellulosic crops have the potential to reduce these N losses. We measured N losses and cycling in establishing miscanthus (*Miscanthus* × *giganteus*), switchgrass (*Panicum virgatum* L. fertilized with 56 kg N ha^{-1} yr^{-1}), and mixed prairie, along with a corn (*Zea mays* L.)–corn–soybean [*Glycine max* (L.) Merr.] rotation (corn fertilized at 168–202 kg N ha^{-1}). Nitrous oxide emissions, soil N mineralization, mid-profile nitrate leaching, and tile flow and nitrate concentrations were measured. Perennial crops quickly reduced nitrate leaching at a 50-cm soil depth as well as concentrations and loads from the tile systems (year 1 tile nitrate concentrations of 10–15 mg N L^{-1} declined significantly by year 4 in all perennial crops to <0.6 mg N L^{-1}, with losses of <0.8 kg N ha^{-1} yr^{-1}). Nitrous oxide emissions were 2.2 to 7.7 kg N ha^{-1} yr^{-1} in the corn–corn–soybean rotation but were <1.0 kg N ha^{-1} yr^{-1} by year 4 in the perennial crops. Overall N balances (atmospheric deposition + fertilization + soybean N_2 fixation – harvest, leaching losses, and N_2O emissions) were positive for corn and soybean (22 kg N ha^{-1} yr^{-1}) as well as switchgrass (9.7 kg N ha^{-1} yr^{-1}) but were −18 and −29 kg N ha^{-1} yr^{-1} for prairie and miscanthus, respectively. Our results demonstrate rapid tightening of the N cycle as perennial biofuel crops established on a rich Mollisol soil.

In light of the world's population now exceeding 7 billion, renewable and environmentally sustainable energy sources are increasingly desired to support the world's escalating energy demands. Global demand for liquid fuel is projected to increase more than 25% by 2035 (DOE, 2011). Biofuel feedstock crops have been widely promoted as a renewable fuel source that will reduce greenhouse gas (GHG) emissions and dependence on foreign oil (Perlack et al., 2005; Heaton et al., 2008a; Searchinger et al., 2008). Current U.S. legislation requires the increased use of biofuel feedstocks to help meet this demand. The Energy Independence and Security Act (2007) requires the annual production of 36 billion gallons of ethanol by 2022. No more than 15 of the 36 billion gallons is to be ethanol produced from corn or sugarcane, and at least 16 billion gallons must be produced from cellulosic feedstocks. As of 2010, approximately 30% of U.S. corn production was used to create ethanol (NASS, 2011), and the United States produced over 13 billion gallons of ethanol (Renewable Fuels Association, 2011). As the United State reaches the 15 billion gallon limit on corn ethanol, it is envisaged that further expansion will be in the form of cellulosic biofuels from perennial feedstocks.

There is significant variability among biofuel crops in terms of their environmental impacts or economic incentives and consequences. First-generation biofuels such as corn require costly inputs and management, whereas second-generation biofuels (e.g., perennial grasses, woody biomass, agricultural wastes) can produce larger yields and have little to no requirements for fertilizer (Lewandowski et al., 2000; McLaughlin and Kszos, 2005; Hoskinson et al., 2007; Heaton et al., 2008b; Seguin, 2011). Considering current biofuel conversion technologies, approximately 50% of the 2009 U.S. corn crop would be required to meet the renewable energy requirements (NASS, 2010). Such extensive use of cropland for biofuel production may drive up food prices and potentially trigger land use change in other parts of the world (Rajagopaul et al., 2007; Searchinger et al.,

J. Environ. Qual. 42:219–228 (2013)
doi:10.2134/jeq2012.0210
Received 18 May 2012.
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Abbreviations: GHG, greenhouse gas; UAN, urea ammonium nitrate.

2008; Tilman et al., 2009; Fargione et al., 2010). However, the production of perennial feedstock crops such as miscanthus and switchgrass, with potential yields greatly exceeding that of corn or soybean, may help to meet energy demands while diverting less land from food production (Heaton et al., 2008a; Heaton et al., 2010). As research and breeding of these crops continues, it is expected that yield and range will also increase (Perlack et al., 2005; Heaton et al., 2008b). Thus, second-generation biofuels from perennial crops have the potential to meet bioenergy goals with less land, thereby reducing risks of increased food prices and land use change.

Concerns have also been raised over the sustainability and environmental impacts of biofuel feedstock crops, particularly corn (Donner and Kucharik, 2008; Fargione et al., 2008; Hill et al., 2009; Melillo et al., 2009). Conventional row crop agriculture relies on intensive management (tillage, fertilizer, and herbicide application) to achieve large grain yields. These management practices can reduce soil C and N pools and increase the loss of available N through nitrate leaching and soil N_2O emissions (Mitchell et al., 2000; David et al., 2009; Hernandez-Ramirez et al., 2009). Nitrate leaching from row crop agriculture in the U.S. Corn Belt is the major contributor to Gulf of Mexico hypoxia, which has large environmental and economic consequences (Goolsby et al., 2001; David et al., 2010). An average of 1.2 million tons of N is supplied to the Gulf of Mexico from the Mississippi River watershed each year, and 80% of that N is from nonpoint sources, primarily agriculture (USEPA, 2007). There are a wide range of management techniques that can better utilize N and decrease N leaching losses (Meisinger and Delgado, 2002), ranging from cover crops and legumes to water table management, along with perennial grasses in Conservation Reserve Program lands. Perennial grasses have been shown to have much smaller nitrate losses compared with traditional row crops (Randall et al., 1997; Mitchell et al., 2000; Jordan et al., 2007; Costello et al., 2009). For example, in a side-by-side field trial of switchgrass, miscanthus, and a corn–soybean rotation, McIsaac et al. (2010) reported significantly less nitrate leaching at a soil depth of 50 cm from the perennial grasses compared with the conventional row crop treatment.

Row crop agriculture is also a major contributor of nitrous oxide (N_2O) to the atmosphere (IPCC, 2007; Ma et al., 2010; Hoben et al., 2011). Crutzen et al. (2008) warned that N_2O emissions from first-generation biofuel crops (e.g., corn) could contribute more CO_2–equivalent GHG to the atmosphere than would be removed from biomass production, thus negating the GHG benefit of biofuels. Low-input perennial crops hold promise for reducing N_2O emissions because N fertilizer is a key driver of N_2O emissions (Dusenbury et al., 2008). This difference is largely driven by fertilizer input; Hernandez-Ramirez et al. (2009) reported significantly larger N_2O fluxes from fertilized corn than unfertilized soybean or restored prairie grasses. Even in the semiarid region of the northern Great Plains, where N_2O fluxes are expected to be small, unfertilized wheat and alfalfa emitted significantly less N than the various fertilized wheat regimes (Dusenbury et al., 2008). Although a first-order estimate that 1% of applied N is lost as N_2O by either nitrification or denitrification (IPCC, 2006) implies that N_2O emissions will be greatly reduced under low- or no-input bioenergy crops, further research is required to accurately quantify N_2O fluxes from perennial bioenergy crops.

Due to a longer growing season and minimal nutrient requirements, perennial grasses have the potential for large yields and reduced environmental impacts. Perennial grasses typically emerge 1 mo before corn or soybean planting, and senescence occurs approximately 1 to 2 mo after the row crops. This extended growing season allows for the accumulation of more biomass and for extended inorganic N uptake. A mature *Miscanthus* × *giganteus* crop can produce a yield nearly double that of corn (Heaton et al., 2008a). Perennial crops have deep, productive rooting systems that help increase soil C and N stocks (Anderson-Teixeira et al., 2009). Perennial grasses are also able to recycle nutrients by removing nutrients from aboveground tissues at senescence for winter storage in the roots or rhizomes. This reduces the need for fertilizer additions and the cost of production. The conservation of N in perennial grasses compared with corn–soybean rotations could be a major benefit contributing to their sustainability as a biofuel feedstock.

The impacts and benefits of perennial bioenergy crops are likely to vary by species and geographic location; consequently, comprehensive data on the resulting N fluxes when row crop agriculture is converted to perennial biofuel crops are needed. Therefore, the objective of this study was to quantify the major pools and fluxes of N in row crop agriculture compared with three perennial biofuel feedstock crops to evaluate the productivity, sustainability, and environmental impacts of each crop during their establishment phase. Central Illinois provides a landscape dominated by tile-drained row crop agriculture, an ecosystem that dominates the upper Mississippi River Watershed. We conducted a side-by-side trial of three perennial biofuel feedstock crops (*Miscanthus* × *giganteus*, *Panicum virgatum*, and restored prairie) against a conventionally managed corn–corn–soybean (C-C-S) rotation to evaluate changes in the N cycle.

Materials and Methods

Site Description and Experimental Design

Our study site is located at the University of Illinois Energy Farm (40°3′46″ N, 88°11′46″ W, ~220 m above sea level). Four biofuel feedstock crops were chosen for study: miscanthus, switchgrass, restored prairie (28 species; see Zeri et al. [2011] for species composition), and a corn–corn–soybean (C-C-S) rotation. Research plots were established in 2008 in a randomized block design with five replicates (one large plot of 4 ha and four small plots of 0.7 ha for each treatment). Before the establishment of this project, this site supported alfalfa (large plots) and corn and soybeans (small plots), although the site was historically in a traditional corn–soybean rotation. Average annual precipitation for the region from 1981 to 2010 was 1051 mm, and average annual temperature was 11°C (Illinois State Water Survey historic climate data). During the time of this study, precipitation was 1335, 1302, 931, and 759 mm for 2008, 2009, 2010, and 2011, respectively. Average annual temperatures for 2008 to 2011 were between 10 and 11°C. Wet N deposition (NH_4–N + NO_3–N) was obtained from the National Atmospheric Deposition Program at Bondville, Illinois, which is approximately 15 km from the study site. Dry deposition was estimated as 70% of wet (McIsaac et al., 2002; USEPA, 2007).

Soils are all Argiudolls, predominantly Dana silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls), with some Flannagan silt loam (fine, smectitic, mesic Aquic Arguidolls) and Blackberry silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls). All soil series are very deep and formed in loess, but Dana and Blackberry are moderately well drained, and Flanagan is somewhat poorly drained. These soil series at this location are quite similar with small differences (R. Darmody, personal communication), and conventional drainage management reduces the importance of hydrology. Crops were planted and managed according to standard agricultural practices for the region (corn and soybean) or best-known management practices (perennial grasses). Specifically, before planting all crops in 2008, diammonium phosphate, potash, and lime were added by variable-rate technology to achieve uniform soil fertility (pH 6.0; P: 50.4 kg ha^{-1}; K: 336 kg ha^{-1}).

These biofuel feedstock crops are managed to simulate those practices of a typical farm in central Illinois. Corn and miscanthus were planted in 75-cm rows, whereas prairie and switchgrass were broadcast. In 2008, crops were planted on 6 May (corn), 28 May (prairie), 29 May (switchgrass), and 2 to 16 June (miscanthus). Corn was planted using conventional tillage and was fertilized with 28% urea ammonium nitrate (UAN) at time of planting (year 1 = 168 kg N ha^{-1}; year 2 = 202 kg N ha^{-1}; year 4 = 180 kg N ha^{-1}). Fertilization rates were based on recommendations from the Iowa State University Agronomy Extension calculator using the central Illinois dataset (http://extension.agron.iastate.edu/soilfertility/nrate.aspx). Switchgrass and prairie were planted with an oat cover crop in the first growing season (2008) to encourage establishment. Granular urea was broadcast at 56 kg ha^{-1} on 21 Apr. 2010 and 14 Apr. 2011 to the switchgrass plots. This rate was chosen to maximize yield and minimize N losses following Mulkey et al. (2006) and Mitchell et al. (2008). According to the Illinois State Water Survey, 2008 and 2009 were, respectively, the fourth and fifth wettest years on record for Champaign-Urbana, Illinois since recording began in 1889. Due to the unusually heavy spring rainfall in 2008 pushing back the planting date to mid-June, the miscanthus crop in the small plots was poorly established and required replanting the following spring (21–27 May 2009). First-year *Miscanthus* × *giganteus* is sensitive to cold conditions and is not likely to survive soil temperatures below −3°C (Clifton-Brown and Lewandowski, 2000; Farrell et al., 2006). The winter of 2008/2009 had several notably cold weeks that damaged miscanthus rhizomes, leading to extensive death in the large plot. Therefore, replanting of miscanthus in the large plot occurred on 14 to 19 Apr. 2010. Due to replanting of miscanthus in the small and large plots and the growth pattern of the plants, there was not a regular spacing or row pattern for this crop. The second year of corn was planted on 12 May 2009. Soybean was planted on 25 May 2010, and corn was again planted on 11 May 2011. The miscanthus and prairie were not fertilized.

Herbicides were applied to reduce weeds during the early establishment phase. In corn, Lumax (*S*-metolachlor, atrazine, and mesotrione) was incorporated each year at planting. Roundup (glyphosate) was also applied to corn on 2 July 2008. Prowl (endimethalin) and 2,4-D were applied to miscanthus on 16 June 2008 and 16 July 2008, respectively. In 2009, 2,4-D was applied to miscanthus on 5 May, and Bicep II Magnum (metolachlor and atrazine) was applied on 23 May. Miscanthus continued to receive Bicep treatments in the following 2 yr (14 Apr. 2010 and 14 Apr. 2011). Switchgrass plots received 2,4-D on 8 Aug. 2008 and 17 June 2009. The switchgrass did not require herbicide treatment once it was established and matured. The restored prairie plots have not received herbicide treatments.

Corn was harvested on 28 Oct. 2008, 3 Nov. 2009, and 6 Oct. 2011, with soybean harvested on 12 Oct. 2010. The soil was chisel plowed after harvest on 29 Oct. 2008, 12 Nov. 2009, and 11 Oct. 2011. Miscanthus, prairie, and switchgrass were not harvested in the first year; the latter two crops were mowed in 2008 (30 June, 17 July, and 4 Sept.) to aid establishment of the target species. For the 2009 growing season, prairie, miscanthus, and switchgrass were harvested between 19 and 26 Mar. 2010. In 2010, switchgrass was harvested on 17 Nov. 2010, prairie on 19 Nov. 2010, and miscanthus on 19 Mar. 2011. In 2011, prairie was harvested on 18 Nov. 2011, switchgrass on 12 Dec. 2011, and miscanthus on 10 Jan. 2012.

Soil Nitrogen Pools and Fluxes

Baseline soil samples were collected during early spring of 2008 before planting. Samples were collected to a depth of 100 cm and divided by depth (0–10, 10–30, 30–50, and 50–100 cm). To fully characterize the soils in each plot, 10 locations were randomly chosen in each small plot and 40 locations were chosen in each large plot, for a total of 320 total locations. At each location, five 3.2-cm-diameter core samples were taken with a hand corer to a depth of 30 cm and divided into 0- to 10-cm and 10- to 30-cm depths. The five cores were then composited by depth. Additionally, a tractor-mounted Giddings probe was used with plastic tube liners (4.5 cm diameter) and a 3.8-cm-diameter tip. One core was taken at each location and divided by depth as described above. Each sample was weighed after air-drying to calculate bulk density, with a subsample oven dried at 105°C to correct for moisture content. Soil samples were air dried, crushed, and sieved (2 mm). All composited hand core samples (0- to 10-cm and 10- to 30-cm depths) and half of the 30- to 50-cm and the 50- to 100-cm Giddings probe samples (half from each plot were randomly selected) were analyzed for total C and N. Subsamples were finely ground with a modified coffee grinder (Sunbeam Products Inc.) and oven-dried at 65°C for at least 48 h. These samples were analyzed for total C and N concentrations with an elemental analyzer (Elemental Combustion System auto-analyzer, Costech Analytical Technologies, Inc.). Particle size (soil texture) was analyzed on 440 samples by hydrometer for clay and silt, with sand determined by wet sieving through a standard USDA sieve set (Gee and Bauder, 1986). For particle size analysis, we randomly selected three samples per depth in each small plot and 10 samples per depth in each large plot, with 36 samples analyzed in duplicate.

Nitrogen mineralization and nitrification rates were determined using the buried bag technique following Gentry et al. (2001). Paired soil cores were taken at two depths (0–10 cm and 10–30 cm). One set was promptly analyzed for inorganic N. The other set was individually sealed in polyethylene bags, placed back in the soil at the proper depth, and retrieved after approximately 2 wk to 1 mo depending on soil moisture conditions. Net mineralization was estimated from the difference in exchangeable nitrate and ammonium for each incubation period after extraction from field moist soil with 1 mol L^{-1} KCl and colorimetric analysis by flow injection (Lachat QuikChem 8000). All values were corrected for moisture content by oven-drying subsamples at 105°C for 48 h.

Patterned tile drains were installed during the fall of 2007 in the large plots (4 ha) at a spacing of 30.5 m between laterals and a depth of 1 to 1.5 m to allow the collection of drainage water from each crop type. Each of the tile outlets was routed through an Agri-Drain structure with a pressure transducer installed to measure continuous flow (15-min basis). Autosamplers (American Sigma 900MAX Portable Sampler) were used to collect flow-proportional water samples for inorganic N analysis. Nitrate and NH_4 concentrations were determined colorimetrically with flow injection analysis (Lachat QuikChem 8000). Volume-weighted annual nitrate concentrations and annual loads were calculated by linear interpolation of nitrate concentrations and flow.

To examine nitrate leaching in all plots, resin lysimeters (Susfalk and Johnson, 2002) were installed at a depth of 50 cm in the soil profile. Four lysimeters were placed in each small plot, and eight lysimeters were placed in each large plot. Each lysimeter holds 10 g dry resin and has a trapping capacity of 42.5 mEq. Lysimeters were first installed on 20 May 2008 and then removed annually each April in 2009, 2010, and 2011, with a new lysimeter installed to replace the removed one. Due to the cropping characteristics of the bioenergy crops, it is not possible to retrieve the lysimeters more than once a year (spring) without damaging the plants, and longer incubation times (annually) may increase signal-to-noise ratios (Langlois et al., 2003). Nitrate concentrations were obtained using a KCl extraction followed by colorimetric flow injection analysis (Lachat QuikChem 8000). Nitrate leaching rates were calculated as above using flow measurements from the tile drainage.

Nitrous oxide was collected using vented static chambers following Hutchinson and Mosier (1981) and as described in Behnke et al. (2012). The chambers had a base diameter of 20 cm and a volume of 3.6 L. Chambers were placed on PVC ring bases (20 cm diameter, 10 cm height) and inserted in to the soil approximately 5 cm deep. These chamber bases remained in the soil throughout the year and were repositioned annually or as needed for crop management. All vegetation was removed from inside the rings, and rings were positioned between rows in the C-C-S treatment. Six rings were placed in each big plot and two were placed in each small plot for a total of 14 rings per treatment. For gas efflux measurements, chambers were secured on the rings for 30-min incubations. Gas samples were collected every 10 min at 0, 10, 20, and 30 min. Fifteen-milliliter samples were then transferred to previously evacuated 10-mL vials sealed with molded gray butyl rubber, Teflon-coated septa (Sun SRI). Sampling occurred midday to minimize variability due to soil temperature. Soil moisture and temperature were concurrently measured with chamber incubations. Gas samples were analyzed within 1 wk by gas chromatography with a Shimadzu 2014 Greenhouse Gas Analyzer (Shimadzu Scientific Instruments). Gas fluxes were calculated from chamber gas concentrations. Sampling began in early spring during the first significant thaw event (early March). Sampling continued throughout the springtime and in to summer at a rate of once every 2 wk or more frequently surrounding fertilization events. Sampling slowed in the summer and into fall to once every 3 to 4 wk. An average of 20 sampling events occurred each year (22 in 2009, 26 in 2010, and 18 in 2011). Cumulative N_2O emissions were calculated by linear interpolation of the days between our field measurements.

Aboveground Biomass

Vegetation samples were collected during harvest (dates are provided above and varied due to crop physiology and environmental conditions). Bulk samples were collected from a Cibus S plot harvester (Wintersteiger) that made passes through the plots that were 20 m in length and ~1 m in width and measured the fresh weight of the biomass. In 2009, there were two or three passes per plot; in 2010 and 2011, there were four passes per plot. Subsamples were collected during this harvest and oven dried at 60°C until constant mass. Subsamples were ground to a powder with the Genogrinder 2000 laboratory mill (OPS Diagnostics) followed by elemental analysis as described above for soils. Nitrogen fixation was not measured in any of the cropping systems. Soybean N_2 fixation was estimated at 60% of above-ground biomass N following David et al. (2010).

Statistical Analyses

Annual harvest yields, resin lysimeter nitrate fluxes within crop types and across years, and annual cumulative N_2O fluxes were analyzed for significant differences using the general linear models procedure in SAS 9.2 (SAS Institute, 2008), with differences in means accepted at a probability level of $p < 0.05$.

Results

Initial Soils and Crop Yields

Soils in each plot had a silt loam texture in the top 30 cm, with a silty clay loam texture below 30 cm, and were consistent across all treatments (Table 1). Surface soil had organic C concentrations of 15.4 to 17.5 g C kg^{-1} in the upper 30 cm, declining to as low as 4.3 g kg^{-1} at 50 to 100 cm depth. Total N followed the same pattern as organic C, with C:N ratios (mass $mass^{-1}$) of 11.6 in the top 30 cm, declining to 8.3 in the deeper soil. Organic C pools averaged 134 Mg C ha^{-1} in the top meter of soil, with a total N pool for the same depth of 13.2 Mg N ha^{-1}.

Crop yields steadily increased for each of the perennial biofuel crops through years 2 to 4 after establishment, except for prairie, which decreased slightly in 2011 (Table 2). Miscanthus biomass lagged switchgrass and prairie in 2009 due to establishment problems but by 2011 was significantly outyielding the other perennials. By 2010, the C:N ratio of harvested miscanthus was 143, nearly twice that of switchgrass and prairie. The C:N ratio of miscanthus increased to 256 for the 2011 harvest, with switchgrass and prairie also increasing (135 for both crops).

Environmental Nitrogen Losses

Nitrate leaching under each biofuel crop at a soil depth of 50 cm showed that in the first year of growth (2008–2009) all crops had large leaching fluxes, ranging from 23 to 75 kg N ha^{-1} yr^{-1} (Fig. 1). Miscanthus had the largest flux due to the poor establishment and lack of plant uptake. Corn had a flux of 49 kg N ha^{-1} yr^{-1}, with switchgrass and prairie having less than half that amount. By the next year, all switchgrass and prairie plots had significantly declined to between 3.5 and 8 kg N ha^{-1} yr^{-1},

Table 1. Mean soil organic C and total N concentrations, particle size, bulk density, and mass of C and N by depth at the start of the study in 2008.

Treatment	Depth	Organic C	Total N	Sand	Silt	Clay	Bulk density	Organic C	Total N
	cm	g kg^{-1}			%		g cm^{3}	Mg ha^{-1}	
Corn	0–10	16.6	1.43	18	61	22	1.2	19.5	1.7
	10–30	15.4	1.36	16	61	23	1.3	40.3	3.6
	30–50	10.6	1.04	12	58	30	1.5	32.1	3.1
	50–100	4.4	0.54	17	52	32	1.7	36.3	4.5
Miscanthus	0–10	17.1	1.50	16	62	22	1.2	20.5	1.8
	10–30	15.7	1.36	15	60	24	1.3	40.9	3.6
	30–50	11.0	1.07	12	58	30	1.5	33.1	3.2
	50–100	4.7	0.55	11	56	33	1.7	38.9	4.6
Prairie	0–10	17.4	1.53	17	60	22	1.2	21.4	1.9
	10–30	17.1	1.46	14	62	25	1.4	46.9	4.0
	30–50	11.5	1.08	16	55	29	1.5	34.8	3.3
	50–100	4.9	0.56	15	51	35	1.6	39.6	4.6
Switchgrass	0–10	17.5	1.50	18	60	23	1.2	20.3	1.7
	10–30	16.1	1.39	16	59	26	1.3	42.8	3.7
	30–50	10.7	1.02	13	58	30	1.6	33.6	3.2
	50–100	4.3	0.51	15	54	31	1.7	35.8	4.3

Table 2. Crop yields, total C and N, and C:N ratio of harvested above-ground biomass.

Year	Crop	Yield	Total C	Total N	C:N Ratio
		Mg ha^{-1}	g kg^{-1}		
2009	switchgrass	3.22 (0.3)b†‡	447 (2)	6.3 (0.2)	72.3 (2.3)
	prairie	2.85 (0.3)b	443 (3)	7.3 (0.3)	61.6 (2.5)
	miscanthus	1.12 (0.4)c	421 (23)	6.8 (0.6)	63.2 (3.2)
	corn	9.54 (1.1)a	422 (1)	14 (0.3)	30.7 (0.8)
2010	switchgrass	7.61 (0.3)a	452 (1)	6.0 (0.4)	77.6 (6.0)
	prairie	5.36 (0.4)b	441 (2)	6.0 (0.2)	73.5 (1.8)
	miscanthus	7.76 (1.2)a	457 (1)	3.6 (0.5)	143 (13.7)
	soybean	2.76 (0.2)c	500 (3)	56 (1.5)	9.0 (0.2)
2011	switchgrass	7.74 (0.3)b	441 (1)	3.4 (0.2)	135 (9.8)
	prairie	3.98 (0.3)c	425 (2)	3.2 (0.1)	135 (3.8)
	miscanthus	11.8 (1.1)a	449 (5)	1.8 (0.1)	257 (11.4)
	corn	8.83 (0.1)b	413 (4)	13 (1.0)	32.2 (2.8)

† Values are means with SE in parentheses.

‡ Within a year, yields with the same lowercase letter are not significantly different at the 0.05 level.

and miscanthus had significantly declined to 30 kg N ha^{-1} yr^{-1}, a little less than corn. In 2010, the soybean treatment was at 43 kg N ha^{-1} yr^{-1}, with miscanthus declining to 17 kg N ha^{-1} yr^{-1}, switchgrass at 8.3 kg N ha^{-1} yr^{-1}, and prairie at 2.8 kg N ha^{-1} yr^{-1}. In the fourth year, nitrate leaching fluxes at 50 cm were <5 kg N ha^{-1} yr^{-1} in all perennial crops, but corn was nearly 50 kg N ha^{-1} yr^{-1}. These results show the rapid decline in mid-soil profile nitrate leaching in perennial crops, with a lag in miscanthus due to establishment problems and steady leaching in corn and soybeans.

Nitrate concentrations in tile drainage showed a striking pattern of decline from production of perennial biofuel crops from 2008 through 2011 (Fig. 2). In 2008, concentrations increased in all crops during the growing season, likely reflecting the previous cropping history (alfalfa) and the preparation (plowing), planting, and initial establishment of all crops. Concentrations reached as high as 25 mg N L^{-1} in the corn plot in 2008. In 2009, miscanthus and corn remained at about 20 mg N L^{-1} for much of the growing season, with miscanthus beginning to decline by August. In 2010, soybean nitrate concentrations were steady at about 8 mg N L^{-1}, miscanthus at 5 mg N L^{-1}, with switchgrass and prairie at <1 mg N L^{-1}. By the fourth year of production, corn again showed a steady

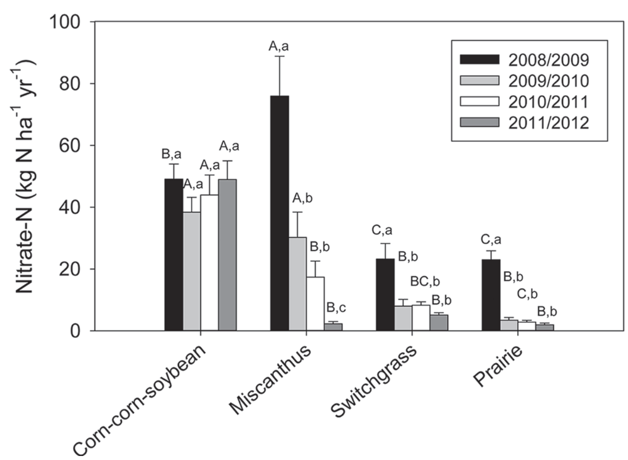

Fig. 1. Annual nitrate N leaching (April to April) at 50 cm soil depth using resin lysimeters for all biofuel crops (mean ± SE). Means by year across crop types with the same capital letter are not significantly different at the 0.05 level. Means by crop type across years with the same lowercase letter are not significantly different at the 0.05 level.

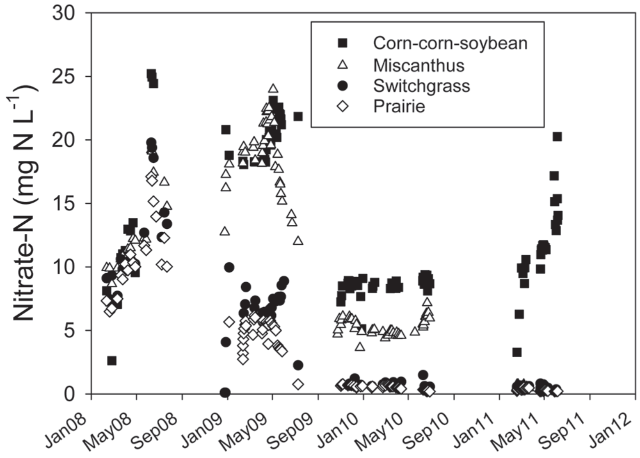

Fig. 2. Nitrate concentrations in tile water during 2008 through 2011 by biofuel crop. Rotation was in corn in 2008 and 2009, soybean in 2010, and corn in 2011.

increase early in the year to >20 mg N L^{-1} with a volume-weighted concentration of 11.8 mg N L^{-1}, whereas all perennial crops were <0.5 mg N L^{-1} (volume-weighted concentrations were 0.5, 0.6, and 0.3 for miscanthus, switchgrass, and prairie, respectively). Nitrate leaching rates vary annually due to the amount, timing, and intensity of precipitation events and are often driven outside the growing season (Randall and Mulla, 2001). Annual precipitation varied from 1335 mm in 2008 to 759 mm in 2011. Tile drainage flow also varies in response to the timing and intensity of precipitation events. In 2009, the year of highest annual precipitation, prairie tile drainage was 24.2 cm; switchgrass, C-C-S, and miscanthus drained similar amounts (15.4, 13.8, and 17.9 cm, respectively). In 2010, drainage values were 12.1, 10.5, 7.0, and 13.9 cm in prairie, switchgrass, C-C-S, and miscanthus, respectively. We found that a small swale was directing some surface water onto the prairie plot, which was redirected off the plot after the 2010 growing season. In 2011 drainage values were 15.0, 8.3, 5.9, and 16.3 cm in prairie, switchgrass, C-C-S, and miscanthus, respectively.

Chamber-based gas sampling for soil N_2O emissions was initiated in 2009. Little N_2O emission was measured in any of our samples from the perennial grasses in each year of measurement, with the exception of miscanthus in 2010 (Fig. 3). Large fluxes were measured in June 2010 in miscanthus, and this likely reflected disturbance (with increased nitrate concentrations) from the replanting that took place. The largest fluxes during the study were the 2 wk after corn fertilization in 2009, with several days of fluxes above 10 μg N m^{-2} min^{-1}. A secondary pulse of N_2O also was measured for corn in late June after a period of high rainfall. Although switchgrass was fertilized in 2010 and 2011, we did not measure high N_2O fluxes on any of our sampling dates.

On a mass basis calculated for 2009, C-C-S (corn) emitted significantly more N_2O (7.7 kg N ha^{-1} yr^{-1}) than miscanthus, switchgrass, or prairie (1.4, 1.4, and 0.7 kg N ha^{-1} yr^{-1}, respectively), which were not significantly different from each other (Fig. 4). More than 80% of the 2009 annual N_2O emission for C-C-S (corn) occurred within 14 d after fertilization. All annual N_2O emission rates were lower in 2010 but followed similar trends as the previous year. The C-C-S (soybean)

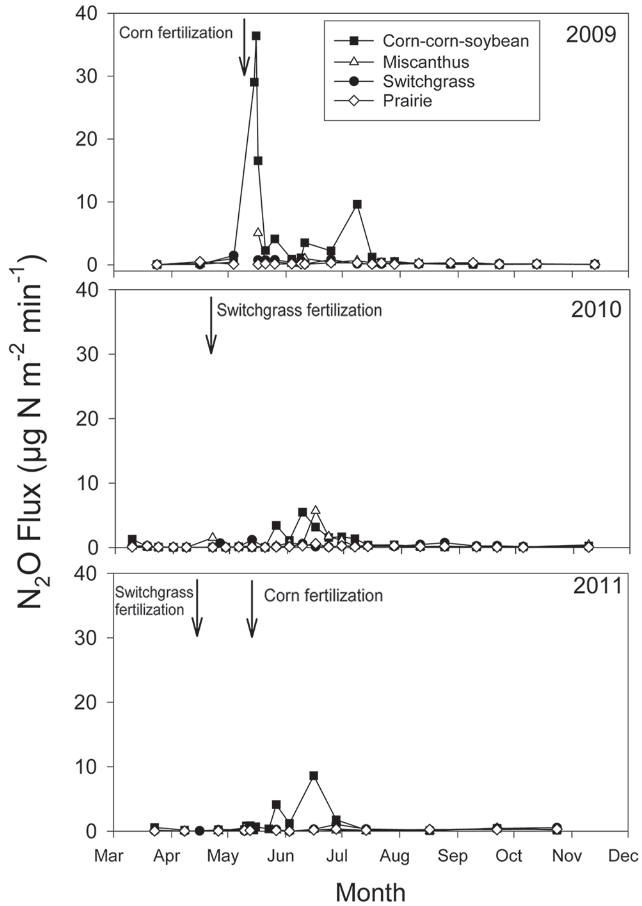

Fig. 3. Nitrous oxide fluxes from chamber measurements during 2009 through 2011 for each biofuel crop. Rotation was in corn in 2009, soybean in 2010, and corn in 2011.

emitted the largest amount of N_2O (2.2 kg N ha^{-1} yr^{-1}), whereas fertilized switchgrass emitted less than half as much N_2O (0.8 kg N ha^{-1}). Miscanthus and prairie emitted 1.4 and 0.4 kg N ha^{-1} yr^{-1}, respectively. Because of variability, the only significant differences were between soybean and prairie in 2010. For 2011, C-C-S (corn) again significantly emitted the largest flux of N_2O (3.5 kg N ha^{-1} yr^{-1}). Switchgrass was the highest perennial feedstock N_2O producer (1.0 kg N ha^{-1} yr^{-1}), followed by miscanthus and prairie (0.6 kg N ha^{-1} yr^{-1} for both), with none of the perennial crops significantly different from each other.

Overall Nitrogen Fluxes

We developed overall N budgets for each crop, with most measurements made in 2009 through 2011 (Table 3). Fertilizer dominated the inputs for C-C-S and switchgrass, followed by estimated N_2 fixation in soybean, with atmospheric deposition ranging from 5.8 to 10.4 kg N ha^{-1} yr^{-1} for all crops. Nitrate leaching losses, as measured by tile drainage, were greatest for C-C-S (7–20 kg N ha^{-1} yr^{-1}) and declined through establishment to 0.4 to 0.8 kg N ha^{-1} yr^{-1} for miscanthus, switchgrass, and prairie. These fluxes were much less than the resin lysimeter data at 50 cm, the latter of which are useful for comparing N cycling within plots but are typically much greater than tile losses. In addition, the C-C-S always had the smallest drainage water flux (see above) but still had a larger nitrate flux in 2010 and 2011 compared with all perennials. Nitrous oxide fluxes were 0.4 to

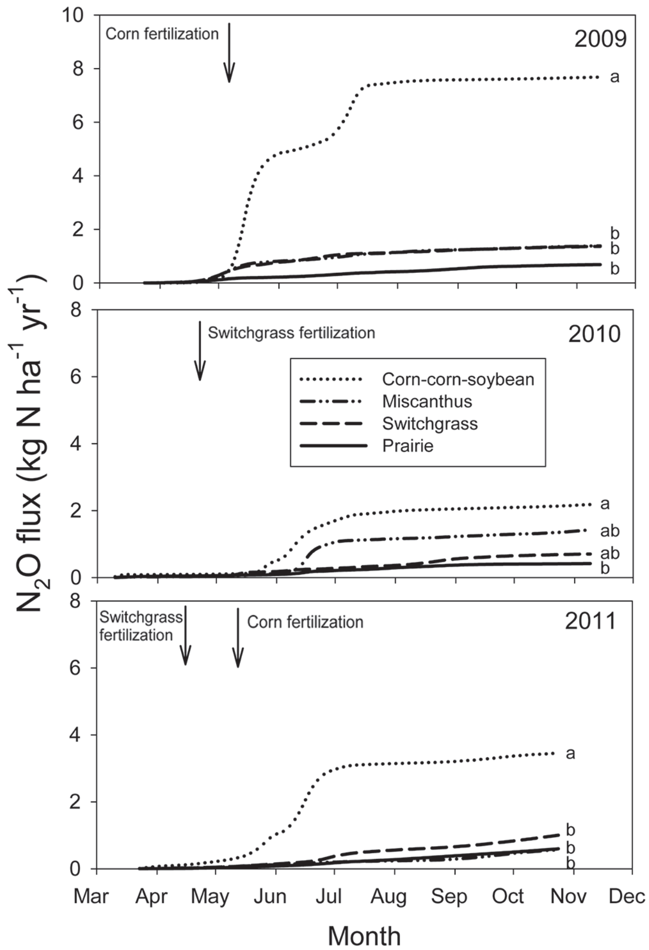

Fig. 4. Cumulative N_2O fluxes during 2009 through 2011 by biofuel crop. Rotation was in corn in 2009, soybean in 2010, and corn in 2011. Final cumulative means by crop type with the same lowercase letter are not significantly different at the 0.05 level.

7.7 kg N ha^{-1} yr^{-1}, again with C-C-S having much greater fluxes. The largest N output was harvested biomass, with >120 kg N ha^{-1} yr^{-1} removed in C-C-S but only 8 to 46 kg N ha^{-1} yr^{-1} in the perennial grasses, reflecting their wide C:N ratios. We also measured net mineralization of soil organic N and nitrification (Fig. 5). Nitrification was much greater in C-C-S due to N fertilization, with much greater mineralization after the soybean year (Table 3). As a result of the disturbance from replanting, net mineralization and nitrification were much greater in miscanthus in 2009 compared with switchgrass and prairie. In addition, our N mineralization results shows that microbial release of N occurs throughout the growing season, and the longer active growing period of the perennial crops was able to take advantage of this nitrate, thus limiting leaching losses. However, the problems with miscanthus establishment showed how mineralized soil organic N can lead to greater leaching of nitrate as well as enhanced N_2O release, without fertilizer additions, until plant uptake can take advantage of the available N.

Discussion

Our results clearly demonstrate that environmental N fluxes from row crop agriculture can be greatly reduced after establishment of perennial biofuel crops. Nitrate leaching and N_2O emissions were much less in miscanthus, switchgrass, or prairie compared with corn and soybeans. At harvest, all the perennial feedstocks contained less N than corn or soybeans because perennial grasses efficiently recycle nutrients by removing them from the aboveground biomass to the roots or rhizomes at senescence. All perennial feedstocks contained less than 8 g kg^{-1} N in above-ground harvest biomass, whereas 2009 corn had 14 g kg^{-1} N, 2010 soybean had 56 g kg^{-1} N, and 2011 corn had 13 g kg^{-1} N. Even taking into consideration yields, corn and soybean removed much more N in the harvested biomass than all three perennial grasses. The 3-yr (2009–2011) N balance was 22 kg N ha^{-1} yr^{-1} for the corn and soybean rotation and averaged 9.7 kg N ha^{-1} yr^{-1} for switchgrass but was −18 and −29 kg N ha^{-1} yr^{-1} for the unfertilized perennial crops (prairie and miscanthus, respectively). The source of the N in prairie and miscanthus biomass could be from mineralized soil organic N, where the pool size is large (~13,000 kg N ha^{-1} in the top m of soil), or from biological N_2 fixation (Davis et al., 2010). Therefore, although the establishment of perennial grasses for biofuel feedstock can greatly reduce N losses and the

Table 3. Nitrogen fluxes for the first (2008) through the fourth (2011) year of biofuel feedstock crop establishment.

	Corn-corn-soybean				Miscanthus				Switchgrass				Prairie			
	C	C	S	C												
	2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
	kg N ha^{-1} yr^{-1}															
Inputs																
Fertilizer	168	202	0	180	0	0	0	0	0	0	56	56	0	0	0	0
Atm. deposition	9.5	10.4	7.0	5.8	9.5	10.4	7.0	5.8	9.5	10.4	7.0	5.8	9.5	10.4	7.0	5.8
N_2 fixation			114													
Total in	178	212	121	186	9.5	10.4	7.0	5.8	9.5	10.4	63	61.8	9.5	10.4	7.0	5.8
Outputs																
N Leaching	NA†	21	9.8	7.0	NA	22	11	0.8	NA	8.1	1.7	0.5	NA	8.8	1.1	0.4
N_2O efflux	NA	7.7	2.2	3.4	NA	1.4	1.4	0.6	NA	1.4	0.8	1.0	NA	0.7	0.4	0.6
Harvest biomass	NA	133	152	116	NA	8.0	28	37	NA	20	46	26	NA	21	32	13
Total out	NA	162	164	126	NA	31	40	38	NA	30	48	28	NA	30	34	14
Other																
Net mineralization	NA	40	97	186	NA	107	79	58	NA	46	28	70	NA	42	56	70
Nitrification	NA	134	113	208	NA	114	83	56	NA	48	32	73	NA	44	40	63

† Not available in 2008.

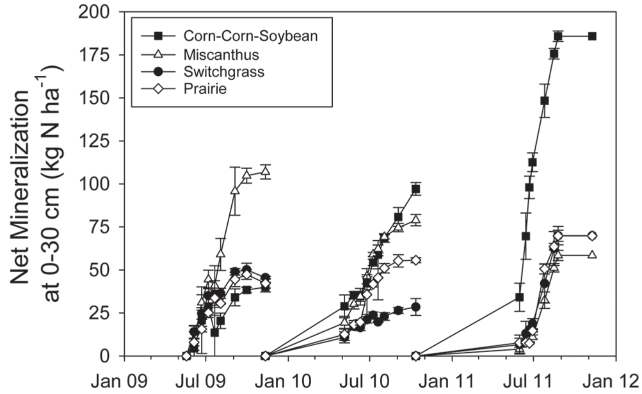

Fig. 5. Soil net mineralization of N during 2009 through 2011 by biofuel crop for the top 30 cm of soil. Rotation was in corn in 2009, soybean in 2010, and corn in 2011.

need for large fertilizer applications, without some additional N source, sustainability in terms of N inputs and outputs would not be achieved.

Environmental variability also provided us with the opportunity to demonstrate how quickly perennial biofuel crops decrease nitrate leaching from tile-drained agricultural fields. Miscanthus failed to adequately establish during the first 2 yr of the project, allowing a comparison of annual row crops (C-C-S) with establishing and non-established perennial biofuel feedstocks. Although nitrate leaching remained relatively high in the non-established miscanthus crop for the first 2 yr, the tile nitrate flux quickly decreased to <1 kg N ha^{-1} yr^{-1} once a dense, productive crop was established in the second year of growth, as did the nitrate flux at 50 cm. Switchgrass and prairie established more quickly, and their nitrate leaching fluxes at both 50 cm and in tile drainage quickly decreased in the second year of growth. Behnke et al. (2012) measured nitrate leaching using resin lysimeters in fertilized miscanthus in central Illinois (60 and 120 kg N ha^{-1} yr^{-1}) and found increased nitrate leaching with larger amounts of fertilizer compared with a much smaller flux in the unfertilized control. This indicates that even with this perennial crop, nitrate concentrations in soil solution at 50 cm depth can exceed the uptake ability of miscanthus when fertilized (Behnke et al., 2012). We were also able to examine leaching rates from fertilized switchgrass (56 kg ha^{-1} yr^{-1} granular urea beginning in 2010); no measureable increase in N loss was detected compared with the previously unfertilized switchgrass. McIsaac et al. (2010) reported similar results of significantly less nitrate leaching (using resin lysimeters at 50 cm) from mature switchgrass and miscanthus crops (1.4 and 3.0 kg N ha^{-1} yr^{-1}) compared with a corn–soybean rotation (40.4 kg N ha^{-1} yr^{-1}). Hernandez-Ramirez et al. (2011) also observed a significant decrease in nitrate from tile drained restored prairie compared with corn fertilized with UAN. In their study, UAN was applied at several different rates and timings, yet the difference in N leaching between these treatments was insignificant. Prairie lost an average of 2.5 kg N ha^{-1} yr^{-1}, whereas corn treatments lost 19.9 kg N ha^{-1} yr^{-1}. Randall et al. (1997) conducted a study comparing N leaching from conventional row crop systems to perennial grasses on Conservation Reserve Program land and alfalfa. Continuous corn and the corn–soybean rotation had approximately 35 and 37 times more nitrate leaching than the perennial crops. The results of these studies are within the same range as our results and demonstrate that unfertilized perennial plant systems can quickly decrease nitrate leaching fluxes in tile drainage.

Excess N and P flowing down the Mississippi and Atchafalaya Rivers is largely responsible for the Gulf of Mexico hypoxia (Goolsby et al., 2001; USEPA, 2007). The majority of these nutrients originate from the Corn Belt, which is dominated by tile drainage (Goolsby et al., 2001; Costello et al., 2009; David et al., 2010). David et al. (2010) showed that the majority of nitrate entering the Gulf of Mexico is from tile drained agricultural fields in the Upper Midwest. Recently, Sprague et al. (2011) presented evidence suggesting that elevated N concentrations in groundwater (likely resulting from annual row crop leaching) may also be responsible for much of the Mississippi River nitrate load and is a source that is increasing in importance. They argue that it could take decades or longer to remediate riverine nitrate concentrations originating from groundwater. Our findings show that a large amount of nitrate was exported from the C-C-S treatment, consistent with other studies, especially after large precipitation events just after planting/fertilization. More importantly, we show how rapidly nitrate concentrations can decrease in tile drainage from conversion to perennial crops. Several other studies have also reported that perennial grasses reduce nitrate leaching rapidly (Randall et al., 1997; Mitchell et al., 2000; Jordan et al., 2007; Hernandez-Ramirez et al., 2009; McIsaac et al., 2010). Therefore, we believe the establishment of perennial biofuel feedstock crops would quickly reduce N exports from tile-drained fields and, if widely planted in the Midwest United States, could mitigate hypoxia in the Gulf of Mexico (Davis et al., 2011).

Nitrous oxide emissions play a large role in determining the sustainability of biofuel crops through GHG emissions. The primary GHG benefit of biofuels is the conversion of CO_2 to biomass and the subsequent combustion of ethanol produced by that biomass. This reduces GHGs in the atmosphere if more GHGs are not emitted during crop growth or production processes. However, agricultural soils are the dominant source of terrestrial N_2O emissions (IPCC, 2007). The primary drivers of N_2O fluxes in this region are available N as nitrate, temperature, and moisture. The largest N_2O fluxes measured in this study were in the spring after fertilization and a substantial, prolonged precipitation event. Annual cumulative N_2O emissions from the three perennial biofuel crops in this study were greatly reduced compared with the conventional row crop (C-C-S), even though switchgrass was fertilized (beginning in 2010). However, in 2011 when both switchgrass and corn were fertilized, 1.8 and 1.9%, respectively, of the fertilizer was emitted as N_2O, both much greater than the IPCC default value of 1% (IPCC, 2006). In the much wetter year of 2009, 3.8% of the corn fertilizer was measured as N_2O; switchgrass was not fertilized that year. However, cumulative switchgrass N_2O emissions were not significantly different from the unfertilized miscanthus and prairie, so we could not detect a difference due to fertilization. Behnke et al. (2012) measured N_2O fluxes from fertilized miscanthus and measured greater N_2O emissions from fertilized plots (1.3–2.9 kg N ha^{-1} yr^{-1}), with unfertilized N_2O fluxes <1.0 kg N ha^{-1} yr^{-1}, similar to our results in 2011 miscanthus. Our results also agree with

Hernandez-Ramirez et al. (2009), who compared a restored prairie with continuous corn and a corn–soybean rotation with numerous fertilization treatments. Restored prairie produced <1 kg N ha^{-1} yr^{-1} N_2O, whereas the corn–soybean rotation fertilized with 135 kg N ha^{-1} UAN (the most similar treatment to our study) averaged 5 kg N ha^{-1} yr^{-1}. In a fertilizer trial on corn, Hoben et al. (2011) reported exponentially increasing N_2O emissions from increasing fertilization rates for corn in Michigan. Their study highlights one of the potential negative consequences of corn ethanol that can undermine the original GHG benefits of biofuels.

Conclusions

A great deal of attention has been paid to the C benefits of biofuel feedstocks, but the reductions of N loss (as shown here) from certain second-generation biofuel feedstocks (e.g., perennial grasses) in the US corn belt are equally important for reducing GHG emissions and could be an important factor for reducing N entering the Gulf of Mexico. Concerns have been raised over sustainability, environmental degradation, and food security associated with the use of traditional row crops for biofuels. Our study is in agreement with a review by Delgado et al. (2011) that examined conservation practices to mitigate and adapt to climate changes. They proposed several principles for soil and water conservation for climate change mitigation and adaptation, especially the key principle of valuing perennial bioenergy crops such as switchgrass. As our results demonstrate, high-yielding perennial grasses used for biofuel feedstock have the potential to greatly reduce N losses that create important environmental and economic problems. Although current biofuel policies create incentives for the establishment of these crops, they do not take into consideration the economic benefits of reduced N losses. Incentivizing the reduction of agricultural N losses through the use of perennial grasses as biofuel feedstocks could have major environmental benefits for water and air quality.

Acknowledgments

The authors thank Tom Voigt and Emily Thomas for providing harvest yield data and Tim Mies for help with Energy Farm logistics. This work was funded by the Energy Biosciences Institute.

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