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Productivity, ^{15}N dynamics and water use efficiency in low- and high-input switchgrass systems

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Abstract

Sustainable and environmentally benign switchgrass production systems need to be developed for switchgrass to become a large-scale dedicated energy crop. An experiment was conducted in California from 2009 to 2011 to determine the sustainability of low- and high-input irrigated switchgrass systems as a function of yield, irrigation requirement, crop N removal, N translocation from aboveground (AG) to belowground (BG) biomass during senescence, and fertilizer ^{15}N recovery (FNR) in the AG and BG biomass (0–300 cm), and soil (0–300 cm). The low-input system consisted of a single-harvest (mid-fall) irrigated until flowering (early summer), while the high-input system consisted of a two-harvest system (early summer and mid-fall) irrigated throughout the growing season. Three N fertilization rates (0, 100, and 200 kg N ha^{-1} yr^{-1}) were applied as subtreatments in a single application in the spring of each year. A single pulse of ^{15}N enriched fertilizer was applied in the first year of the study to micro-plots within the 100 kg N ha^{-1} subplots. Average yields across years under optimal N rates (100 and 200 kg ha^{-1} yr^{-1} for low- and high-input systems, respectively) were 20.7 and 24.8 Mg ha^{-1}. However, the low input (372 ha mm) required 47% less irrigation than the high-input system (705 ha mm) and achieved higher irrigation use efficiency. In addition, the low-input system had 46% lower crop N removal, 53% higher N stored in BG biomass, and a positive N balance, presumably due to 49% of ^{15}N translocation from AG to BG biomass during senescence. Furthermore, at the end of 3 years, the low-input system had lower fertilizer ^{15}N removed by harvest (26%) and higher FNR remaining in the system in BG biomass plus soil (31%) than the high-input system (45% and 21%, respectively). Based on these findings, low-input systems are more sustainable than high-input systems in irrigated Mediterranean climates.

Keywords: ^{15}N recovery, bioenergy, biomass yields, high-input system, irrigation use efficiency, low-input system, N fertilization, N use efficiency, switchgrass

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Introduction

Switchgrass (*Panicum virgatum* L.) is a dominant native perennial warm-season C$_4$ grass in the North American tallgrass prairie (USDA, NRCS, 2006). Switchgrass has received considerable attention as one of the most promising energy crops, due to its high yield potential, excellent conservation attributes, good compatibility with conventional agricultural practices, relative ease of establishment, high seed production, adaptability to marginal areas, and high N use efficiency (McLaughlin *et al.*, 1999; McLaughlin & Kszos, 2005). Nevertheless, switchgrass biomass yields and crop N removal vary widely, depending on varietal selection, N fertilization, precipitation, stand age, and number of harvests per year (Sanderson *et al.*, 1999; Heaton *et al.*, 2004; Pedroso *et al.*, 2011). Observed biomass yields range from 5.5 to 39.4 Mg ha^{-1} and crop N removal from 28 to 234 kg N ha^{-1} yr^{-1} (McLaughlin *et al.*, 1999; Vogel *et al.*, 2002; Pedroso *et al.*, 2013).

In addition to the direct offset of greenhouse gases emissions through reductions in fossil fuel consumption, the use of switchgrass as an energy crop has the benefit of increasing carbon (C) sequestration in soils (Lemus & Lal, 2005; Garten *et al.*, 2010). The rate of C sequestration is particularly related to the belowground (BG) biomass rather than aboveground (AG) biomass, because in energy production systems most of the AG biomass is harvested and removed from the field. Switchgrass has a deep root system that can exceed 3 m deep (Ma *et al.*, 2000). Observed total BG biomass range from 1.24 to 3.63 kg m^{-2} in the first 150 cm of soil (Ma *et al.*, 2000; Garten *et al.*, 2010), with the crowns accounting for up to 50% of total biomass (Frank *et al.*, 2004).

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Nitrogen management is an important consideration in developing sustainable, energy efficient, and environmentally benign cropping systems dedicated to energy production (Guretzky *et al*., 2011). Fertilizer N is the main energy input and source of greenhouse gases emissions during switchgrass cultivation for biofuel purposes (Adler *et al*., 2007; Schmer *et al*., 2008). The N fertilizer requirement of switchgrass is dependent, in part, on the amount of crop N removal at harvest, which is related to the number and timing of annual harvest operations (Reynolds *et al*., 2000; Heaton *et al*., 2009). In addition, harvest practices also affect biomass biofuel quality (Adler *et al*., 2006), such as biomass moisture content and mineral concentration. Biomass moisture content affects transportation costs, safety in storage, and combustion efficiency (Lewandowski & Kicherer, 1997), while high N concentration can cause char formation (Rejai *et al*., 1992), corrosion and increased NO_x emissions (Jorgensen, 1997) during the thermochemical conversion of biomass into ethanol.

Multiple-harvest systems remove more N than a single harvest following plant senescence (Vogel *et al*., 2002). Warm-season perennial grasses translocate N during senescence from AG to BG biomass (Clark, 1977). For example, Heckathorn & DeLucia (1994) reported that 30% of the N was translocated BG in C_4 perennial grasses in response to drought stress. Translocation of N during senescence reduces the concentration of N in the AG biomass, thereby decreasing crop N removal through harvest. Nitrogen translocation within the plant is also indicated by seasonal fluctuations of N concentration and storage in BG biomass of switchgrass (Lemus *et al*., 2008; Dohleman *et al*., 2012). However, fluctuations in BG biomass N concentration could be a result of N dilution due to BG biomass increases or N uptake from the soil (Lemus *et al*., 2008). The use of ^{15}N enriched fertilizer allows for the determination of flow and fate of applied N in different sinks, such as in the AG biomass, BG biomass, and soil. In addition, it allows for the quantification of N losses from the plant–soil system, a key indicator of cropping system N use efficiency and sustainability (Dourado-Neto *et al*., 2010).

Switchgrass has high yield potential in California, with yields up to 39.4 Mg ha^{-1} yr^{-1} in irrigated two- and three-harvest systems (Pedroso *et al*., 2011, 2013). However, these multi-harvest systems resulted in high crop N removal up to 364 kg N ha^{-1} yr^{-1} and required use of irrigation throughout the entire growing season. In addition, the second and third harvests accounted for only 30% of the annual biomass yield. Reports have shown 8–36% higher yields in two- vs. single-harvest systems during the first 5 years of switchgrass production (Fike *et al*., 2006a, b). However, differences between harvest systems decreased over time (Fike *et al*., 2006a). While yields are generally lower in single-harvest systems, a single harvest performed in fall would allow for N translocation during senescence, decreasing crop N removal, and potentially reducing fertilizer N requirements (Sanderson *et al*., 1999; Vogel *et al*., 2002; Parrish & Fike, 2005; Guretzky *et al*., 2011). In addition, the translocation of N may result in higher N storage in BG biomass and in the soil, further decreasing N input requirements and increasing long-term sustainability. Moreover, biomass accumulation in switchgrass ceases after floral development (Parrish & Fike, 2005). Therefore, the use of irrigation could be stopped without decreases in biomass yield, potentially leading to significant water savings.

The main objective of this study was to develop more sustainable switchgrass production systems for energy production. We hypothesized that a partially irrigated single-harvest system (i.e., 'low-input system') would be more sustainable than a two-harvest system irrigated during the entire growing season (i.e., 'high-input system') due to relatively high yields, lower irrigation requirements, and significantly lower crop N removal and fertilizer N requirements. The specific objectives of this study were to (i) measure biomass yields and crop N removal of low- and high-input switchgrass grown under different rates of N fertilization; (ii) determine N translocation during senescence and N storage in BG biomass; and (iii) determine irrigation use efficiency (IUE) and fertilizer ^{15}N recoveries (FNR) in the plant–soil system.

Materials and methods

Crop establishment and management

The experiment was conducted at the UC Davis Agronomy Field Headquarters in Davis (38.5°N; 121.7°W), situated in the Central Valley of California. Davis is located in a warm Mediterranean climate (CSa) (Peel *et al*., 2007), exhibiting warm and dry summers and cool and wet winters, with average annual precipitation of 445 mm. Precipitation and potential evapotranspiration (ETo) data for the duration of the experiment were obtained from the California Irrigation Management Information System - CIMIS (Table 1). The soil type at the experiment is classified as a very deep well-drained Brentwood silty clay loam, with a water table at more than 200 cm. Four soil samples of 15 cm deep were taken prior to field establishment in 2008 and composited into one sample. The samples were air dried, ground through a 2 mm mesh screen, and analyzed for pH (saturated paste extract), NO_3-N, Olsen-P, extractable-K, cation exchange capacity (CEC), organic matter (Walkley-Black), particle size, and total N (Table 2).

The field was disked prior to field establishment in July of 2008 to provide a firm and fine seedbed for the seeding operation. In August of 2008, the lowland southern switchgrass variety 'Alamo' was broadcast seeded at a rate of 7.2 kg ha^{-1} of pure live seeds. The field was sprinkler irrigated following

Table 1 Monthly precipitation and evapotranspiration from 2008 to 2011 at Davis, CA

	Precipitation (mm)				Evapotranspiration (mm)			
Month	2008	2009	2010	2011	2008	2009	2010	2011
January	209	29	160	43	29	42	17	26
February	58	141	70	77	58	35	34	61
March	1	40	0	139	111	98	92	65
April	32	14	76	2	154	137	113	138
May	0	47	11	22	205	171	171	168
June	0	4	0	40	230	174	214	176
July	0	0	2	0	204	212	207	203
August	0	0	0	16	193	196	185	177
September	0	0	0	0	146	153	149	144
October	16	13	21	27	112	97	92	91
November	49	8	51	28	46	60	62	50
December	43	53	131	9	24	27	18	55
Mean/Total	406	350	522	404	1512	1402	1355	1355

California Irrigation Management Information Systems (CIMIS).

Table 2 Soil properties (0–15 cm) at the location used to evaluate low- and high-input switchgrass systems

Soil name	Brentwood silty clay loam
Clay (g kg^{-1})	280
Silt (g kg^{-1})	480
Sand (g kg^{-1})	240
pH (saturated paste extract)	7.2
CEC (cmol$_c$ kg^{-1})	35.4
Olsen-P (mg kg^{-1})	13.6
NO_3-N (mg kg^{-1})	9.9
Extractable-K (mg kg^{-1})	375
Organic matter (Walkley-Black) (g kg^{-1})	19.1
Total N (combustion) (g kg^{-1})	1.3

seeding and kept under sprinkler irrigation during the establishment year (2008) to ensure proper seed germination and crop establishment. No fertilizers were applied in the establishment year and the field was harvested in the fall of 2008.

In March of 2009, soil cores (3.8 cm diameter) down to 3 m deep were taken from 16 points using a Geoprobe direct push machine (GEOprobe systems; KEJR Engineering Inc., Salina, KS, USA). The soil cores were divided into nine sections representing different soil depths (0–15, 15–33, 33–66, 66–100, 100–133, 133–166, 166–200, 200–250, and 250–300 cm), weighed, subsampled for moisture content, and oven dried to constant weight at 105 °C for dry weight and bulk density determination.

The experimental design was laid out in April 2009 as a split plot randomized complete block design replicated four times. The field was divided into eight main plots measuring 8 by 15 m each that received the main plot treatments (low- and high-input system). The low-input system consisted of a single harvest performed in fall at post-anthesis developmental stage (R5) (Moore *et al.*, 1991), and irrigated only until mid-July at inflorescence emerged stage (R3). The high-input system consisted of a two-harvest system irrigated throughout the entire growing season. The high-input treatment harvests were performed between spikelets fully emerged (R2) to inflorescence emerged stage (R3) in early summer and at post-anthesis stage (R5) in fall simultaneous to the low-input treatment harvest. The main plots contained three subplots (8 by 5 m) for three N fertilization rates (0, 100, and 200 kg N ha^{-1} yr^{-1}). The N fertilizer was applied as ammonium sulfate [$(NH_4)_2SO_4$] in a single application in the spring of each year. Phosphorus and potassium were applied annually to all plots at a rate of 100 kg ha^{-1} of P_2O_5 and K_2O to ensure these nutrients were not limiting crop production (Table 3).

Each main plot was surrounded by levees to allow for independent irrigation regimes through flood irrigation from 2009 till 2011. The main treatments were irrigated at the same dates until early summer, when the irrigation of the low-input treatment ceased. The irrigation was measured with a turbine flow meter installed before the irrigation pump. The amount of water applied at each irrigation event was 120 ha mm, determined based on crop effective rooting depth (160 cm), available water capacity of the soil (153 mm of water per 100 cm of soil), and allowable water depletion (50% of available water capacity). Potential evapotranspiration (ETo) data were obtained from a nearby meteorological station (Table 1) and the main plots were flood irrigated when accumulated ETo (sum of daily ETo) reached ca. 120 mm.

Biomass yields and N concentration were determined from 2009 to 2011. All harvests were performed using a walk-behind tractor (BCS 750 model) equipped with a 76 cm wide sickle bar mower set at a cutting height of 10 cm. The borders were cleaned and the area harvested from each subplot was ca. 10 m^2 for the 0 and 200 kg N ha^{-1} subtreatments and 5 m^2 for the 100 kg N ha^{-1} subtreatment (for reasons discussed below). At all harvests the plants were weighed and a subsample was taken for dry matter determination and nutrient analysis. Subsamples were dried to constant weight at 60 °C, ground, and analyzed for N concentration.

Table 3 Primary management practices from 2008 to 2011

Year	Management	Low input	High input
2008	Planting	8/8	8/8
	Harvest	11/13	11/13
2009	Soil sampling	4/20	4/20
	Fertilization	4/27	4/27
	^{15}N fertilization	4/27	4/27
	Irrigation	4/28; 5/8; 6/7; 7/2	4/28; 5/8; 6/7; 7/2; 7/23; 8/15; 9/8
	Harvest	11/4	7/10; 11/4
	^{15}N sampling	11/6	11/6
2010	Fertilization	4/7	4/7
	Irrigation	4/20; 5/20; 6/14, 7/5	4/20; 5/20; 6/14, 7/5; 7/27; 8/20; 9/15
	Harvest	10/20	6/30; 10/20
	^{15}N sampling	10/22	10/22
2011	Fertilization	4/8	4/8
	Irrigation	4/20; 5/14; 6/20	4/20; 5/14; 6/20; 7/16; 8/11; 9/6
	Harvest	10/17	7/11; 10/17
	^{15}N sampling	10/19	10/19

^{15}N microplots

Sixteen ^{15}N microplots measuring 1.5 by 1.5 m (2.25 m^2) were established in April of 2009 within the 100 kg N ha^{-1} yr^{-1} subplots (two microplots per subplot). The microplots were set up longitudinally to the left-side half of the subplots, distant 1 m away from the south and north borders and 0.5 from the west border, and with 3 m between microplots. This design allowed for the subplots to be harvested for biomass yield on the right-side half without disturbing the microplots. In addition, this design left an area between microplots of ca. 4.5 m^2 that was used for destructively determination of BG biomass without compromising either sub- or microplots.

A single application of ^{15}N enriched fertilizer was applied in April of 2009 to the microplots as ^{15}N enriched $(NH_4)_2SO_4$ at 10 atom% ^{15}N at the same N fertilization rate as the subplots (100 kg N ha^{-1}). The ^{15}N-enriched fertilizer was diluted in 7.5 l of water and immediately applied uniformly onto the microplots with a sprinkling can. The sprinkler can was refilled with clean water, and emptied uniformly onto the microplots. In the subsequent years (2010 and 2011), the microplots were fertilized with unlabeled $(NH_4)_2SO_4$ at the same rate and time as the subplots.

Micro-plots AG and BG biomass yield determination

The AG biomass was divided into harvested biomass and residues. The harvested biomass and residues yields were determined at every harvest event by placing a 0.8 × 0.8 m (0.64 m^2) square in the center of the microplots. Plants were hand-cut at 10 cm above the soil surface, weighed, and subsampled for dry matter determination. The residues were collected with a small rake, weighed, subsampled, and returned to the hand-cut microplots. The remaining plants within the microplots were hand-harvested and removed from the field.

The BG biomass yield was determined after the fall harvest of each year from the region between microplots. Two cubes of soil measuring 0.2 × 0.2 × 0.15 m (0.006 m^3) per subplot were manually dug out and composited into one sample representing the 0–15 cm soil layer. The crown and rhizomes were included in the 0–15 cm BG biomass. Two soil cores (3.81 cm diameter) per subplot (one core per cube) were taken down to 3 meters from the bottom of the pits representing the 15–300 cm soil layer. The soil cores were divided into sections representing different soil depths (15–33, 33–100, 100–200, and 200–300 cm). Samples from the same subplot and depths were composited into one sample. The crown and roots were separated from the soil by soaking the samples in water for 30 min. followed by hand agitation and manipulation of the soil–root–water mixture. The mixture was poured over a 1 mm sieve placed on top of a 0.5 mm sieve to recover all roots. Water from a hose was used to wash down the roots and remove all the soil. The materials collected in both sieves were combined into one sample, oven dried to constant weight at 60 °C, and weighed.

^{15}N atom% enrichment and natural abundance determination

The microplots were used to determine the ^{15}N atom% in the AG biomass, BG biomass (crown plus roots), and soil. The AG biomass (harvested biomass and residues) subsamples collected to determine dry matter and biomass yields were subsequently used to determine the ^{15}N atom%. The ^{15}N atom% in the BG biomass and soil were determined by taking a 3.81 cm diameter soil core from the center of the microplots down to 3 m with a Geoprobe direct push machine. The BG biomass and soil were divided into five depths (0–15, 15–33, 33–100, 100–200, and 200–300 cm). Samples were subsequently oven dried, ground, and analyzed for N concentration and ^{15}N atom% content at the Stable Isotope Laboratory at UC Davis.

The ^{15}N natural abundance in the soil at different depths was determined from soils cores taken in March of 2009 prior to the ^{15}N fertilization. The ^{15}N natural abundance in the AG biomass was determined at the summer harvest of the high-input treatment and at the final harvest of both main treatments during the first growing season by sampling the zero N subplots. The ^{15}N natural abundance in the BG biomass was measured after the fall harvest of the first growing season by taking soil cores down to 3 m deep from the zero N subplots.

Calculations

Irrigation use efficiency. Irrigation use efficiency was used to express biomass yields per unit of water applied. The calculation was performed as follows.

$$\mathrm{IUE} = \frac{\text{Biomass yield}}{\text{Irrigation}}, \tag{1}$$

where IUE is expressed in kg biomass ha mm^{-1}, biomass yield in kg ha^{-1}, and irrigation in ha mm.

Fertilizer N recovery. Total N in the AG and BG biomass and soil was calculated as follows:

$$\text{AG and BG biomass}: \mathrm{TN}_{\mathrm{AG,BG}} = \frac{\mathrm{NC} \times \mathrm{BY}}{100} \tag{2}$$

$$\text{soil}: \mathrm{TN}_{\mathrm{soil}} = \mathrm{NC} \times \mathrm{BD} \times \mathrm{SV} \times 10, \tag{3}$$

where TN is total N (kg ha^{-1}), NC is N concentration (%), BY is biomass yield (kg ha^{-1}), BD is bulk density (g cm^{-3}), and SV is the soil layer volume (m^3 ha^{-1}).

The calculation of ^{15}N atom% excess was performed as follows:

$$\mathrm{ANEx} = \mathrm{ANEn} - \mathrm{NNA}, \tag{4}$$

where ANEx is ^{15}N atom% excess (%), NNA is ^{15}N natural abundance (%), and ANEn is ^{15}N atom% enriched concentration (%).

Fertilizer N recovery (FNR, in %) was calculated as follows:

$$\mathrm{FNR} = \frac{\mathrm{ANEx}_{\mathrm{AG,BG,soil}}}{\mathrm{ANEx_f}} \times \frac{\mathrm{TN}_{\mathrm{AG,BG,soil}}}{\mathrm{TN_f}}, \tag{5}$$

where $\mathrm{ANEx}_{\mathrm{AG,\ BG,\ soil}}$ is the ^{15}N atom% excess in the AG and BG biomass and soil (%), $\mathrm{ANEx_f}$ is the ^{15}N atom% excess in the fertilizer (%), $\mathrm{TN}_{\mathrm{AG,\ BG,\ soil}}$ is the total N in the AG and BG biomass and soil (kg ha^{-1}), and $\mathrm{TN_f}$ is the total N applied in the N fertilization (kg ha^{-1}).

Statistical analysis

Data were analyzed for normality and constant variance of errors, and data transformations were performed when assumptions were violated. Analysis of variance on biomass yields, biomass N concentration, crop N removal, and IUE were performed using the Mixed Procedure in SAS (SAS Institute Inc., Cary, NC, USA). Year, input system, N treatment, and the interactions between year × input system, year × N treatment, input system × N treatment, and year × input system × N treatment were considered to be fixed effects, while block was considered a random effect. Analysis of variance on BG biomass yields, N concentration, and total N were performed with year, input system, depth, and the interactions between year × input system, year × depth, input system × depth, and year × input system × depth were considered to be fixed effects, while block was considered a random effect. Analysis of variance on the fertilizer N recovery in the compartments of AG biomass (harvested biomass and residues), were performed using the Mixed Procedure is SAS, with year, input system, and the interactions between year × input system as fixed effects, and block as a random effect. Analysis of variance on the FNR in the compartments of BG biomass and soil were performed with year, input system, depth, and the interactions between year × input system, year × depth, input system × depth, and year × input system × depth were considered fixed effects, while block was considered a random effect.

The significance of fixed effects and their respective interactions are shown in Tables 4–6. Due to the significant interactions, the effect of input system on biomass yield and crop N removal was analyzed at each year and N treatment, while the effect of N fertilization was analyzed by input system across years. The effect of input system on biomass N concentration was analyzed by N fertilization rate across years. The effect of input system on BG biomass yields, N concentration, and total N was analyzed for each depth across years. The effect of input system on FNR in the AG biomass was analyzed for each year, while the effect of input system on FNR in the BG biomass and

Table 4 Summary statistics with significance of fixed effects on biomass yields, biomass N concentration, crop N removal, and irrigation use efficiency (IUE). *P*-values lower than 0.05 are considered statistically significant

Harvested biomass	Biomass yield			N concentration		Crop N removal		IUE	
Fixed effects	df	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Year (Y)	2	11.30	<0.0001	14.18	<0.0001	13.90	<0.0001	2.23	0.118
Input system (IS)	1	21.80	<0.0001	235.94	<0.0001	229.23	<0.0001	87.62	<0.0001
N treatment (N)	2	27.97	<0.0001	52.41	<0.0001	73.30	<0.0001	25.17	<0.0001
Y × IS	2	5.27	0.0083	1.01	0.3718	9.14	0.004	2.71	0.0761
Y × N	4	0.48	0.7514	1.00	0.4154	0.33	0.8535	0.32	0.864
IS × N	2	7.15	0.0019	2.85	0.0673	13.12	<0.0001	5.18	0.009
Y × IS × N	4	0.90	0.4691	0.45	0.7714	0.14	0.9651	1.67	0.1723

Table 5 Summary statistics with significance of fixed effects on belowground (BG) (crown plus roots) biomass yield, BG biomass N concentration, and total N in BG biomass. *P*-values lower than 0.05 are considered statistically significant

Below-ground biomass	Biomass			N concentration		Total N	
Fixed effects	df	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Year (Y)	2	1.28	0.2833	1.84	0.1663	0.98	0.3783
Input system (IS)	1	1.4	0.2394	19.31	<0.0001	16.46	0.0001
Depth (D)	4	44.01	<0.0001	2.22	0.0744	29.90	<0.0001
Y × IS	2	2.14	0.1243	1.57	0.2153	0.14	0.8687
Y × D	8	4.59	0.0001	0.54	0.8211	1.12	0.356
IS × D	4	0.57	0.6867	2.56	0.0449	0.90	0.4698
Y × IS × D	8	1.02	0.4291	1.44	0.1913	1.37	0.223

Table 6 Summary statistics with significance of fixed effects on fertilizer ^{15}N recovery (FNR) in belowground (BG) (crown plus roots) biomass, soil, harvested biomass, residues, and fertilizer ^{15}N unaccounted for. *P*-values lower than 0.05 are considered statistically significant

Fixed effects	BG biomass			Soil			Harvested biomass			Residues		Unaccounted	
FNR	df	*F*-value	*P*-value	df	*F*-value	*P*-value	df	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Year (Y)	2	42.39	<0.0001	2	11.85	<0.0001	2	70.24	<0.0001	5.65	0.0148	3.09	0.075
Input system (IS)	1	100.29	<0.0001	1	7.11	0.0087	1	9.35	0.008	2.2	0.1586	4.6	0.0488
Depth (D)	4	28.19	<0.0001	6	19.57	<0.0001	–	–	–	–	–	–	–
Y × IS	2	1.77	0.1778	2	1.12	0.3291	2	12.68	0.0006	1.65	0.2256	1.74	0.2099
Y × D	8	1.36	0.2266	12	0.2	0.9983	–	–	–	–	–	–	–
IS × D	4	4.28	0.0035	6	0.14	0.9911	–	–	–	–	–	–	–
Y × IS × D	8	1.25	0.2799	12	0.26	0.9941	–	–	–	–	–	–	–

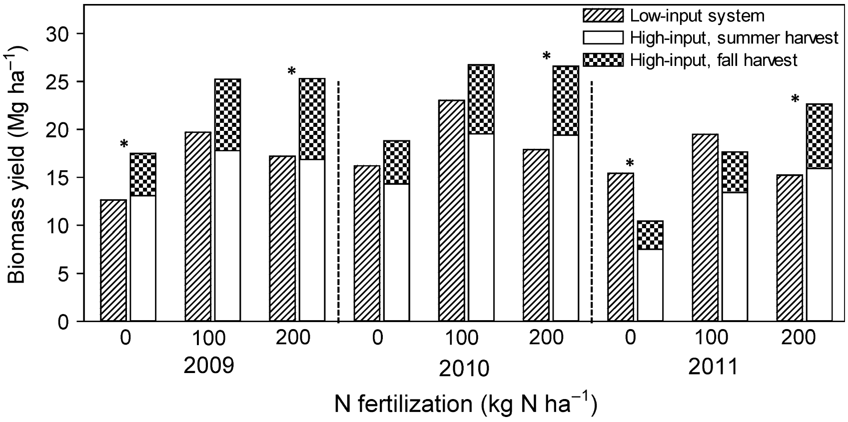

Fig. 1 Biomass yield by year, input system, N fertilization rate, and harvest. Input system mean separation was performed at each year and N rate on year-total biomass yield (sum of summer and fall harvests). The symbol * indicates that differences between input systems are statistically significant ($P > 0.05$) within the same year and N rate.

soil was analyzed for each depth and year. All differences were considered to be significant at the 5% probability level.

Results

Biomass yields and response to N fertilization

Unfertilized switchgrass yield was initially higher in the high-input system (17.5 Mg ha^{-1}) than in the low-input system (12.6 Mg ha^{-1}), but by the third year it was higher in the low-input system (15.4 Mg ha^{-1}) than in the high-input system (10.4 Mg ha^{-1}) (Fig. 1). The addition of 100 kg N ha^{-1} yr^{-1} resulted in similar yields between input systems for all years. In contrast, the high-input system (24.8 Mg ha^{-1}) had higher yields than the low-input system (16.8 Mg ha^{-1}) when fertilized with 200 kg N ha^{-1} yr^{-1} in all years. Averaged across N rates and years, the summer harvest of the high-input system accounted for 72% of the annual biomass yield (Fig. 1). A significant decrease in yields of all N treatments over time was observed with the high-input system, while the low-input system had similar yields among years (Table 4; Fig. 1).

Significant responses to N fertilization were observed in both input systems (Table 4). The high-input system had a more pronounced response to N inputs, with yields increasing up to 200 kg N ha^{-1} yr^{-1} by the third year (Fig. 1); although the differences between the 100 and 200 kg N ha^{-1} yr^{-1} rate were not significant across years. On average, yields increased by 7.6 and 9.3 Mg ha^{-1} in the 100 and 200 kg N ha^{-1} N rates. In contrast, the low-input system had the highest yields when fertilized with 100 kg N ha^{-1} yr^{-1}, with yields increasing by 6.0 Mg ha^{-1} relative to the zero N. The addition of 200 kg N ha^{-1} resulted in declining biomass yields due to plant lodging (lodging data not shown).

Irrigation requirement and IUE

On average across years, the irrigation required for the low-input system (372 ha mm yr^{-1}) was 47.3% lower than for the high-input system (705 ha mm yr^{-1}) (Fig. 2). The low-input system had greater IUE values at all N rates compared to the high-input system, averaging across years and N rates, 48 and 30 kg biomass ha mm^{-1} of irrigation, respectively. As there was an increase in biomass yields of the N-fertilized treatments, a significant effect of N fertilization on IUE was observed with both input systems.

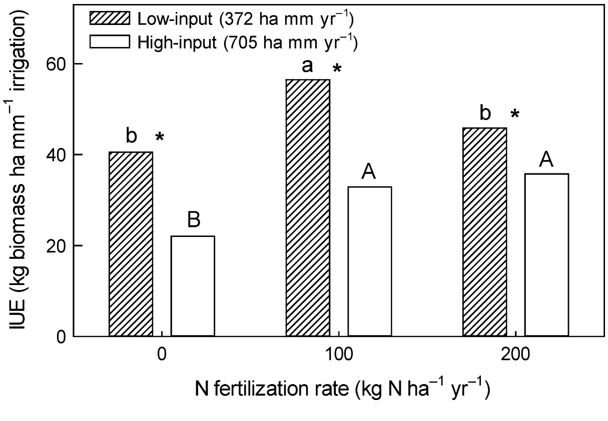

Fig. 2 Total irrigation use by input system averaged across years and irrigation use efficiency by N rate and input system averaged across years. The symbol * indicates that differences between input systems are statistically significant ($P < 0.05$) within the same N rate. The lower case letters refer to the N fertilization rates means separation within the low-input system, whereas the upper case letters refer to the N rates means separation within the high-input system. Different letters indicate that differences between N fertilization rates are statistically significant ($P < 0.05$) within the same input system

Biomass N concentration and crop N removal

The low-input system had significantly lower biomass N concentration than both summer and fall harvests of the high-input system at all N rates (Table 7). While N fertilization increased biomass N concentration, averaged across years and N rates, biomass N concentration of the low-input system was 4.0 g kg^{-1}, while biomass N concentration of the summer and fall harvests of the high-input system were 7.6 and 5.8 g kg^{-1}, respectively.

The high-input system had significantly higher rates of crop N removal than the low-input system for all N rates and years, except for the zero N treatment in the third year (Table 7). In addition, greater increases in crop N removal due to N fertilization were observed with the high-input system in comparison to the low-input system. Average crop N removal across years of the 0, 100, and 200 kg N ha^{-1} yr^{-1} treatments were 43, 79, and 90 kg N ha^{-1} yr^{-1} (low input) and 95, 158, and 212 kg N ha^{-1} yr^{-1} (high input), respectively. The summer harvest of the high-input system was responsible for 77% of annual crop N removal. The high-input system had a significant decrease in crop N removal over time from all N treatments, while the low-input system had similar N removal rates across years (Tables 4 and 7).

Overall, the high-input system removed more N than what was applied for both N-fertilized treatments, resulting in a cumulative negative N balance at the end of the 3 years of −174 and −36 kg N ha^{-1} for the 100 and 200 kg N ha^{-1} yr^{-1} treatments (Table 7). In contrast, the low-input system removed less N than what was applied, resulting in a cumulative positive N balance of +64 (100 kg N ha^{-1} yr^{-1}) and +332 kg N ha^{-1} (200 kg N ha^{-1} yr^{-1}). The cumulative negative N balance of the zero N treatment was also lower in the low input (−128 kg N ha^{-1}) than in the high-input system (−284 kg N ha^{-1}).

Belowground biomass: yield, N concentration, and total N

Belowground biomass did not change significantly over time during the 3 year study in either the low- or high-input system (Table 5). The low- and high-input systems had similar BG biomass yields, with an average across years of 2.82 (low input) and 2.63 kg biomass m^{-2} (high input) (Fig. 3), translating to 28.2 (low input) and 26.3 (high input) Mg ha^{-1}. In both systems, ca. 73% of the BG biomass was found within the first 100 cm of soil, with only 19% and 9% in the 100–200 and 200–300 cm depths, respectively. At the end of each

Table 7 Biomass N concentration by input system and N treatment averaged across years, crop N removal and annual N balance by year, N treatment, and input system, and cumulative N balance (sum of all years) by N treatment and input system

Variable/Year	N rate (kg N ha^{-1} yr$^{-1)}$	Low input	High input	*P*-value (input system)
Biomass N Concentration (g kg^{-1})				
Across years	0	2.9	5.9 (5.8)	*
	100	3.8	7.3 (5.7)	*
	200	5.3	9.7 (5.9)	*
	P-value (N rate)	*	*	–
Crop N removal (kg N ha^{-1})				
2009	0	43	94 (29)	*
	100	76	135 (50)	*
	200	102	193 (53)	*
2010	0	44	82 (21)	*
	100	77	132 (33)	*
	200	88	176 (36)	*
2011	0	39	39 (19)	ns
	100	81	98 (25)	*
	200	78	137 (41)	*
	P-value (N rate)	*	*	–
Annual N balance (kg N ha^{-1})				
2009	0	−44	−123	*
	100	24	−86	*
	200	98	−45	*
2010	0	−45	−103	*
	100	22	−65	*
	200	112	−12	*
2011	0	−39	−58	ns
	100	18	−23	*
	200	122	22	*
	P-value (N rate)	*	*	–
Cumulative N balance (kg N ha^{-1})				
Across years	0	−128	−284	*
	100	64	−174	*
	200	332	−36	*

ns, not significant. The values outside and inside of parenthesis refer to the summer and fall harvests, respectively.
*Differences between input systems and N treatments are statistically significant ($P > 0.05$).

year when samples were taken, the N concentration of BG biomass was higher in the low-input system at all depths except for the 200–300 cm (Fig. 3); averaging across years and depths, 5.02 and 3.44 g kg^{-1} for low- and high-input systems, respectively. Consequently, the low-input system had more N stored in BG biomass (14.7 g m^{-2}) than the high-input system (9.6 g m^{-2}).

Fertilizer ^{15}N recovery

In the first year, the high-input system had significantly greater FNR in the AG biomass than the low-input system where 36.5%, 2.6%, and 2.7% of the applied ^{15}N fertilizer was recovered in the summer and fall harvest and residues, respectively (Fig. 4). In contrast, in the low-input system 18.4% and 2.4% of the applied ^{15}N fertilizer was recovered in the harvested biomass and residues, respectively. In the second year, FNR in the harvested biomass and residues were similar between input systems. In the third year, the FNR of low-input system was significantly higher in the harvested biomass and in the residues (2.2% and 1.1%, respectively) than for the high-input system (0.9% and 0.4%, respectively).

The low-input system had significantly higher FNR in the BG biomass in all years and depths, except for the 200–300 cm depth in the years of 2010 and 2011 (Fig. 4). In the first year, FNR in the BG biomass in low- and

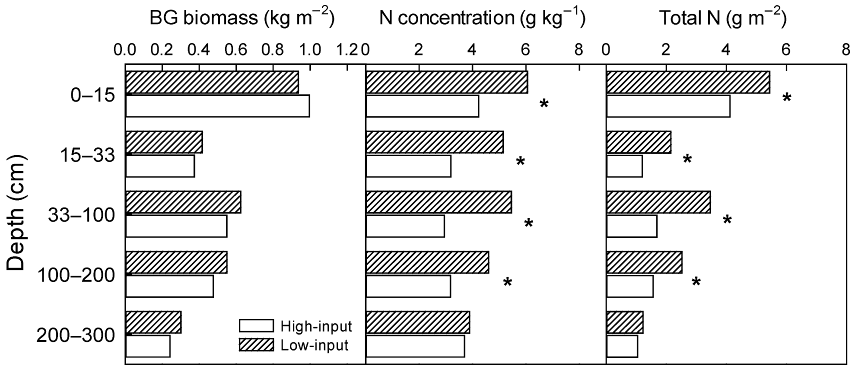

Fig. 3 Vertical distribution in the soil profile of belowground (crown plus roots) biomass yield, N concentration, and total N by depth and input system averaged across years. The symbol * indicates that differences between input systems are statistically significant ($P > 0.05$) within the same depth.

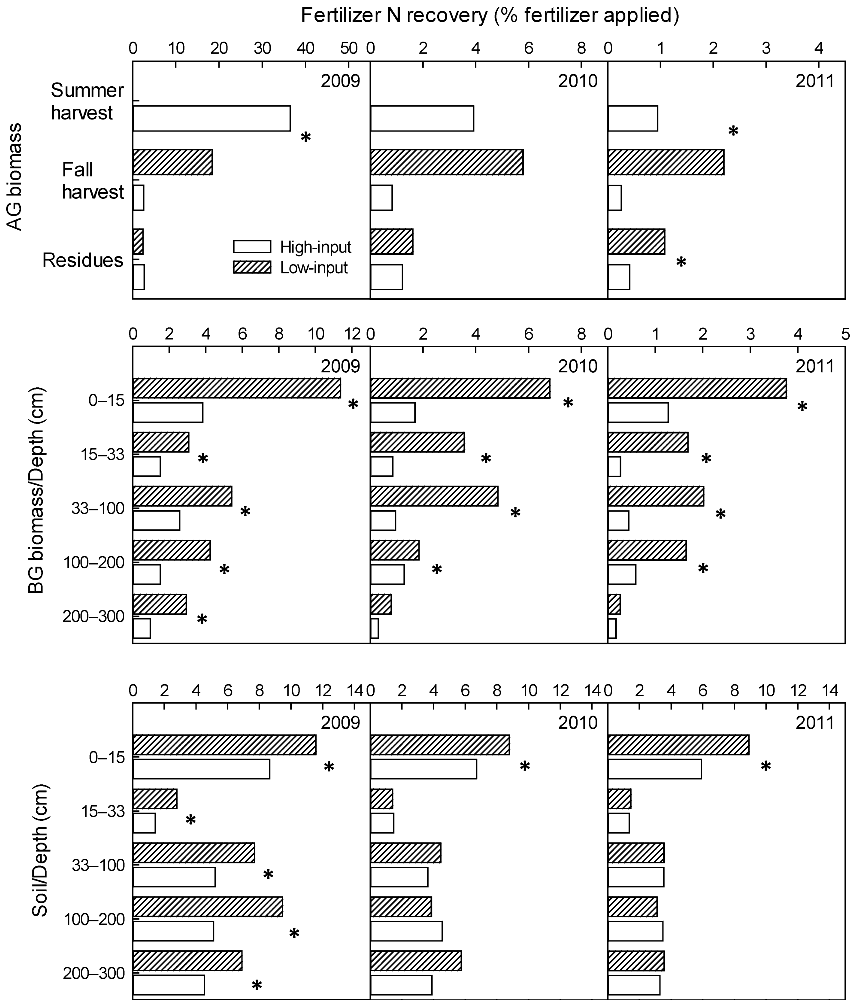

Fig. 4 Fertilizer 15N recovery in aboveground biomass (harvested biomass and residues), belowground biomass (crown plus roots), and soil by depth, input system, and year. The symbol * indicates that differences between input systems are statistically significant ($P > 0.05$) within the same compartment (i.e., harvested biomass, residues, depth).

high-input systems was 27.0% and 10.4%, respectively. In both systems FNR in the BG biomass decreased over time. The FNR in the low-input system was 17.9% and 9.4% in the second and third years, respectively, while the high-input system had 5.1% (second year) and 2.7% (third year). Averaged across years, 76% of FNR in BG biomass was within the upper 100 cm of soil (Fig. 3).

The FNR in the soil of the low-input system was higher at all depths in the first year and in the top layer (0–15 cm) in the last 2 years (Fig. 4). In the first year, the FNR in the soil across all depths was higher in the low- (38.4%) than in the high -input system (24.9%). However, this difference became nonsignificant after the first year. On average across years, 61.1% of the N applied in the soil was recovered within the first 100 cm of soil.

After 3 years cumulative FNR in the AG biomass (harvested biomass plus residues) was higher in the high-input system (50.2%) than in the low-input system (31.5%) (Fig. 5). Cumulative FNR in the harvested biomass that was removed from the field was 45.1% (high input) and 26.4% (low input). Furthermore, at the end of the third year, more fertilizer was recovered in the BG biomass of the low- (9.4%) than the high- (2.7) input system. Also, more fertilizer N was recovered in the soil of the low- (20.6%) than in the high-(17.6%) input system. In total, after 3 years, the percentage of applied ^{15}N fertilizer remaining in the plant–soil system (BG biomass, soil, and residues) was also higher in the low input (31%) than in the high-input system (20.3%). The total cumulative FNR at the end of 3 years was 61.5% (low input) and 72.2% (high input); consequently, the fertilizer ^{15}N unaccounted for was 38.5% and 27.8% in low and high-input systems, respectively.

Discussion

Biomass yield, IUE, and crop N removal

The average yields across years and N rates achieved of 17.4 (low input) and 21.2 Mg ha^{-1} yr^{-1} (high input) are significantly higher than the average yield reported by Heaton *et al.* (2004) of 10.3 ± 0.7 Mg ha^{-1} yr^{-1} based on 21 studies, representing 174 observations. However, the highest yields achieved of 23.0 (low input) and 26.7 Mg ha^{-1} yr^{-1} (high input) in this study are similar to those found in other parts of the United States. Switchgrass yields of 26.1 Mg ha^{-1} (Jung & Lal, 2011; -Ohio), 22.2 Mg ha^{-1} (Guretzky *et al.*, 2011; -Oklahoma), as well as much higher yields of 39.1 Mg ha^{-1} (West & Kincer, 2011; -Tennessee) have been reported. In a previous study in California, yields of up to 27.9 and 39.4 Mg ha^{-1} were found for upland and lowland ecotype varieties, respectively (Pedroso *et al.*, 2011).

Irrigation is a valuable and scarce resource globally. In California, depending on the watershed and irrigation district, allocation of surface water to irrigation has been reduced by 30–65% over the last 10 years (Sanden *et al.*, 2012). Therefore, the lower water requirement of the low-input system is an advantage over the high-input system. However, the water applied to the high-input system (705 ha mm yr^{-1}) remains low when compared to other crops such as alfalfa, in which irrigation requirements range from 840 to 1400 ha mm yr^{-1} in California (Hanson *et al.*, 2008; Sanden *et al.*, 2012). Although the high-input treatment had greater biomass yields than the low-input system (Fig. 1), the increase in irrigation requirement was higher than the gain in

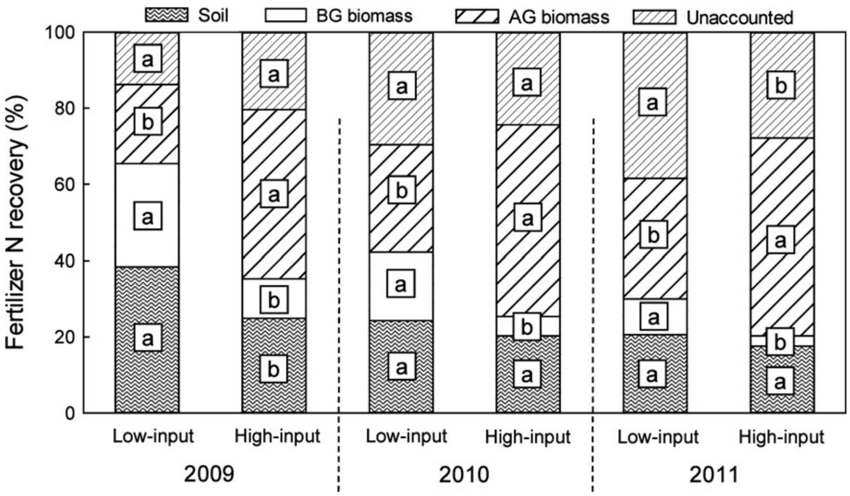

Fig. 5 Fertilizer ^{15}N recovery (FNR) in soil and belowground (BG) biomass (crown plus roots) by input system and year, cumulative FNR in aboveground (AG) biomass and unaccounted for by input system and year. Different letters within the same compartment (i.e., soil, BG and AG biomass) and year indicate that differences between input systems are statistically significant ($P < 0.05$).

yields, resulting in lower IUE values at all N fertilization rates compared to the low-input system.

Crop N removal is a concern for dedicated energy crops because high rates of crop N removal indicate high N input requirements to replenish soil and plant N reserves to sustain high yields. The high rates of crop N removal by the summer harvest of the high-input system were a function of high yields (Fig. 1) and high biomass N concentration (Table 7), while the low crop N removal by the fall harvest of the high-input system was primarily a function of low yields. In contrast, the lower crop N removal observed with the low-input system was mostly due to low biomass N concentration at harvest (Table 7). The low biomass N concentration and low crop N removal showed by the low-input system was a result of N translocation from AG to BG during senescence (further discussed below). The low biomass N concentration observed with the low-input system is another advantage of the low-input system, because high N concentration in the biomass feedstock during the thermochemical ethanol production can cause char formation (Rejai *et al.*, 1992), corrosion and increased NO_x emissions (Jorgensen, 1997). However, it is likely that the first harvest had high biomass quality for methane production due to low fiber concentration and high digestibility, which are related to methane yields from anaerobically digested switchgrass silage (Bélanger *et al.*, 2012). Therefore, the desired biomass quality will be dependent on the conversion technology adopted by the industry.

The high rates of crop N removal and negative N balance of all N treatments in the high-input system (Table 7) may explain the decline in yields across all N treatments (Fig. 1), as the plots became limited for available N leading to a decline in biomass yields over time. In contrast, the low-input system had a positive N balance when N fertilizer was applied, which explains the similar biomass yields across years for each treatment. In the low-input system, the high N rate resulted in a high positive N balance (+332 kg N ha^{-1}), and lower yields due to lodging.

The low return in yields for increased use of resources in the high-input system is an important consideration. The fall harvest of the high-input system produced on average 5.9 Mg ha^{-1} yr^{-1} (Fig. 1), but required an additional 333 ha mm of irrigation (Fig. 2) resulting in an IUE of 17.8 kg biomass ha mm^{-1} of irrigation. In addition, averaged across years and N rates, the high-input system had 2.2 times higher crop N removal resulting in a negative N balance and higher N input requirements (Table 7), and required increased use of harvest operations, increasing the energy input and greenhouse gases emissions during switchgrass cultivation (Adler *et al.*, 2007; Schmer *et al.*, 2008). However, a more detailed analysis is required to assess whether the increased energy input and greenhouse gases emissions in high-input systems will override the value of yield gains.

BG biomass

The BG biomass yields reported here are similar to reports of 1.78 kg biomass m^{-2} in the top 90 cm of soil (Garten *et al.*, 2010) and of 1.5 kg biomass m^{-2} in the top 35 cm of soil (Al Kaisi & Grote, 2007). Although total BG biomass yields were similar among years, a significant increase in BG biomass yields over time was observed for both input systems in the top 15 cm, with BG biomass yields increasing from 0.72 kg biomass m^{-2} to 1.1 kg biomass m^{-2} between 2009 and 2011 (data not shown). This increase in BG biomass reflects the increasing size of crowns and rhizomes as the switchgrass matures (Heaton *et al.*, 2004). Similar to other reports (Porter, 1966; Ma *et al.*, 2000), switchgrass roots were found at least down to 300 cm deep (Fig. 3). The high BG biomass yields and vertical distribution suggests that switchgrass has good potential for net soil C sequestration, especially in deeper soil layers where organic matter decomposition is slower (Post & Kwon, 2000). In addition, the potential for net soil C sequestration under switchgrass is similar for both input systems due to similar BG biomass. While we only examined BG root biomass in the 100 kg treatment, Ma *et al.* (2000) reported that switchgrass BG biomass was not affected by N fertilization rates, suggesting equal C sequestration potential across N rates in our study. However, Heggenstaller *et al.* (2009) have reported decreasing BG biomass yields with N rates greater than 150 kg N ha^{-1} yr^{-1}, suggesting a lower C sequestration potential with the highest N rate in our study.

Although the low- and high-input systems had equal BG biomass yields, the low input system had significantly greater BG biomass N concentration and total N, a result of N translocation from AG to BG biomass during senescence and lower crop N removal (Table 7). In addition, the higher total N in the BG biomass of low-input systems indicates higher N storage that may be re-allocated in the following growing season. Such re-allocation of N from the BG to the AG plant components may further decrease N fertilizer requirements and reduce yield responses to N fertilization.

N translocation during senescence

The amount of N translocated from the AG to the BG plant components during senescence can be estimated from the difference in FNR between the summer harvested biomass of the high-input system (36%) and the fall harvested biomass of the low-input system (18%) in the first year of the study (2009) (Fig. 4). Since both input systems were managed similarly until that time, we can assume that the FNR of both systems was the same (i.e., 36%) at the time of the summer harvest. The timing of the summer harvest was between developmental stages R2 and R3 (Moore *et al*., 1991) and coincides with maximum N in AG biomass (Vogel *et al*., 2002). It is also assumed for the sake of this calculation that no N leached from the AG biomass and there was no N loss as a result of NH_3 volatilization from leaves (Schjoerring & Mattsson, 2001) due to the lower NH_3 compensation point of old leaves (Hill *et al*., 2002). The difference in FNR in the harvested biomass between the summer harvest (high-input system) and fall harvest (low-input system) was 18%, suggesting that 49% of the N taken up in the low-input system had been translocated to BG biomass by harvest. This result is further supported by the crop N removal (Table 7) and FNR in the BG biomass data (Fig. 4). In the first year, crop N removal by the summer harvest of the high-input system and fall harvest of the low-input system were 135 and 76 kg N ha^{-1}, respectively, suggesting that 44% of N uptake had translocated during senescence. In addition, FNR in BG biomass at the end of the first year was 17% higher in the low-input system and similar to the difference in FNR (18%) between the summer harvest (high-input system) and fall harvest (low-input system) (discussed above).

Our estimate of N translocation (44–49%) is higher than the 30% reported for other perennial C_4 grasses in response to senescence (Clark, 1977) and drought (Heckathorn & DeLucia, 1994). However, calculations based on switchgrass biomass N concentration and crop N removal data from Heaton *et al.* (2009) and Dohleman *et al.* (2012) suggest that up to 66% of N was translocated to BG biomass during senescence. In addition, our findings are similar to the average N translocation reported by Aerts (1996) of 58% for graminoids and 50% for evergreen and deciduous shrubs and trees. The translocation of N within the plant played an important role in the sustainability of switchgrass systems by maintaining a positive N balance and lowering the overall fertilizer N requirement to maintain yields.

Fertilizer ^{15}N recovery in the plant–soil system

Switchgrass systems appear substantially more efficient in the recovery of fertilizer N than other cropping systems. Total cumulative FNR in the crop (AG plus BG biomass) and soil at the end of 3 years were 61% for the low-input system and 72% for the high-input system (Fig. 5). As a consequence, at the end of 3 years we could not account for 39% and 28% of the fertilizer N in low- and high-input systems, respectively. Dourado-Neto *et al.* (2010) investigated the recovery of a single ^{15}N application in 13 diverse tropical cropping systems, and found an average cumulative FNR recovery in the crop and soil of 53%, and 47% of the N being lost during the initial 3 years. Karlen *et al.* (1996) studied FNR in two crop rotation systems involving maize, wheat, and cotton, and reported that FNR in the crop and soil accounted for 30–56%, respectively, with 44–70% of applied N lost from the system at the end of 2 years. The higher FNR and lower ^{15}N losses observed in our study is likely due to the presence of an established and extensive root system and active plant growth at the time of the N fertilization. These two factors may have contributed to a rapid uptake of available N, thus increasing crop FNR and reducing losses from the plant–soil system. The high FNR exhibited by switchgrass systems has the advantage of reducing fertilizer N requirement, thus reducing the energy input and greenhouse gases emissions during switchgrass production.

Although the low-input system had higher FNR in the plant–soil system, this system also had higher fertilizer ^{15}N losses at the end of 3 years (39% vs. 28%) (Fig. 5). The seemingly contradictory result of higher ^{15}N losses in the most N conservative input system is explained by the high amount of fertilizer N removed by harvest in the high-input system in the first year (42% of total N applied vs. 18% in low input system). Therefore, the removal of fertilizer N makes it not subject to further N losses in the system.

Our study suggests that low-input systems are more sustainable than high-input systems for several reasons: (i) relatively high yields, (ii) lower irrigation use and higher IUE, and (iii) efficient N translocation within the plant resulting in lower fertilizer N removal by harvest, higher N storage in BG biomass, lower N input requirements, and higher FNR in the plant–soil system at the end of 3 years. In contrast, the high-input system had higher irrigation and N input requirements, and higher biomass N concentration due to the additional harvest with limited yield benefit.

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