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Response of "Alamo" switchgrass tissue chemistry and biomass to nitrogen fertilization in West Tennessee, USA☆

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ABSTRACT

Switchgrass (*Panicum virgatum*) is a perennial, warm-season grass that has been identified as a potential biofuel feedstock over a large part of North America. We examined above- and belowground responses to nitrogen fertilization in "Alamo" switchgrass grown in West Tennessee, USA. The fertilizer study included a spring and fall sampling of 5-year old switchgrass grown under annual applications of 0, 67, and 202 kg N ha^{-1} (as ammonium nitrate). Fertilization changed switchgrass biomass allocation as indicated by root:shoot ratios. End-of-growing season root:shoot ratios (mean ± SE) declined significantly ($P \leq 0.05$) at the highest fertilizer nitrogen treatment (2.16 ± 0.08, 2.02 ± 0.18, and 0.88 ± 0.14, respectively, at 0, 67, and 202 kg N ha^{-1}). Fertilization also significantly increased above- and belowground nitrogen concentrations and decreased plant C:N ratios. Data are presented for coarse live roots, fine live roots, coarse dead roots, fine dead roots, and rhizomes. At the end of the growing season, there was more carbon and nitrogen stored in belowground biomass than aboveground biomass. Fertilization impacted switchgrass tissue chemistry and biomass allocation in ways that potentially impact soil carbon cycle processes and soil carbon storage.

1. Introduction

Switchgrass is a perennial, warm-season grass that is wide-ranging over North America and a potential biofuel feedstock for production of lignocellulosic ethanol (McLaughlin and Kszos, 2005; Gunderson et al., 2008). It has been widely established that nitrogen fertilization increases production of aboveground biomass in switchgrass (e.g., Haferkamp and Copeland, 1984; Muir et al., 2001; Vogel et al., 2002; Lemus et al., 2008; Heggenstaller et al., 2009), but the effect of nitrogen fertilization on switchgrass root chemistry and belowground biomass is less well studied (Ma et al., 2000, 2001; Sanderson and Reed, 2000; Heggenstaller et al., 2009). Depending on location, different studies indicate variable belowground responses. For example, Ma et al. (2001) found that fertilization (224 kg N ha^{-1}) of 4-year old switchgrass stands in Alabama had no effect on root biomass, but reduced root:shoot ratios by about 70% relative to control stands (0 kg N ha^{-1}). In contrast, Heggenstaller et al. (2009) found that high rates of fertilization (220 kg N ha^{-1}) tended to reduce root biomass (relative to its maximum under

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more moderate levels of fertilization) in 3- to 4-year old stands of switchgrass in Iowa, but had relatively little effect on root:shoot ratios.

Although hidden, and more difficult to quantify, belowground plant responses to nitrogen fertilization are important to soil carbon cycle processes under switchgrass, including soil carbon storage. The amount of carbon that can be sequestered beneath perennial bioenergy crops depends on what land use is replaced, but studies indicate increased soil carbon storage following switchgrass establishment (Marquez et al., 1999; Frank et al., 2004; Liebig et al., 2005, 2008; Anderson-Teixeira et al., 2009; Blanco-Canqui, 2010; Collins et al., 2010), and nitrogen fertilization can increase soil carbon storage (Lee et al., 2007). Fertilization can impact belowground carbon cycle processes through at least two mechanisms: (1) changes in tissue chemistry that alter root decomposition or otherwise affect the decomposition of soil organic matter produced through root mortality, and (2) changes in plant biomass or carbon allocation that result in increased soil carbon inputs belowground.

Increased root biomass in response to nitrogen fertilization would be especially important to maintain soil organic matter and sustainable plant yield in switchgrass where most of the aboveground production is annually removed to produce biofuel. To accomplish soil carbon sequestration, carbon inputs must exceed carbon losses via decomposition. Root decomposition rates are affected by root tissue chemistry (Silver and Miya, 2001; Johnson et al., 2007). Changing root C:N ratios, that come about as a result of nitrogen fertilization, have the potential to alter root decomposition and thereby affect carbon transfer to pools of labile soil organic matter.

The purpose of our research was to examine above- and belowground responses of 5-year old "Alamo" switchgrass to nitrogen fertilization in West Tennessee, USA. The State of Tennessee has promoted alternative fuels, like production of ethanol from switchgrass, as a means for rural economic development and regional energy independence. Alamo is a high producing variety of switchgrass with biomass yields that average 14 Mg ha^{-1} yr^{-1} in favorable settings throughout the southeastern United States (Fike et al., 2006). Numerous studies have presented data on belowground biomass in switchgrass (Tufekcioglu et al., 1999, 2003; Ma et al., 2000, 2001; Sanderson and Reed, 2000; Zan et al., 2001; Sanderson, 2008; Heggenstaller et al., 2009; Collins et al., 2010; Garten et al., 2010; Xu et al., 2010), but few have examined the effects of nitrogen fertilization on roots. More research is needed to develop a better understanding of changes in root tissue chemistry, root biomass, and switchgrass biomass allocation in response to nitrogen fertilization.

2. Materials and methods

2.1. Study site and field sampling

The fertilizer experiment was located at the University of Tennessee's Research and Education Center near Milan, TN (35°55′31″N latitude; 88°42′57″W longitude). Soil at the site is classified as a moderately well drained Grenada silt loam (Alfisol; thermic Oxyaquic Fraglossudalf). Cropping history included corn–soybean rotations prior to the planting of "Alamo" switchgrass (a lowland variety) in the spring of 2004. The experiment had a randomized complete block design with four treatments (0, 67, 134, and 202 kg N ha^{-1} yr^{-1}) and five seeding rates (2.8, 5.6, 8.4, 11.2, and 13.5 kg pure live seed ha^{-1}). At the start of the second growing season, each treatment plot (4.6 m × 7.3 m) received a single springtime application of ammonium nitrate at its assigned rate. Three treatment levels (0, 67, and 202 kg N ha^{-1} yr^{-1}) at the 8.4 kg ha^{-1} seeding rate were selected for study during the 2008 – growing season. There were four replicate plots per fertilizer treatment. Management of the switchgrass included an annual harvest following the first killing frost (October or November). Mean annual temperature and precipitation during 2008 at Milan was 14.7 °C and 146 cm, respectively.

Above- and belowground biomass was sampled during the spring (April 22–24) and fall (November 10–12). Four sampling points were randomly chosen in each treatment plot. A sickledrat (Kennedy, 1972) was used to harvest aboveground biomass and surface litter from a 0.1-m^2 area at each point. The four sickledrat samples were pooled to yield one sample of aboveground biomass and one sample of surface litter per treatment plot (total sampling area was 0.4 m^2 per plot). A soil core (5.0 cm diameter) was removed from each treatment plot to a 15 cm depth using a core sampler with hammer attachment. Samples from the same depth increment (0–5, 5–10, and 10–15 cm) in each treatment plot were composited in a zip-lock bag. Deeper soil samples were obtained using a bucket auger (7.8 cm diameter) and samples from each treatment plot were composited by sampling depth (15–30, 30–60, and 60–90 cm). The spring and fall sampling events each produced 12 samples of aboveground biomass, 12 surface litter samples, and 72 soil samples (3 fertilizer treatments × 6 depth increments × 4 replicates).

2.2. Sample processing and chemical analysis

In the laboratory, samples of aboveground biomass and surface litter were oven dried (70 °C) and weighed to determine their dry mass per unit area. After mixing each dry sample by hand, a subsample was withdrawn, ground, and homogenized in a Foss Tecator Cyclotec™ 1093 sample mill. A subsample (20–50 g) was removed from each bag of fresh soil and weighed, then oven-dried and reweighed to determine the gravimetric water content for each soil sample. Approximately half each soil sample from the field was used to recover switchgrass roots. After weighing, the soil sample was soaked in a bucket of water for 10–20 min. Roots were then recovered by gentle hand washing and by pouring the mixture through two sieves (1 mm and 0.5 mm). The roots recovered on each sieve were thoroughly washed with water to remove attached soil particles.

Roots from the two sieves were combined in a shallow tray and hand sorted into five different classes: (1) rhizomes or root crowns, (2) coarse live roots, (3) coarse dead roots, (4) fine live roots, and (5) fine dead roots. Coarse and fine roots were >1 mm and ≤1 mm in diameter, respectively. Both color and turgor were used to separate living and dead roots. Roots were oven dried (70 °C), weighed, and ground in the sample mill. Ground samples of aboveground biomass, surface litter, and roots were stored in airtight glass jars. Because the amounts of root biomass from the April sampling event were small, roots collected from the four replicate plots were pooled prior to chemical analysis. Samples were analyzed for carbon and nitrogen concentrations using a LECO TruSpec® CN analyzer (LECO Corporation, St. Joseph, MI). The instrument was calibrated using LECO standards (EDTA, alfalfa, and barley) traceable to the National Institute of Standards and Technology (Gaithersburg, MD).

2.3. Calculations

Measurements of aboveground biomass, surface litter mass, and root biomass (all g m^{-2}) were multiplied by carbon concentrations (g C g^{-1}) to calculate carbon stocks on an area basis (g C m^{-2}). Nitrogen stocks (g N m^{-2}) were calculated in a similar manner. Root biomass (g m^{-2}) in each soil depth increment was calculated from root density (g roots kg^{-1} dry soil), soil bulk density (kg m^{-3}), and increment depth (m). Stocks of root biomass, carbon, and nitrogen

Table 1
Mean (±SE) amounts of dry matter, nitrogen concentrations, nitrogen and carbon stocks, and C:N ratios in aboveground biomass of 5-year old Alamo switchgrass grown under three different fertilizer application rates at Milan, TN.

Variable	Month	Fertilizer applied (kg N ha^{-1} yr^{-1})			*Fp*
		0	67	202	
Dry mass (kg m^{-2})	April	0.18 ± 0.05	0.20 ± 0.05	0.10 ± 0.03	ns
	November	0.72 ± 0.05	1.34 ± 0.16	3.21 ± 1.31	
Nitrogen concentration (g N kg^{-1})	April	6.85^{a} ± 0.27	8.22^{a} ± 0.72	13.5^{b} ± 0.36	0.001
	November	2.95^{a} ± 0.18	3.99^{ab} ± 0.68	5.46^{b} ± 0.46	
Nitrogen stock (g m^{-2})	April	1.19^{a} ± 0.28	1.59^{a} ± 0.29	1.32^{a} ± 0.41	0.05
	November	2.11^{a} ± 0.11	5.43^{a} ± 1.14	16.8^{b} ± 6.06	
Carbon stock (g m^{-2})	April	79 ± 21	90 ± 22	42 ± 13	ns
	November	330 ± 23	611 ± 72	1462 ± 601	
C:N ratio	April	64.8^{a} ± 3.1	54.8^{ab} ± 5.1	32.5^{b} ± 0.85	0.001
	November	157^{a} ± 9.9	123^{b} ± 17	84.7^{c} ± 6.1	

Sample size for each mean is four replicates. Means with different alphabetic superscripts are significantly different ($P \leq 0.05$) among fertilizer treatments. *Fp* is the probability that the difference among fertilizer treatments occurred by chance; "ns" indicates that differences among fertilizer treatments are not statistically significant.

were summed over 0–30, 0–60, and 0–90 cm soil depths. The net seasonal change in root biomass (g m^{-2}) was calculated only when the difference in root biomass between the spring and fall sampling periods was statistically significant.

Belowground biomass and carbon or nitrogen stocks were calculated for coarse live roots (CLR), fine live roots (FLR), coarse dead roots (CDR), and fine dead roots (FDR). Biomass and stocks were also calculated for total belowground biomass (TBG) and total live belowground biomass (TLB) according to the following mass balance:

$$\mathrm{TBG} = \mathrm{RHZ} + \mathrm{CLR} + \mathrm{FLR} + \mathrm{CDR} + \mathrm{FDR}$$

$$\mathrm{TLB} = \mathrm{RHZ} + \mathrm{CLR} + \mathrm{FLR}$$

$$\mathrm{TLR} = \mathrm{TLB} - \mathrm{RHZ} = \mathrm{CLR} + \mathrm{FLR}$$

$$\mathrm{TDR} = \mathrm{TBG} - \mathrm{TLB} = \mathrm{CDR} + \mathrm{FDR}$$

where RHZ, TLR, and TDR denote rhizomes/crowns, total live roots, and total dead roots, respectively.

2.4. Statistical analysis

Means and standard errors (SE) were used to summarize the data. Differences among fertilizer treatments and time (spring or fall) were tested using a two-way analysis of variance (ANOVA) with a fertilizer × time interaction. Because we were primarily interested in differences among fertilizer treatments, a Bonferroni test was used in post-ANOVA comparisons of paired means when the effect of fertilizer was statistically significant in the ANOVA. Unless otherwise stated, statistically significant differences were indicated by the probability (P) of a Type I error ≤0.05. Measurements from all plots were retained in the ANOVA despite a large amount of variation during the November sampling event in some measurements at the highest fertilizer treatment (202 kg N ha^{-1}). Depth profiles of cumulative root carbon and root nitrogen were fitted mathematically using logarithmic regression: $Y = a + b\,[\ln(X)]$, where Y is the cumulative measurement and X is soil depth (cm). Statistical analyses were performed using GraphPad Prism 4.0c (GraphPad Software, Inc., La Jolla, CA 92037).

3. Results

3.1. Aboveground biomass

Dry biomass, tissue nitrogen concentrations, calculated aboveground nitrogen and carbon stocks, and tissue C:N ratios were all significantly different between the April and November sampling events. Switchgrass biomass and carbon and nitrogen stocks, as well as tissue C:N ratios, increased over the growing season while tissue nitrogen concentrations declined (Table 1). Carbon concentrations in aboveground biomass were relatively invariant over both time and fertilizer treatment (0.45 ± 0.002 g C g^{-1}, $n = 24$).

Differences in plant tissue chemistry among the fertilizer treatments were statistically significant, but not differences in aboveground biomass (Table 1). Nitrogen concentrations in aboveground biomass increased with nitrogen fertilization. Nitrogen stocks were significantly higher at the highest fertilizer treatment (202 kg N ha^{-1} yr^{-1}) in November, but not April. Differences among fertilizer treatments in C:N ratios of aboveground biomass were statistically significant and inversely related to fertilizer application rate.

Table 2
Mean (±SE) amounts of dry matter, nitrogen and carbon stocks, and C:N ratios in surface litter beneath 5-year old Alamo switchgrass grown under three different fertilizer application rates at Milan, TN.

Variable	Month	Fertilizer applied (kg N ha^{-1} yr^{-1})			*Fp*
		0	67	202	
Dry mass (kg m^{-2})	April	0.80 ± 0.09	0.89 ± 0.08	0.87 ± 0.06	ns
	November	0.73 ± 0.12	0.94 ± 0.16	1.07 ± 0.11	
Nitrogen concentration (g N kg^{-1})	April	3.92^{a} ± 0.36	5.09^{ab} ± 0.39	6.73^{b} ± 0.51	0.001
	November	5.86^{a} ± 0.13	6.82^{a} ± 1.06	10.1^{b} ± 0.25	
Nitrogen stock (g m^{-2})	April	3.23^{a} ± 0.59	4.61^{a} ± 0.73	5.87^{a} ± 0.57	0.001
	November	4.29^{a} ± 0.73	6.16^{a} ± 1.12	10.8^{b} ± 1.13	
Carbon stock (g m^{-2})	April	293 ± 20	324 ± 42	332 ± 14	ns
	November	299 ± 45	390 ± 77	432 ± 49	
C:N ratio	April	98.2^{a} ± 14.0	71.2^{ab} ± 2.7	58.2^{b} ± 5.9	0.01
	November	70.6^{a} ± 2.8	66.7^{ab} ± 13.2	39.8^{b} ± 1.6	

Sample size for each mean is four replicates. Means with different alphabetic superscripts are significantly different ($P \leq 0.05$) among fertilizer treatments. *Fp* is the probability that the difference among fertilizer treatments occurred by chance; "ns" indicates that differences among fertilizer treatments are not statistically significant.

Table 3
Mean (±SE) switchgrass biomass ($g\ m^{-2}$) in coarse live roots (CLR), fine live roots (FLR), total live belowground biomass (TLB), coarse dead roots (CDR), fine dead roots (FDR), and total belowground biomass (TBG) for two soil depths under three fertilizer regimes (0, 67, and 202 $kg\ N\ ha^{-1}$) at two different times in Milan, TN.

Root category	Depth (cm)	April 2008			November 2008		
		0	67	202	0	67	202
Coarse live	0–30	478 ± 62	502 ± 116	435 ± 172	440 ± 31	512 ± 21	723 ± 374
	0–90	587 ± 102	666 ± 145	471 ± 178	620 ± 54	628 ± 21	861 ± 396
Fine live	0–30	425^{a} ± 37	372^{a} ± 73	226^{b} ± 41	172^{a} ± 9.7	153^{a} ± 7.8	115^{a} ± 40
	0–90	580 ± 67	592 ± 123	388 ± 76	247 ± 12	280 ± 20	246 ± 101
Total live belowground	0–30	1240 ± 281	1160 ± 337	1130 ± 522	672 ± 53	1440 ± 415	1240 ± 757
	0–90	1520 ± 360	1550 ± 357	1320 ± 518	929 ± 71	1680 ± 410	1510 ± 839
Coarse dead	0–30	61 ± 11	200 ± 136	138 ± 48	187 ± 23	311 ± 90	457 ± 217
	0–90	82 ± 13	225 ± 141	165 ± 51	254 ± 23	350 ± 91	533 ± 227
Fine dead	0–30	43 ± 12	67 ± 24	65 ± 19	300 ± 49	357 ± 74	441 ± 152
	0–90	57 ± 13	128 ± 38	89 ± 25	373 ± 55	693 ± 59	841 ± 280
Total belowground	0–30	1340 ± 296	1430 ± 480	1330 ± 549	1160 ± 116	2100 ± 554	2140 ± 1122
	0–90	1660 ± 368	1900 ± 491	1580 ± 562	1560 ± 125	2770 ± 469	2890 ± 1337

Each mean is based on measurements from four replicate plots. Means in the same row with different alphabetic superscripts are significantly different among fertilizer treatments in April or November ($P \leq 0.05$).

3.2. Surface litter

Surface litter chemistry varied seasonally. Nitrogen concentrations and stocks in surface litter increased significantly over the growing season, and litter C:N ratios were significantly less in November than in April at the highest fertilizer treatment (Table 2). Measured surface litter mass and calculated carbon stocks in surface litter did not change significantly between the April and November sampling events. Carbon concentrations in surface litter were relatively invariant over both time and fertilizer treatment ($0.39 \pm 0.01\ g\ C\ g^{-1}$, $n = 24$).

Similar to aboveground biomass, surface litter chemistry was changed by nitrogen fertilization. Nitrogen concentrations in surface litter increased with increasing rates of fertilization (Table 2). Surface litter C:N ratios declined with increasing fertilization, and C:N ratios at the highest treatment were significantly less than those in surface litter from the control plot. The dry mass of surface litter and the surface litter carbon stock were not significantly different among the three fertilizer treatments.

3.3. Belowground biomass

Root biomass in the 5-year old switchgrass stand was concentrated near the soil surface. Approximately 70–80% of total belowground biomass and 70–90% of the total live belowground biomass (to a 90 cm soil depth) resided in the top 30 cm of mineral soil (Table 3). Aside from soil depth, season of the year was the factor that most affected measurements of switchgrass root biomass, root carbon stocks, and root nitrogen stocks (Table 4). There was a significant loss of cumulative fine live root biomass over the growing season (losses were 333, 312, and 142 $g\ m^{-2}$, respectively, under 0, 67, and 202 $kg\ N\ ha^{-1}$ for the 0–90 cm depth).

In contrast to fine live root biomass, fine dead root biomass increased significantly from April to November (Tables 3 and 4). Over the entire depth profile (0–90 cm), there was also a significant net increase in coarse dead roots during the growing season (a change of 172, 125, and 368 $g\ m^{-2}$, respectively, under 0, 67, and 202 $kg\ N\ ha^{-1}$). The net change in coarse dead root biomass was approximately half, or less, of the seasonal change in fine dead root

Table 4
Results of two-way analysis of variance to test for significant fertilizer (F) and time of sampling (T) effects on cumulative switchgrass biomass, cumulative carbon stocks, and cumulative nitrogen stocks in coarse live roots (CLR), fine live roots (FLR), total live belowground biomass (TLB), coarse dead roots (CDR), fine dead roots (FDR), and total belowground biomass (TBG) at Milan, TN.

Variable	Depth (cm)	CLR		FLR		TLB		CDR		FDR		TBG	
		F	T	F	T	F	T	F	T	F	T	F	T
Cum. biomass	5	ns	ns	*	***	ns	ns	ns	ns	ns	***	ns	ns
	10	ns	ns	*	***	ns	ns	ns	ns	ns	***	ns	ns
	15	ns	ns	*	***	ns	ns	ns	ns	ns	***	ns	ns
	30	ns	ns	*	***	ns	ns	ns	ns	ns	***	ns	ns
	60	ns	ns	ns	***	ns	ns	ns	*	ns	***	ns	ns
	90	ns	ns	ns	***	ns	ns	ns	*	ns	***	ns	ns
Cum. carbon stock	5	ns	ns	*	***	ns	ns	ns	ns	ns	***	ns	ns
	10	ns	ns	*	***	ns	ns	ns	ns	ns	***	ns	ns
	15	ns	ns	**	***	ns	ns	ns	ns	ns	***	ns	ns
	30	ns	ns	*	***	ns	ns	ns	ns	ns	***	ns	ns
	60	ns	ns	ns	***	ns	ns	ns	*	ns	***	ns	ns
	90	ns	ns	ns	**	ns	ns	ns	*	ns	**	ns	ns
Cum. nitrogen stock	5	ns	ns	ns	***	ns	ns	ns	ns	ns	***	ns	ns
	10	ns	ns	ns	***	ns	ns	ns	ns	ns	***	ns	ns
	15	ns	ns	ns	***	ns	ns	ns	ns	ns	***	ns	ns
	30	ns	ns	ns	***	ns	ns	ns	*	ns	***	ns	ns
	60	ns	ns	ns	***	ns	ns	ns	*	*	***	ns	ns
	90	ns	ns	ns	***	ns	ns	ns	*	*	***	ns	ns

In all cases, the F × T interaction was not statistically significant. Depth indicates the cumulative soil depth. ns: not statistically significant.
* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

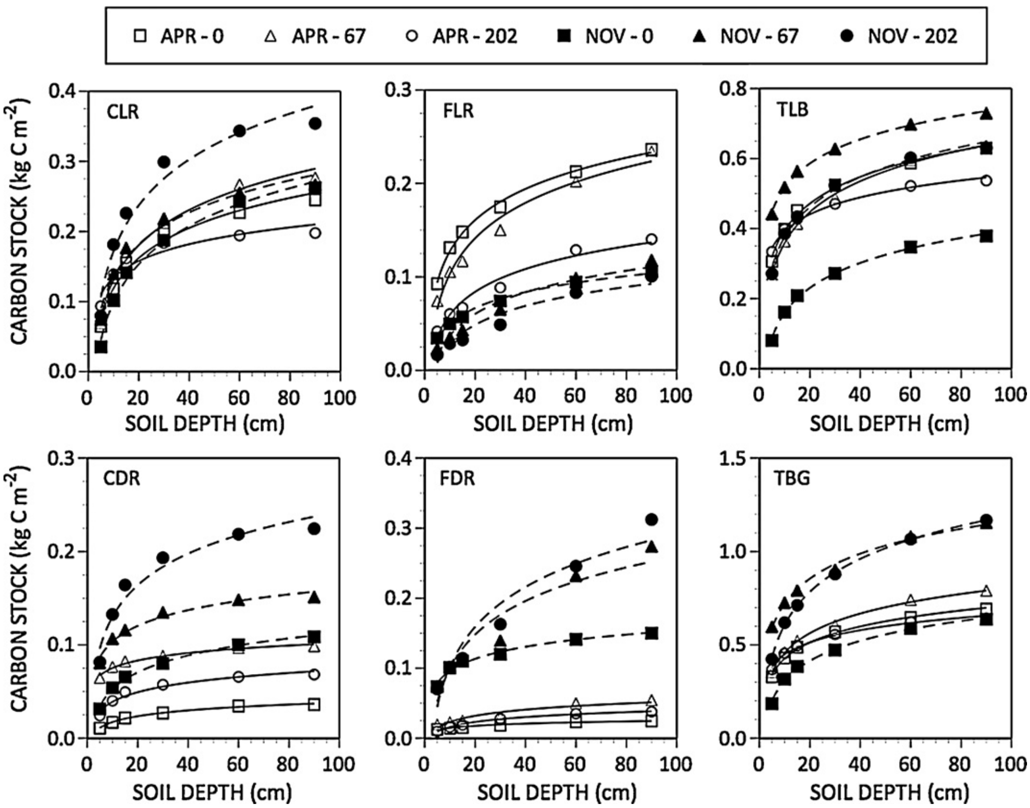

Fig. 1. Mean cumulative belowground carbon stocks as a function of soil depth for coarse live roots (CLR), fine live roots (FLR), total live belowground biomass (TLB), coarse dead roots (CDR), fine dead roots (FDR), and total belowground biomass (TBG) during April (APR – open symbols with solid lines) and November (NOV – filled symbols with dashed lines) in 5-year old switchgrass field trials grown under three different regimes of nitrogen fertilization (0, 67, and 202 kg N ha^{-1}) at Milan, TN. Each line is based on a logarithmic regression ($Y = a + b$ [ln(X)] and has a coefficient of determination ≥0.88.

biomass (316, 565, and 752 g m^{-2}, respectively, under 0, 67, and 202 kg N ha^{-1}) (Table 3).

With the exception of fine live roots, nitrogen fertilization had no detectable effect on cumulative root biomass (Table 4). Only the near surface (0–30 cm) fine live root biomass changed with fertilization. In April, there was significantly less fine live root biomass at the highest fertilizer application in the 0–30 cm depth increment (the same trend was evident in November, but not statistically significant). There was no significant interaction between the effects of fertilization and time of sampling on measurements of cumulative root biomass or cumulative carbon or nitrogen stocks.

End of the growing season root-to-shoot ratios, based on cumulative total belowground biomass, declined significantly ($P \leq 0.001$) at the highest rate of fertilization. Mean root-to-shoot ratios in November were 2.16 ± 0.08, 2.02 ± 0.18, and 0.88 ± 0.14, respectively, at the 0, 67, and 202 kg N ha^{-1} fertilization rates. Mean root-to-shoot ratios were markedly lower when total live belowground biomass was substituted for total belowground biomass in the ratio's numerator: 1.29 ± 0.05, 1.21 ± 0.18, and 0.42 ± 0.08, respectively, at 0, 67, and 202 kg N ha^{-1}.

3.4. Belowground carbon stocks

Mean (±SE) carbon concentrations in coarse dead roots, coarse live roots, fine dead roots, and fine live roots were 0.43 ± 0.02, 0.42 ± 0.01, 0.41 ± 0.03, and 0.41 ± 0.03 g C g^{-1}, respectively. Because carbon concentrations were relatively invariant across different root categories, from spring to fall, and across different rates of nitrogen fertilization, it was variation in measurements of root biomass that primarily determined differences in root carbon stocks. Therefore, effects of season and fertilization on cumulative root carbon stocks were similar to previously described effects of these factors on root biomass (Table 4).

Most of the carbon stored in root biomass, to a depth of 90 cm, was found near the soil surface. Carbon stocks in fine live roots declined significantly from spring to fall while stocks in both coarse and fine dead roots increased significantly over the same time period (Fig. 1). The ratio of carbon stocks in fine live roots to carbon stocks in coarse live roots was unaffected by fertilization (data not shown). Over the entire depth profile (0–90 cm), the net increase in coarse dead root carbon stocks during the 2008 growing season (73, 52, and 156 g C m^{-2}, respectively, under 0, 67, and 202 kg N ha^{-1}) was approximately half, or less, of the net increase in fine dead root carbon stocks (125, 220, and 274 g C m^{-2}, respectively, under 0, 67, and 202 kg N ha^{-1}).

Whole plant carbon stocks in spring and fall were examined in detail at the lowest rate of nitrogen fertilization (67 kg N ha^{-1}) (Fig. 2). The net carbon accrual by switchgrass over the growing season, based on the change in above- and belowground plant carbon stocks, was 952 g C m^{-2}. In both spring and fall, belowground plant carbon stocks exceeded carbon stocks in living and dead biomass aboveground. Rhizomes and/or root crowns were an important component of belowground carbon stocks (Fig. 2) even though they were limited to the upper 5 cm of mineral soil and were only sporadically present during soil sampling.

3.5. Belowground nitrogen stocks

Nitrogen concentrations in roots generally declined with increasing soil depth (Table 5), sometimes by as much as 50% or more (see e.g., fine dead roots). Coarse live roots growing in the control plots exhibited the least change in tissue nitrogen concentrations with depth. For many depth increments, root nitrogen

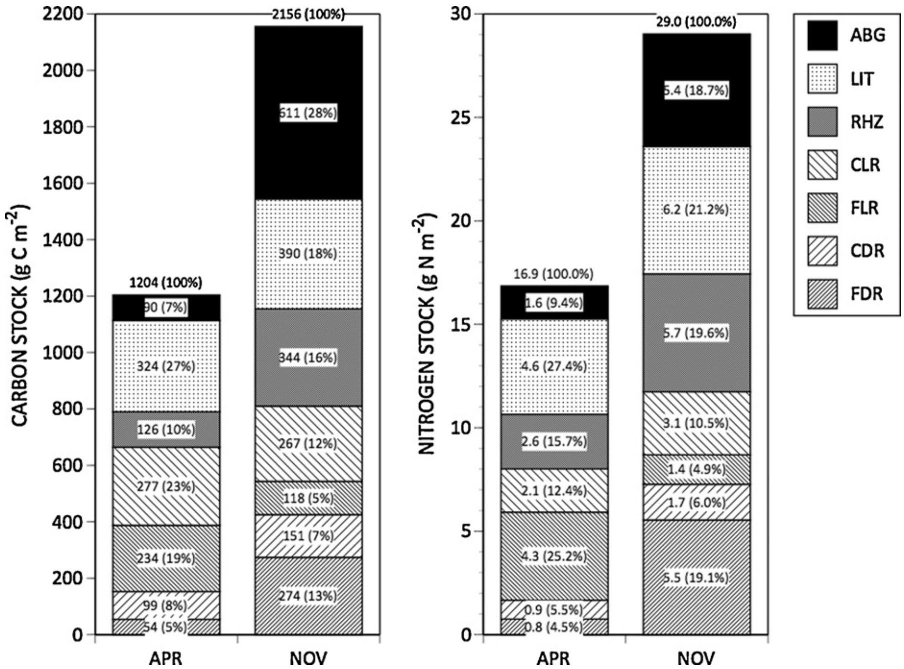

Fig. 2. Mean carbon and nitrogen stocks in aboveground biomass (ABG), surface litter (LIT), rhizomes/crowns (RHZ), coarse live roots (CLR), fine live roots (FLR), coarse dead roots (CDR), and fine dead roots (FDR) during April and November, 2008, under 5-year old switchgrass fertilized at 67 kg N ha^{-1} yr^{-1} in Milan, TN. The percentage of whole plant carbon or nitrogen stock for each plant component is shown in parenthesis.

concentrations increased with fertilizer application rate, although the difference between 67 kg N ha^{-1} and the control was often not statistically significant (Table 5). With the exception of fine dead roots, the effect of fertilization on root nitrogen stocks was not statistically significant (Table 4 and Fig. 3). Nitrogen stocks in fine live roots declined from spring to fall, while those in fine dead roots increased over the same time period (Fig. 3).

There was a significant amount of nitrogen stored in switchgrass roots at the end of the growing season (Fig. 2). Nitrogen stocks in total belowground biomass under 0 and 67 kg N ha^{-1} were more than three times the measured, end-of-season nitrogen stocks in aboveground biomass. Plant nitrogen demand, calculated as the difference between nitrogen in live plant biomass at the end and start of the growing season, increased with the amount of nitro-

Table 5
Mean (±SE) nitrogen concentrations and C:N ratios in switchgrass coarse live roots, fine live roots, coarse dead roots, and fine dead roots from six soil depths under three fertilizer regimes (0, 67, and 202 kg N ha^{-1}) in Milan, TN, at the end of the growing season (November).

Root category	Depth (cm)	Concentrations (g N kg^{-1})			P	C:N ratios			P
		0	67	202		0	67	202	
Coarse live	0–5	$3.6^a \pm 0.1$	$5.2^b \pm 0.5$	$8.7^c \pm 0.4$	***	$123^a \pm 4$	$86^b \pm 7$	$49^c \pm 2$	***
	5–10	$3.6^a \pm 0.3$	$5.2^a \pm 0.6$	$9.9^b \pm 0.6$	***	$123^a \pm 11$	$86^b \pm 10$	$42^c \pm 3$	***
	10–15	$3.3^a \pm 0.2$	$5.3^a \pm 0.5$	$8.0^b \pm 0.8$	***	$128^a \pm 7$	$81^b \pm 10$	$51^b \pm 7$	***
	15–30	$2.9^a \pm 0.2$	$5.3^{ab} \pm 0.8$	$6.7^b \pm 0.7$	**	$145^a \pm 10$	$86^b \pm 15$	$62^b \pm 6$	**
	30–60	$2.8^a \pm 0.2$	$4.1^{ab} \pm 0.7$	$6.3^b \pm 0.9$	*	$151^a \pm 8$	$111^{ab} \pm 16$	$68^b \pm 12$	**
	60–90	$3.2^a \pm 0.4$	$4.2^{ab} \pm 0.8$	$6.2^b \pm 0.9$	*	$137^a \pm 14$	$110^{ab} \pm 18$	$72^b \pm 10$	*
Fine live	0–5	$6.2^a \pm 0.5$	$7.3^a \pm 0.6$	$9.4^b \pm 0.2$	**	$71^a \pm 6$	$61^{ab} \pm 5$	$46^b \pm 1$	*
	5–10	$4.4^a \pm 0.1$	$5.4^a \pm 0.4$	$8.7^b \pm 0.4$	***	$99^a \pm 4$	$81^a \pm 8$	$49^b \pm 3$	***
	10–15	$4.4^a \pm 0.7$	$5.2^{ab} \pm 0.6$	$7.5^b \pm 0.8$	*	105 ± 14	86 ± 14	58 ± 6	ns
	15–30	$3.6^a \pm 0.7$	$4.9^{ab} \pm 0.8$	$6.6^b \pm 0.3$	*	132 ± 25	94 ± 17	63 ± 3	ns
	30–60	$3.2^a \pm 0.1$	$4.1^{ab} \pm 1.0$	$6.2^b \pm 0.8$	*	$133^a \pm 5$	$119^{ab} \pm 24$	$68^b \pm 8$	*
	60–90	3.2 ± 0.4	4.4 ± 0.6	5.5 ± 0.9	ns	$137^a \pm 16$	$99^{ab} \pm 13$	$79^b \pm 11$	*
Coarse dead	0–5	$4.9^a \pm 0.4$	$5.6^a \pm 0.6$	$8.6^b \pm 0.5$	**	$89^a \pm 8$	$82^a \pm 9$	$50^b \pm 3$	**
	5–10	$4.1^a \pm 0.4$	$4.7^a \pm 0.4$	$8.0^b \pm 0.7$	**	$110^a \pm 14$	$92^{ab} \pm 10$	$52^b \pm 5$	**
	10–15	$3.8^a \pm 0.1$	$4.8^{ab} \pm 0.4$	$7.2^b \pm 1.0$	*	$112^a \pm 5$	$89^{ab} \pm 11$	$59^b \pm 9$	**
	15–30	$3.0^a \pm 0.2$	$4.9^b \pm 0.7$	$6.3^b \pm 0.3$	**	$148^a \pm 11$	$92^b \pm 11$	$68^b \pm 4$	***
	30–60	$3.2^a \pm 0.1$	$4.3^{ab} \pm 0.9$	$6.0^b \pm 0.6$	*	$133^a \pm 4$	$111^{ab} \pm 20$	$70^b \pm 7$	*
	60–90	3.6 ± 0.6	4.6 ± 0.3	5.7 ± 0.7	ns	$129^a \pm 17$	$93^{ab} \pm 6$	$77^b \pm 9$	*
Fine dead	0–5	$11^a \pm 0.3$	$11^a \pm 0.7$	$16^b \pm 0.6$	***	$36^a \pm 2$	$37^a \pm 2$	$24^b \pm 2$	**
	5–10	$8.2^a \pm 0.7$	$10^{ab} \pm 1.0$	$12^b \pm 0.7$	*	50 ± 5	39 ± 6	31 ± 3	ns
	10–15	$7.2^a \pm 0.7$	$8.0^{ab} \pm 0.5$	$11^b \pm 1.0$	*	58 ± 7	53 ± 6	37 ± 6	ns
	15–30	$4.9^a \pm 0.4$	$8.3^b \pm 1.0$	$7.9^b \pm 0.9$	*	$91^a \pm 9$	$51^b \pm 6$	$50^b \pm 5$	**
	30–60	$5.2^a \pm 0.4$	$6.1^a \pm 1.1$	$9.4^b \pm 0.7$	*	$81^a \pm 7$	$74^a \pm 12$	$41^b \pm 3$	*
	60–90	$4.5^a \pm 0.7$	$6.6^{ab} \pm 0.6$	$7.4^b \pm 1.1$	*	$100^a \pm 15$	$60^{ab} \pm 6$	$56^b \pm 7$	*

Means in the same row with different alphabetic superscripts are significantly different at the indicated probability (P) level. ns: not significantly different.
* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

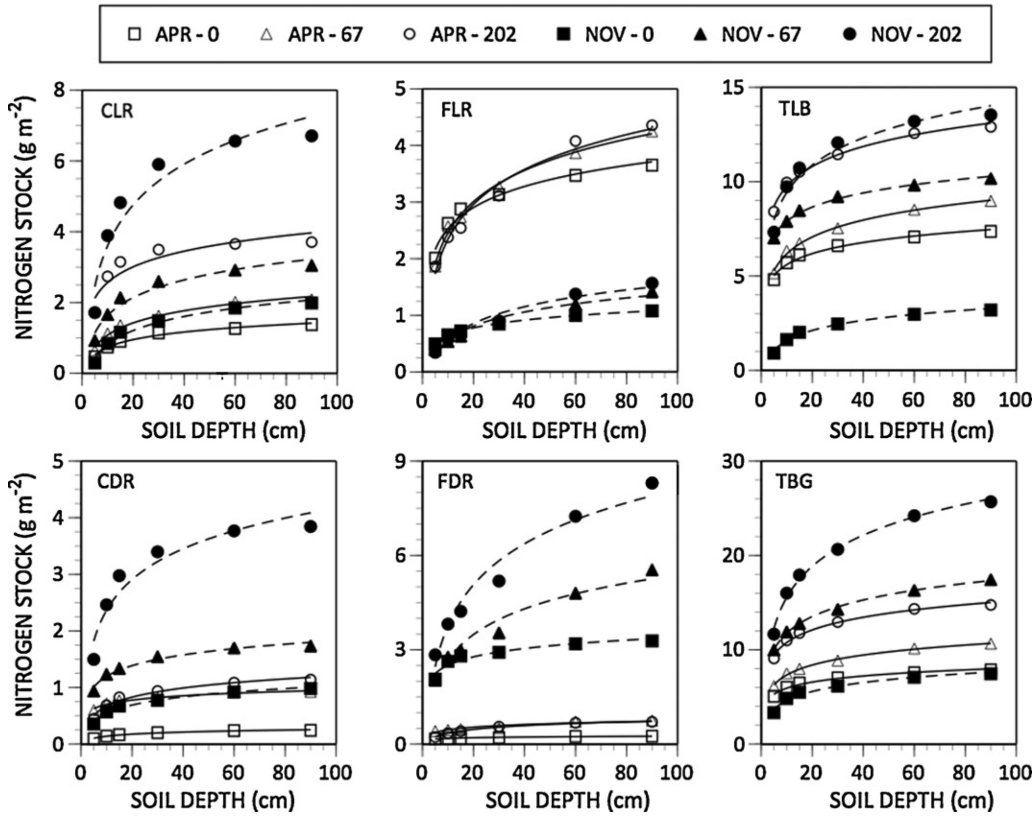

Fig. 3. Mean cumulative belowground nitrogen stocks as a function of soil depth for coarse live roots (CLR), fine live roots (FLR), total live belowground biomass (TLB), coarse dead roots (CDR), fine dead roots (FDR), and total belowground biomass (TBG) during April (APR – open symbols with solid lines) and November (NOV – filled symbols with dashed lines) in 5-year old switchgrass field trials grown under three different regimes of nitrogen fertilization (0, 67, and 202 kg N ha^{-1}) at Milan, TN. Each line is based on a logarithmic regression ($Y = a + b$ [ln(X)] and has a coefficient of determination ≥0.85.

gen applied at the start of the growing season. At the highest rate of fertilization, and assuming no loss of fertilizer nitrogen, nitrogen supply appeared to exceed the estimated nitrogen demand for production of switchgrass biomass (Table 6).

Root C:N ratios generally increased with depth and declined with an increasing rate of nitrogen fertilization (Table 5). Although fertilization with 67 kg N ha^{-1} yr^{-1} tended to increase root nitrogen concentrations and decrease root C:N ratios, the changes were often not statistically significant (coarse live roots in the surface mineral soil being the exception). It was the highest fertilizer treatment (202 kg N ha^{-1} yr^{-1}) that significantly reduced root C:N ratios relative to the control plot.

4. Discussion

Nitrogen fertilization of switchgrass in West Tennessee changed the chemistry of above- and belowground biomass, as indicated by C:N ratios, and also biomass allocation, as indicated by root:shoot ratios. These results emerged despite considerable within treatment variation in measured biomass. Variability in biomass at our site was primarily a result of within treatment variation the highest rate of nitrogen fertilization during November 2008. Random sampling in fields of heterogeneously distributed and clumped bunch grasses, like switchgrass, can sometimes yield higher than expected measurements of plant biomass if all sampling points within a plot randomly fall into the center of several bunches. Measurements of plant nitrogen and C:N ratios were unaffected by variation in biomass data. Even though amounts of above- and belowground biomass in different fertilizer treatments were often not statistically significant because of high measurement variability, root:shoot ratios indicated a significant effect of nitrogen fertilization on switchgrass biomass allocation.

4.1. Root tissue chemistry

Nitrogen fertilization decreased root C:N ratios in switchgrass, and the implication for soil carbon cycle processes is that fertilization may accelerate switchgrass root decomposition. Several

Table 6
Nitrogen in aboveground switchgrass biomass (AGB), total live belowground biomass (TLB), and total live biomass at the end of the growing season, plant nitrogen stock at the start of the growing season, and estimated plant nitrogen demand under different levels of nitrogen fertilization.

Fertilization (kg N ha^{-1} yr^{-1})	End of growing season			Start of growing season N stock[a]	Plant nitrogen demand
	AGB	TLB[b]	Total live plant N stock		
0	2.1	3.2	5.3	7.4	−2.1
67	5.4	10.2	15.6	9.0	6.6
202	16.8	13.5	30.3	12.9	17.4

Unless indicated otherwise, all values are g N m^{-2}.
[a] Based on TLB at the beginning of the growing season (April).
[b] TLB = total live belowground = rhizomes + live coarse roots + live fine roots.

aspects of root tissue chemistry (e.g., ash content, lignin, hemicellulose, and calcium) influence decomposition, but roots with higher C:N ratios usually decompose more slowly than those with lower C:N ratios (Silver and Miya, 2001). In switchgrass, both fast and slow components of root decomposition can be positively correlated with increasing tissue nitrogen concentrations, and the decomposition rate of rapidly decomposing root litter is negatively correlated with C:N ratio (Johnson et al., 2007). Lower root C:N ratios may decrease soil carbon storage if accelerated root decomposition leads to carbon loss as CO_2 prior to the incorporation of root litter into physically or chemically protected pools of soil organic matter. Nitrogen fertilization, through its impacts on root tissue chemistry, has definite implications for soil carbon storage beneath switchgrass, but there are multiple complexities that remain to be untangled. Our results that show changing switchgrass root nitrogen concentrations and C:N ratios in response to fertilization are qualitatively in agreement with prior studies at other locations (Ma et al., 2000; Sanderson and Reed, 2000; Vogel et al., 2002; Lemus et al., 2008; Heggenstaller et al., 2009).

4.2. Biomass allocation

We were unable to detect a significant effect of fertilizer treatment on aboveground biomass, carbon stocks, or surface litter. Similarly, there was no effect of nitrogen fertilization on root biomass measured over 0–30 and 0–90 cm depth increments, with the exception of fine live roots in April. Despite the general absence of statistically significant changes in biomass and carbon stocks with changing nitrogen fertilization, changing root:shoot ratios indicated an altered plant biomass or carbon allocation pattern (favoring aboveground over belowground production) at the highest fertilizer treatment tested. Other research reports mixed results for the effects of nitrogen fertilization on switchgrass root biomass. Prior studies indicate that switchgrass root biomass is either increased (Sanderson and Reed, 2000), unchanged (Ma et al., 2001), or responds in a complex manner (Heggenstaller et al., 2009) to increasing rates of fertilization. Our data are consistent with those of Ma et al. (2001) that indicate declining root:shoot ratios with high rates of nitrogen fertilization.

Two other patterns that are important to belowground carbon cycle processes beneath switchgrass are demonstrated by our study. First, there was more switchgrass biomass and carbon belowground than aboveground in mature stands. Given that most of the aboveground production is removed annually, belowground root production and turnover is essential to the maintenance of soil organic matter. Second, most (75% or more) of the root biomass, carbon, and nitrogen were found near the soil surface, generally in the top 30 cm of mineral soil. Although prolific rooting by switchgrass does contribute both carbon and nitrogen to deeper soil (Liebig et al., 2005), where organic matter may be more protected from decomposition, root distributions with depth indicate that the majority of soil carbon inputs occur in the top 30 cm of mineral soil.

4.3. System nitrogen balance

The dissection of whole plant nitrogen stocks (Fig. 2) indicated (1) rhizomes were an important part of total biomass and accounted for 16–20% of total plant nitrogen, (2) there was a sizable return of plant nitrogen to soil organic matter pools from spring to fall via fine dead roots, and (3) surface litter accounted for 21–27% of whole plant nitrogen, depending on season of the year. Prior studies of switchgrass at Milan (Garten et al., 2010), and at other locations (Tufekcioglu et al., 2003), have also reported substantial carbon and nitrogen stocks in surface litter that could be important to system nitrogen balance. End-of-season nitrogen stocks in surface litter were slightly greater than those measured in standing biomass and comparable to nitrogen added annually at the lowest fertilizer treatment (Table 2). Both nitrogen and carbon in surface litter beneath switchgrass plantations deserve additional attention from the standpoint of accrual rates, disappearance, and transfer into the surface mineral soil.

Calculations of whole plant nitrogen demand, based on differences between spring and fall, were conservative because they did not account for plant nitrogen turnover during the growing season through root mortality. The assumption that dead biomass does not contribute fully to the estimation of whole plant nitrogen demand can be defended based on the widely documented remobilization of nitrogen to living tissue during plant senescence (Yang et al., 2009). When both above- and belowground nitrogen demands were considered, data from the fertilizer trial indicated that switchgrass nitrogen demand increased in proportion to nitrogen supplied through annual fertilization.

Nitrogen supply appeared to closely match plant nitrogen demand at the lowest fertilizer treatment (6.7 g N m^{-2}) where plant uptake was 6.6 g N m^{-2}. Plant nitrogen uptake by 5-year old Alamo switchgrass at the lowest fertilizer treatment in 2008 was similar to calculated nitrogen uptake by Alamo, and other 4-year old switchgrass cultivars, that received the same amount of nitrogen fertilizer in 2007 in a different field at Milan (Garten et al., 2010). Although uptake can vary from year-to-year, depending on biomass production, the reported nitrogen uptake by 4- to 5-year old Alamo switchgrass in East Tennessee (Reynolds et al., 2000) was similar to that calculated for the lowest fertilizer application rate in West Tennessee.

At the highest fertilizer treatment, nitrogen supply exceeded plant nitrogen demand by possibly 5–7 g N m^{-2} yr^{-1}. Therefore, the highest level of fertilization would appear to increase the risk of nitrogen export from switchgrass fields to surface receiving waters and/or denitrification. Our calculations with whole plant nitrogen stocks indicated that nitrogen demand would be slightly underestimated by not accounting for belowground nitrogen stocks because there is only a slight change in nitrogen in total live belowground biomass over the growing season at 202 kg N ha^{-1} yr^{-1} (Table 6). In addition, based on data in Tables 1 and 3, calculated nitrogen use efficiency (see Lemus et al., 2008) declined from 213 g of biomass produced per gram of nitrogen applied at 67 kg N ha^{-1} to 83 g of biomass produced per gram of nitrogen applied at 202 kg N ha^{-1}. Thus, over-fertilization of switchgrass fields potentially impacts cost-efficient use of nitrogen fertilizers as well as surface water quality.

5. Conclusions

Season of the year was a consistently important factor affecting biomass and tissue chemistry of Alamo switchgrass grown in West Tennessee, USA. Effects of nitrogen fertilization were more difficult to detect due to variability in field measurements, but fertilization decreased switchgrass root:shoot ratios and root C:N ratios (significantly at the highest rate of 202 kg N ha^{-1} yr^{-1}). The net seasonal increase in fine dead root biomass over the growing season exceeded the net increase in coarse dead root biomass. At the end of the growing season, more carbon and nitrogen were stored in switchgrass root biomass than in aboveground biomass. Considering other research on the effect of tissue chemistry on root decomposition, higher root nitrogen concentrations and lower root C:N ratios at high levels of nitrogen fertilization could accelerate root decomposition and, consequently, impact soil carbon storage beneath switchgrass. The belowground response to nitrogen additions will depend on the interplay between processes leading to protection of soil carbon inputs and the change in root decomposition rate as a function of changing root tissue chemistry.

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