Agriculture, Ecosystems and Environment 136 (2010) 177–184

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Intra-annual changes in biomass, carbon, and nitrogen dynamics at 4-year old switchgrass field trials in west Tennessee, USA☆

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ARTICLE INFO

Article history:
Received 28 July 2009
Received in revised form 14 December 2009
Accepted 16 December 2009
Available online 12 January 2010

Keywords:
Carbon sequestration
Bioenergy crops
Switchgrass
Belowground biomass
Roots
Carbon
Nitrogen

ABSTRACT

Switchgrass is a potential bioenergy crop that could promote soil C sequestration in some environments. We compared four switchgrass cultivars on a well-drained Alfisol to test for differences in biomass, C, and N dynamics during the fourth growing season. There was no difference ($P > 0.05$) among cultivars and no significant cultivar × time interaction in analyses of dry mass, C stocks, or N stocks in aboveground biomass and surface litter. At the end of the growing season, mean (±SE) aboveground biomass was 2.1 ± 0.13 kg m^{-2}, and surface litter dry mass was approximately 50% of aboveground biomass. Prior to harvest, the live root:shoot biomass ratio was 0.77. There was no difference ($P > 0.05$) among cultivars for total biomass, C, and N stocks belowground. Total belowground biomass (90 cm soil depth) as well as coarse (≥1 mm diameter) and fine (<1 mm diameter) live root biomass increased from April to October. Dead roots were <10% of live root biomass to a depth of 90 cm. Net production of total belowground biomass (505 ± 132 g m^{-2}) occurred in the last half of the growing season. The increase in total live belowground biomass (426 ± 139 g m^{-2}) was more or less evenly divided among rhizomes, coarse, and fine roots. The N budget for annual switchgrass production was closely balanced with 6.3 g N m^{-2} removed by harvest of aboveground biomass and 6.7 g N m^{-2} supplied by fertilization. At the location of our study in west Tennessee, intra-annual changes in biomass, C, and N stocks belowground were potentially important to crop management for soil C sequestration.

1. Introduction

Production of aboveground biomass by switchgrass (*Panicum virgatum*) has received much attention from the perspective of cultivar differences, geographic variation, and cultivar × location interactions (Lemus et al., 2002; Casler et al., 2004; Cassida et al., 2005; Fike et al., 2006; Gunderson et al., 2008). Substitution of switchgrass for fossil fuels as an energy feedstock has the direct benefit of offsetting fossil-fuel carbon dioxide emissions to the atmosphere and the potential indirect benefit of sequestering carbon (C) in soil on idle or abandoned agricultural land that is devoted to switchgrass production (McLaughlin et al., 2002; Lemus and Lal, 2005). During the first part of the 20th century, cultivation throughout North America caused a substantial loss of soil organic C from agricultural land, and soil C is now slowly recovering as a result of practices like no-till agriculture (Lal et al., 1999). Hence, large areas of agricultural and abandoned agricultural land throughout the United States have soil C levels that are below capacity for soil C storage (based on levels of soil C residing under native vegetation characteristic of the land prior to cultivation). Soil C sequestration beneath switchgrass grown in the north central USA is generally positive with rates approaching

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doi:10.1016/j.agee.2009.12.019

120 g C m^{-2} $year^{-1}$ in the top 30 cm of soil at favorable sites (Liebig et al., 2005, 2008). Since most of the aboveground biomass in switchgrass is removed at harvest, the production and dynamics of belowground biomass are of utmost importance to potential soil C storage and the prospect of increasing C in soils with marginal or sub-marginal quality for agriculture.

Although switchgrass rooting depth can extend to more than 3 m (Ma et al., 2000), field studies indicate that switchgrass root biomass declines sharply with depth over the first meter of soil (Bransby et al., 1998; Garten and Wullschleger, 1999; Ma et al., 2000; Frank et al., 2004). Root crowns, the transition point between roots and stems, can represent a significant portion (up to 50%) of the total switchgrass biomass (Frank et al., 2004). Measurements to a 60 cm soil depth in the southeastern US after 4 years of switchgrass growth indicated root biomass in different soil types on the order of 1100–3100 g m^{-2} (Ma et al., 2000). Differences among ecotypes in belowground biomass have not been well studied even though there is some indication of variation in root biomass among switchgrass cultivars (Ma et al., 2000). The most extensive studies of switchgrass root biomass dynamics come from central Iowa (Tufekcioglu et al., 1999, 2003; Al-Kaisi and Grote, 2007). Tufekcioglu et al. (1999, 2003) characterized the distribution of live and dead roots, by two size classes, beneath 6–7-year old plantations and found that live, fine (≤2 mm diameter) roots comprise 80% of total root biomass (1682 g m^{-2}) to a depth of 125 cm. Monthly sampling indicated increasing live, fine root biomass from spring to fall with an overwinter decline between consecutive years (Tufekcioglu et al., 2003). Mature plantations (>20 years old) in central Iowa are reported to have approximately 1500 g m^{-2} root biomass in surface (30 cm deep) soils; significantly more than corn and soybean root biomass on the same soils (Al-Kaisi and Grote, 2007).

Nitrogen fertilization of switchgrass grown for bioenergy will also be an important component of site management from the standpoint of maximizing N use and minimizing N loss to surface receiving waters (Nyakatawa et al., 2006). Moreover, soil C storage beneath switchgrass can be increased at some locations (to as much as 240 g C m^{-2} $year^{-1}$ over a depth of 90 cm) with regular applications of inorganic N fertilizer (Lee et al., 2007). Like most crops, aboveground production in switchgrass is responsive to N fertilization, however fertilizer rates for maximum production can be relatively high (>100 kg N ha^{-1}) at some locations. Muir et al. (2001) found that switchgrass biomass yield in response to fertilizer N varied from year to year and that optimum yields in central and southern Texas occurred at annual rates of 168 kg N ha^{-1}. Vogel et al. (2002) reported optimum switchgrass biomass yields and N balance (i.e., fertilizer N was approximately equal to N removed by plant harvest) at annual rates of 120 kg N ha^{-1} in the midwestern USA. High N requirements for optimum aboveground production may be linked to reports of relatively low fertilizer N use efficiency by switchgrass. For example, Staley et al. (1991) and Stout and Jung (1995), respectively, reported rates of fertilizer N recovery by switchgrass (over 3 years) that ranged from 24 to 50% in central Pennsylvania. Harvest frequency also affects N use by switchgrass. Nitrogen demand is higher in switchgrass that is cut twice annually as opposed to once annually and, based on N uptake in one-cut systems, aboveground N requirement for a stand yielding 2000 g m^{-2} $year^{-1}$ is approximately 60 kg N ha^{-1} in east Tennessee (Reynolds et al., 2000). These studies indicate that N economy in switchgrass can be highly site-specific and the N cycle under switchgrass merits additional research.

The purpose of this research was to compare biomass, C, and N dynamics in four switchgrass cultivars grown in west Tennessee, USA. Previous studies in the southeastern USA indicate that lowland switchgrass ecotypes, like "Alamo", are higher yielding than upland ecotypes (Fike et al., 2006). Based on the experience of various breeders, three switchgrass cultivars with a potential for more vigorous growth and higher aboveground yields than the widely planted lowland ecotype, Alamo, were identified for production field trials at the University of Tennessee Research and Education Center at Milan, Tennessee. Considering that belowground C allocation might increase under the more vigorous lowland varieties, we studied intra-annual changes in above- and belowground biomass, C, and N stocks during the fourth growing season to test a null hypothesis of no differences among cultivars. Net root production and belowground biomass allocation among different root categories as well as the vertical distribution and tissue chemistry of roots through the soil profile were also measured because of their potential importance to soil C sequestration beneath switchgrass grown as a bioenergy crop. Lastly, we calculated the N balance for the one-cut management system in west Tennessee to determine if plant N demands are adequately met through annual N fertilization.

2. Materials and methods

2.1. Study site and field sampling

The experiment was established at the University of Tennessee's Research and Education Center near Milan, Tennessee (35°56′N latitude, 88°43′W longitude). The soil at the site is a moderately well-drained Alfisol (soil series: Grenada silt loam) that is classified as a thermic Oxyaquic Fraglossudalf. The study site was acquired by the University of Tennessee's Research and Education Center in 2001. It had been previously tilled in 2000, and in 2001 the field was plowed to a depth of 10 cm and planted with corn. In 2002, the field was planted with no-tillage soybeans, and in 2003 the field was rotated to no-tillage corn. In spring 2004, four switchgrass cultivars were no-till planted in 5 m × 8 m plots in a randomized, complete block design with four replicate plots for each cultivar. The switchgrass cultivars included Alamo (a lowland ecotype), two varieties from Georgia (GA992 and GA993), and a variety from Oklahoma (SL-93-3). Both of the cultivars from Georgia were lowland ecotypes that had been derived, respectively, from Kanlow and Alamo varieties. The SL-93-3 variety also had an Alamo origin. Fertilizer N (ammonium nitrate) was applied to the field trials at an annual rate of 67 kg N ha^{-1} every spring at the start of the second growing season. The field trials were harvested annually in October or November after the first killing frost. During the year of the study, 2007, the mean annual temperature and mean annual precipitation at Milan were 16.0 °C and 118 cm, respectively.

Above- and belowground biomass was sampled on three occasions in 2007 (fourth growing season) from three replicate plots of each switchgrass cultivar (12 plots total): spring (April 2–4), summer (July 23–25), and fall (October 29–31). At each sampling event, four sampling points were chosen in each plot using a random number table. A sickledrat (Kennedy, 1972) was used to harvest all aboveground biomass from a 0.1 m^2 area at each sampling point and four samples of aboveground biomass were pooled by plot in a paper bag. Following removal of aboveground biomass, all surface litters were removed from the ground (0.1 m^2 per sample) and the 4 litter samples from each plot were pooled in a paper bag. Soil cores (0–15 cm) were taken from each sickledrat patch using a soil core sampler (5.0 cm diameter) with hammer attachment. The soil was extruded from the cores and cut into 0–5, 5–10, and 10–15 cm increments. Four soil samples from the same depth in each plot were composited in a zip-lock plastic bag. Deeper soil samples were obtained from 15–30, 30–60, and 60–90 cm using a bucket auger (6.7–7.8 cm diameter depending on sampling event). One to three deep soil samples were collected

from each sickledrat patch and soil samples from the same depth were pooled by plot in a zip-lock plastic bag. Each field event yielded 12 samples of aboveground biomass, 12 litter samples, and 12 soil samples at 6 different depth increments for sample processing.

2.2. Sample processing and chemical analysis

In the laboratory, samples of aboveground biomass from each plot were separated into live and dead biomass. Live biomass, dead biomass, and surface litter from each plot were placed into tared paper bags, oven dried at 70 °C, and weighed to determine dry mass per unit area. Each dry sample was mixed by hand and a portion was ground and homogenized in a Tecator Cyclotec sample mill (model 1093). Ground materials were stored in airtight glass jars prior to chemical analysis.

Each bag of fresh soil was weighed and oven dry mass-to-fresh mass ratios were determined for each sample using 20–50 g portions of soil. Roughly half of each soil sample, with a known fresh weight, was soaked for approximately 10–20 min in a bucket of water and soil was removed from the roots by hand agitation and manipulation of the soil–water–root mixture. The mixture was poured through a 1 mm sieve stacked over a 0.5 mm sieve to recover roots. Water from a hose was used to wash the roots on each sieve and to remove soil adhered to the roots. Materials captured by the two sieves were combined into a single shallow tray for sorting into five categories: (1) rhizomes or root crowns, (2) live roots $\geq$1 mm diameter, (3) dead roots $\geq$1 mm diameter, (4) live roots $<$1 mm diameter, and (5) dead roots $<$1 mm diameter. For convenience, we refer to roots $\geq$1 mm and $<$1 mm diameter as "coarse" and "fine" roots, respectively. Live and dead roots were distinguished on the basis of color and root turgor. Roots from each soil sample were oven dried (70 °C), weighed, and ground in a sample mill prior to chemical analysis.

Biomass samples were analyzed for C and N concentrations using a LECO CN-2000 elemental analyzer (LECO Corporation, St. Joseph, MI). LECO standards (EDTA) traceable to the National Institute of Standards and Technology (Gaithersburg, MD) were used for instrument calibration. For lignin analysis, coarse or fine roots from each sampling event (April, July, and October) were pooled by cultivar and soil depth increments (0–15 and 15–90 cm). Acid detergent lignin was analyzed by the University of Wisconsin's Soil and Forage Analysis Laboratory in Marshfield, WI.

2.3. Gross nitrogen mineralization and immobilization

Using an isotope dilution technique (Davidson et al., 1991), gross N mineralization and gross nitrification rates were measured in surface soils (5 cm deep) on two occasions in 2007. Twenty-four tubes (5 cm diameter) were inserted into the soil in each plot in April and October. Trace amounts (1–3 mg N kg^{-1} soil) of enriched (99 atom %) NH_4-^{15}N or NO_3-^{15}N were injected into 18 tubes (9 for each form of N) and three tubes were injected with distilled water. Multiple injections that were made to each tube raised the soil–water content by 5–8% (w/w). Three control tubes received no injections.

Following injections (time zero), three labeled tubes that received NH_4-^{15}N or NO_3-^{15}N were destructively sampled for initial soil N concentration and initial percent ^{15}N enrichment. After 24 h, six labeled tubes from both the NH_4-^{15}N and NO_3-^{15}N treatment were collected along with the three tubes receiving water or no injection. The soil from each tube was mixed in a plastic bag and a subsample was extracted by shaking with 2N potassium chloride. An aliqout of the filtered solution was analyzed for total inorganic NH_4-N and NO_3-N using a continuous-flow colorimetric analyzer. To determine the isotope dilution, the remaining solution was diffused onto filter paper (Brooks and Stark, 1989; Stephan and Kavanagh, 2009) and measurements of atom percent ^{15}N were made using an isotope ratio mass spectrometer.

2.4. Calculations

Root density (g roots kg^{-1} dry soil) in each soil increment was multiplied by soil bulk density (kg m^{-3}) and divided by increment depth (m) to obtain standing stocks of root biomass (g m^{-2}). Estimates of net root production (g m^{-2}) over the growing season were calculated as the difference in root biomass between sampling intervals: April to July, July to October, and April to October. Measurements of aboveground biomass, litter mass, and root biomass (all g m^{-2}) were multiplied by C or N concentrations (g element g^{-1}) to obtain stocks of C and N (g element m^{-2}). Stocks of root biomass, C, or N were summed over 0–30, 0–60, and 0–90 cm. Measures of dry mass and stocks of C or N were summed for some root categories. Total belowground biomass refers to all five root categories combined while total live belowground biomass refers to the combination of rhizomes/crowns and live coarse and fine roots.

Atom percent excess values and the total inorganic N values from the isotope dilution study were used to calculate spring and fall soil N transformation rates. Nitrogen mineralization, nitrification, and immobilization rates were calculated from isotope dilution equations (Davidson et al., 1991).

Data were summarized using means and standard errors (SE). Differences among cultivars and months of the year were tested by sample type and/or soil depth using a two-way analysis of variance with a cultivar $\times$ time interaction. Statistical analyses were performed using KaleidaGraph 4.0 (Synergy Software, Reading, PA). Unless indicated otherwise, statistical significance was indicated by the probability (P) of a type I error $\leq$0.05.

3. Results

3.1. Aboveground biomass

At the end of the fourth growing season, an expected difference in yield among the cultivars was not supported by measurements at our study site. There were no statistically significant differences among cultivars and no significant cultivar $\times$ time interactions for amounts of dry mass, C stocks, or N stocks in aboveground biomass and surface litter. In Alamo, switchgrass yields were 0.3, 1.2, and 2.3 kg m^{-2}, respectively, at the end of the 2004, 2005, and 2006 growing seasons (Mooney et al., 2009). Our measured aboveground biomass at the end of the growing season in 2007 (2.1 ± 0.13 kg m^{-2}) was similar to the yield measured at the end of the growing season in 2006. Thus, available data indicated that switchgrass attained maturity at our study site after three growing seasons, and measurements in the fourth season should reflect any potential differences in belowground biomass.

Changes over the growing season were statistically significant for many aboveground measurements. Aboveground biomass increased 10-fold from spring to fall (Table 1). Carbon and N stocks in standing biomass also increased significantly over the same time period. There were statistically significant differences in the C/N ratio of aboveground biomass over the growing season: 71.9 ± 3.8 in April, 102.3 ± 4.6 in July, and 155.0 ± 9.4 in October. Dry mass and C stock in surface litter did not change significantly from April to October and was approximately 50% of standing biomass at the end of the growing season (Table 1). Surface litter C/N ratios were significantly higher in summer (101.1 ± 5.4) than in spring (72.0 ± 4.8) or fall (79.4 ± 4.0) reflecting a significant mid-year decline in litter N concentrations.

Table 1
Mean (±SE) biomass, C, and N ($g\,m^{-2}$) in 2007 at the Milan, Tennessee, cultivar field trials.

Component	Measurement	April	July	October
Aboveground	Biomass	$207^{a} \pm 23$	$1534^{b} \pm 133$	$2092^{c} \pm 131$
	Carbon	$92.6^{a} \pm 10.5$	$675^{b} \pm 58$	$946^{c} \pm 59$
	Nitrogen	$1.27^{a} \pm 0.10$	$6.61^{b} \pm 0.51$	$6.25^{b} \pm 0.41$
Surface litter	Biomass	1111 ± 29	1026 ± 63	1066 ± 66
	Carbon	391 ± 21	430 ± 28	429 ± 25
	Nitrogen	$5.54^{a} \pm 0.22$	$4.33^{b} \pm 0.29$	$5.59^{a} \pm 0.45$
Total belowground (live and dead)	Biomass	$1279^{a} \pm 122$	$1243^{a} \pm 160$	$1784^{b} \pm 120$
	Carbon	$492^{a} \pm 47$	$509^{a} \pm 66$	$769^{b} \pm 52$
	Nitrogen	$7.12^{ab} \pm 0.86$	$5.70^{a} \pm 0.83$	$8.91^{b} \pm 0.74$
Total live belowground	Biomass	$1196^{a} \pm 113$	$1168^{a} \pm 148$	$1621^{b} \pm 107$
	Carbon	$472^{a} \pm 47$	$477^{a} \pm 61$	$697^{b} \pm 46$
	Nitrogen	$6.42^{ab} \pm 0.74$	$5.34^{a} \pm 0.77$	$7.93^{b} \pm 0.65$
Coarse live roots	Biomass	$574^{ab} \pm 46$	$531^{a} \pm 59$	$713^{b} \pm 49$
	Carbon	$227^{a} \pm 20$	$219^{a} \pm 25$	$308^{b} \pm 21$
	Nitrogen	$1.94^{ab} \pm 0.16$	$1.55^{a} \pm 0.17$	$2.18^{b} \pm 0.17$
Fine live roots	Biomass	$450^{a} \pm 37$	$475^{a} \pm 46$	$609^{b} \pm 38$
	Carbon	$176^{a} \pm 15$	$190^{a} \pm 18$	$259^{b} \pm 16$
	Nitrogen	3.11 ± 0.23	2.82 ± 0.26	3.62 ± 0.12

Belowground data are summed to a 90 cm soil depth. Means ($n = 12$) in the same row with different alphabetic superscripts are significantly different ($P = 0.05$) over time.

3.2. Belowground biomass

Mirroring measurements aboveground, there was no significant difference among cultivars for total belowground biomass, C stocks, or N stocks in the 0–30, 30–60, and 60–90 cm soil depths at our site. Therefore, there was no basis for presenting or discussing differences among cultivars. We pooled data from all four cultivars by sampling event to examine intra-annual changes in switchgrass biomass belowground.

Throughout the growing season, there were statistically significant changes in biomass, C stocks, and N stocks belowground. Total belowground biomass to a 90 cm soil depth, as well as coarse and fine live root biomass, exhibited the same seasonal trend; all increased significantly from April to October (Table 1). Significant increases in belowground biomass in the 0–5 and 15–30 cm soil depths were strong contributors to seasonal changes in biomass measured over the entire 90 cm soil sampling depth (Fig. 1). Dead coarse root biomass, but not dead fine root biomass, also increased significantly from April ($14 \pm 4\ g\,m^{-2}$) to October ($105 \pm 15\ g\,m^{-2}$). At each sampling event, most of the total belowground biomass was live roots. For example, in October, 40, 34, and 17% of total belowground biomass was coarse live roots, fine live roots, and rhizomes, respectively. Dead roots were consistently <10% of the belowground biomass for the 0–30, 0–60, and 0–90 cm soil depths.

The increase in total belowground biomass, total live belowground biomass, coarse live roots, and fine live roots from spring to the end of the growing season was 505 ± 132, 426 ± 139, 139 ± 67, and $159 \pm 53\ g\,m^{-2}$, respectively, to a 90 cm soil depth. Net production of root biomass occurred late in the growing season between July and October (Table 2). Root-to-shoot ratios, that included total live belowground biomass (rhizomes + coarse and fine roots), changed from 5.8 in April, to 0.76 in July and 0.77 in October.

Root C stocks exhibited the same seasonal pattern as root biomass (i.e., significant change from July to October) because C concentrations in root biomass were relatively invariant among tissue types, cultivars, and sampling events ($0.42 \pm 0.003\ g\ C\ g^{-1}$, $n = 75$). The vertical distribution of total belowground biomass and the C stock in belowground biomass through the soil profile was similar; both biomass and C stocks declined sharply with soil depth. Increasing root biomass and C stocks over the growing season were attributed principally to statistically significant

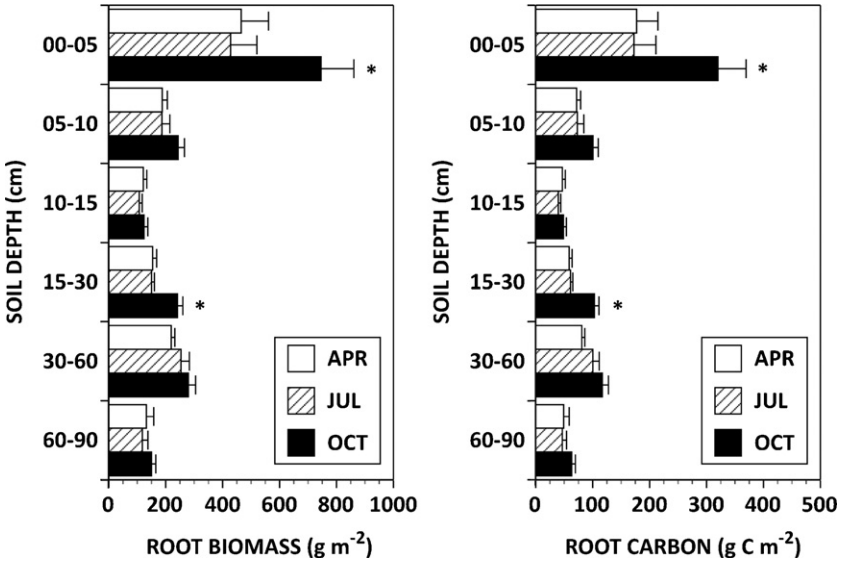

Fig. 1. Vertical profile of mean (±SE) total belowground biomass and total belowground C stocks during the 2007-growing season beneath switchgrass field trials at Milan, Tennessee. An asterisk indicates October means are significantly ($P = 0.05$) different from April and July ($n = 12$).

Table 2
Mean (±SE) cumulative biomass of rhizomes, coarse live and dead roots, and fine live and dead roots for three soil depths in 2007 at the Milan, Tennessee, cultivar field trials.

Depth (cm)	Month	Rhizomes	Coarse Roots		Fine Roots	
			Live	Dead	Live	Dead
0–30	April	171 ± 50	420^{a} ± 42	12^{a} ± 4	262^{a} ± 24	63 ± 51
	July	162 ± 54	372^{a} ± 48	51^{b} ± 15	272^{a} ± 21	14 ± 2
	October	299 ± 70	574^{b} ± 46	93^{c} ± 14	341^{b} ± 23	48 ± 11
0–60	April	171 ± 50	534 ± 45	13^{a} ± 4	359^{a} ± 30	70 ± 51
	July	162 ± 54	491 ± 56	54^{b} ± 15	399^{a} ± 36	18 ± 2
	October	299 ± 70	664 ± 48	103^{c} ± 15	513^{b} ± 36	55 ± 10
0–90	April	171 ± 50	574 ± 46	14^{a} ± 4	450^{a} ± 37	70 ± 51
	July	162 ± 54	531 ± 59	55^{b} ± 15	475^{a} ± 46	20 ± 2
	October	299 ± 70	713 ± 49	105^{c} ± 15	609^{b} ± 38	58 ± 10

Means from the same depth increment and particular root class with different alphabetic superscripts are significantly different over time ($P = 0.05$).

Table 3
Mean (±SE) N concentrations and C/N ratios in rhizomes, coarse live roots, and fine live roots from April, July, and October 2007 at the Milan, Tennessee, cultivar field trials.

Depth (cm)	Month	Rhizomes		Coarse live roots		Fine live roots	
		g N kg^{-1}	C/N	g N kg^{-1}	C/N	g N kg^{-1}	C/N
0–5	April	8.1^{a} ± 0.51	52.6^{a} ± 4.3	4.8^{a} ± 0.21	90.7^{a} ± 4.5	11.6^{a} ± 0.47	35.8^{a} ± 2.6
	July	5.4^{b} ± 0.44	80.2^{b} ± 7.0	3.7^{b} ± 0.14	113^{b} ± 3.8	10.2^{b} ± 0.48	41.2^{b} ± 2.3
	October	7.6^{a} ± 0.58	61.0^{a} ± 3.9	3.8^{b} ± 0.16	121^{b} ± 6.0	10.0^{b} ± 0.47	44.9^{b} ± 2.3
5–10	April	–	–	3.8^{a} ± 0.13	107^{a} ± 3.7	10.3^{a} ± 0.61	40.1^{a} ± 3.1
	July	–	–	3.2^{b} ± 0.14	134^{b} ± 5.3	7.2^{b} ± 0.39	61.0^{b} ± 3.4
	October	–	–	3.2^{b} ± 0.07	134^{b} ± 3.6	6.8^{b} ± 0.33	66.7^{b} ± 3.3
10–15	April	–	–	3.3 ± 0.15	128^{a} ± 4.9	7.7^{a} ± 0.41	54.2^{a} ± 3.7
	July	–	–	3.0 ± 0.10	142^{b} ± 4.6	5.7^{b} ± 0.65	82.7^{b} ± 8.1
	October	–	–	2.9 ± 0.10	150^{b} ± 6.6	5.1^{b} ± 0.41	91.9^{b} ± 6.7
15–30	April	–	–	2.8 ± 0.14	138^{a} ± 5.3	4.9^{a} ± 0.43	86.1^{a} ± 6.7
	July	–	–	2.6 ± 0.14	161^{b} ± 6.6	3.9^{b} ± 0.26	107.8^{b} ± 6.3
	October	–	–	2.7 ± 0.14	164^{b} ± 6.3	5.4^{a} ± 0.41	83.1^{a} ± 5.9
30–60	April	–	–	2.6 ± 0.10	141^{a} ± 8.4	4.0 ± 0.17	95.2 ± 3.7
	July	–	–	2.4 ± 0.06	171^{b} ± 4.2	4.0 ± 0.18	101.1 ± 4.7
	October	–	-	2.3 ± 0.11	186^{b} ± 8.5	4.4 ± 0.37	99.6 ± 7.1
60–90	April	–	-	2.6 ± 0.18	155 ± 12	3.7^{ab} ± 0.21	106.4 ± 6.3
	July	–	–	2.3 ± 0.09	179 ± 7.8	3.4^{a} ± 0.14	118.3 ± 3.9
	October			2.4 ± 0.10	177 ± 6.9	3.9^{b} ± 0.15	109.9 ± 4.0

Means from the same depth increment and root category with different alphabetic superscripts are significantly different over time ($P = 0.05$).

changes from April to October in the 0–5 and 15–30 cm soil increments (Fig. 1).

3.3. Root tissue chemistry

There were strong differences in root tissue chemistry from spring to fall and with rooting depth (Table 3). Nitrogen stocks in total belowground biomass, total live roots, coarse live roots, and fine live roots were not significantly different between April and July even though there was a tendency for declining root N stocks (Table 1). Rhizomes from the surface soil (0–5 cm) had significantly lower N concentrations in July (5.4 ± 0.4 g N kg^{-1}) than in April (8.1 ± 0.5 g N kg^{-1}) or October (7.6 ± 0.6 g N kg^{-1}). Nitrogen concentrations in live roots generally declined over the growing season in near surface (0–15 cm) soil samples (Table 3). With the exception of fine live roots, N stocks in belowground biomass increased significantly from mid-season minimums to end-of-growing season maximums because of high root production (Table 1).

Nitrogen concentrations in fine live roots exceeded N concentrations measured in coarse live roots (Table 3). In addition, root N concentrations decreased with increasing soil depth. Fine live root C/N ratios were less than those measured in coarse live roots, but C/N ratios in both root classes increased 2- to 3-fold from the soil surface to the deepest soil increment sampled. Mean lignin concentrations in coarse and fine live roots from the soil surface (0–15 cm) were significantly elevated in mid-summer (11.7%), but exhibited a narrow range from 9.9 to 13.1% over the entire growing season. Live roots from deeper soil samples (15–90 cm) had lower lignin concentrations than near-surface roots (ranging from 6.7 to 8.9%).

3.4. System nitrogen balance

Two aspects of the dataset indicated significant basipetal translocation of N by switchgrass late in the 2007-growing season. First, a comparison of live and dead switchgrass foliage from July to October indicated that leaf senescence was associated with an approximate 50% decline in N concentration (Fig. 2). Nitrogen concentrations in rhizomes also increased by approximately 40% over the same time period consistent with belowground storage of basipetally translocated foliar N. Second, the switchgrass stand continued to accumulate C aboveground from July to October while N stocks in aboveground biomass appeared to be unchanged over the same time period (Table 1). The standing stock of N at

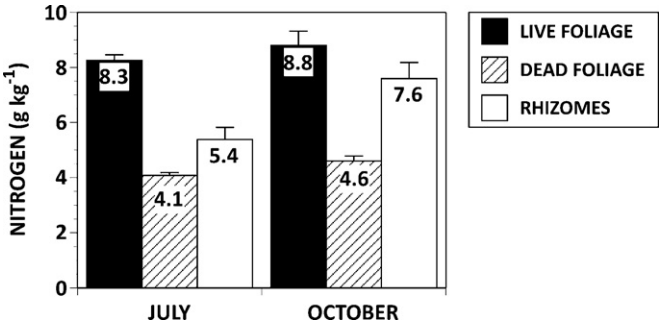

Fig. 2. Mean (±SE) N concentrations in switchgrass tissues collected in the middle of the growing season (July) and prior to harvest (October) in Milan, Tennessee (n = 12 for foliage and 7–11 for rhizomes).

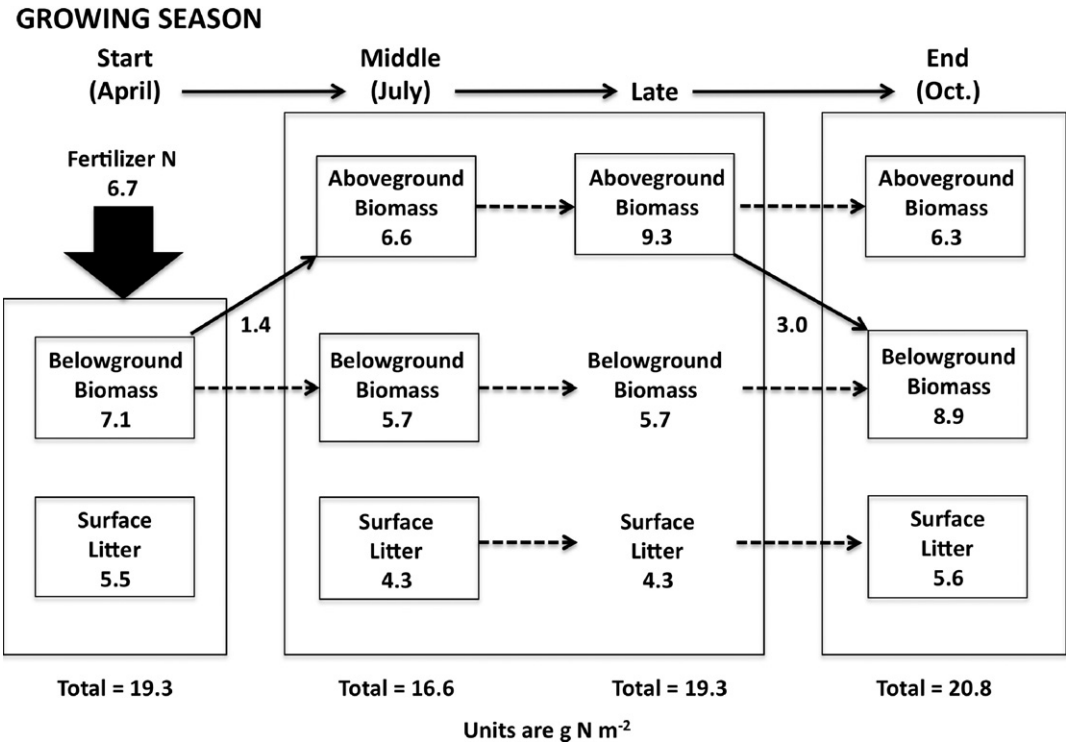

Fig. 3. System N stocks (and translocation) during the 2007-growing season in switchgrass field trials at Milan, Tennessee. Statistics for the stocks are presented in Table 1. The "late" growing season N stock, prior to switchgrass dormancy in October, was estimated from the change aboveground biomass C and a C/N ratio.

plant maturity (9.3 g N m^{-2}), and just prior to dormancy (identified as the "late" part of the growing season in Fig. 3), was calculated on the basis of the change in C stock in aboveground biomass from July (675 g C m^{-2}) to October (946 g C m^{-2}) and July C/N ratios (102). In October, measured N stocks in aboveground biomass (6.3 g N m^{-2}) were 3.0 g m^{-2} less than the estimated stock at plant maturity indicating that approximately 50% of the N in aboveground biomass had been translocated to rhizomes and roots by the time plants had become dormant in October.

Measured mean (±SE) rates of gross soil N mineralization and nitrification in spring, using the isotope dilution technique (n = 18), were 0.27 ± 0.05 and 0.15 ± 0.03 g N m^{-2} d^{-1}, respectively. In contrast, the October rate of gross soil N mineralization was 0.13 ± 0.03 g m^{-2} d^{-1} and the gross nitrification rate was 0.11 ± 0.02 g m^{-2} d^{-1}. The difference between mineralization and nitrification indicated that NH_4-N was the predominant inorganic soil N pool during the spring. This was supported by a higher concentration of NH_4^+ (0.30 ± 0.02 g m^{-2}) than NO_3^- (0.15 ± 0.01 g m^{-2}) in soil extracts. In the fall sampling, the gross nitrification rate was similar to the gross N mineralization rate and the NO_3^- pool was higher (0.41 ± 0.03 g m^{-2}) than in the spring relative to NH_4^+ (0.16 ± 0.02 g m^{-2}). Over the growing season (April to October), the measured gross N mineralization rates were extrapolated to 42 g N m^{-2}. However, net N mineralization is a balance between gross production and consumption (or N immobilization). The calculated N immobilization rate was 0.18 ± 0.03 g N m^{-2} d^{-1} or approximately 38 g N m^{-2} over the growing season. From these data, it was estimated that switchgrass had an available soil N pool of 2–4 g N m^{-2} over the growing season and the calculated net N mineralization rate was 1.3–2.7% per year of organic soil N.

The N budget associated with switchgrass production at Milan was in reasonably close balance (Fig. 3). The difference in system N stock from the start to the end of the growing season was 1.5 g N m^{-2}. Approximately 6.3 g N m^{-2} was removed from the site by harvest of aboveground biomass at the end of the growing season and 6.7 g N m^{-2} was annually supplied in N fertilizer. Basipetal N translocation at the end of the growing season (3.0 g N m^{-2}) was not balanced by acropetal translocation (1.4 g N m^{-2}) at the beginning, leaving a small deficit in system N balance that also had to originate from other sources (such as net soil N mineralization). The calculated available soil N over the growing season (2–4 g N m^{-2}, see above) was only slightly greater than the estimated deficit in system N balance. Calculated basipetal translocation of N (3.0 g N m^{-2}) accounted for most (94%) of the increase in total belowground N (3.2 g N m^{-2}) during the last half of the growing season (Fig. 3).

4. Discussion

4.1. Relative importance seasonal- and cultivar-based differences in belowground biomass

Genetically-based differences in root biomass and net root production can be important attributes when selecting switchgrass cultivars to promote soil C sequestration, but such differences appear to be highly site dependent. Based on prior studies that indicated cultivar-based differences in production of aboveground biomass (Frank et al., 2004; Fike et al., 2006) and standing stocks of belowground biomass (Ma et al., 2000; Sanderson, 2008), along with preliminary data from breeders who were familiar with the varieties used on our field trial, we expected to find differences in belowground biomass among the four switchgrass cultivars grown in west Tennessee. If cultivar differences in belowground biomass existed at the end of the fourth growing season, we were unable to detect them at a 95% probability level due to high variability in measurements of belowground biomass.

Net production and the rapid turnover of live fine roots could be an important input to soil organic C under switchgrass. The production of belowground biomass over the 2007-growing season at Milan did not strongly favor any particular category of live roots in the cultivar field trial. The increase in total live

belowground biomass from April to October was divided almost equally among rhizomes, coarse, and fine roots. Carbon storage in rhizomes at the end of the growing season represented <20% of total C stored in belowground biomass at Milan, even though switchgrass rhizomes can be an important belowground structure for C storage at some sites (Frank et al., 2004).

Intra-annual changes in belowground biomass and root C and N stocks at Milan were of overall greater importance to crop management for C sequestration than differences among cultivars. Switchgrass can be managed as a one- or two-cut production system. The former involves a single autumn harvest and the latter includes both mid-summer and autumn harvests. In the upper southeastern U.S., two-cut systems can yield more biomass than a single harvest (Fike et al., 2006), but with a potential negative effect due to higher N removal and faster soil N depletion (Lemus et al., 2008). Although most aboveground biomass production occurs from spring to mid-summer, our data indicate that the last part of the growing season (mid-summer to autumn) is the time of maximum belowground production. Therefore, two-cut systems may diminish soil C sequestration by directing C allocation aboveground during a time when it would otherwise be directed to root production and turnover. A one-cut system enables a full net production of belowground biomass that is fundamental to enhancing soil C storage.

4.2. Comparisons of belowground switchgrass biomass at Milan with other sites

Measurements of belowground biomass in 4-year old switchgrass field trials at Milan compared favorably with mature fields at other locations. Total belowground biomass in surface soil (30 cm depth) at the end of the growing season was 1354 ± 120 g m^{-2} at Milan (Table 2). Tufekcioglu et al. (2003) and Al-Kaisi and Grote (2007), respectively, reported approximately 1220 and 1500 g m^{-2} root biomass in surface soils (30–35 cm deep) beneath switchgrass stands that were more than 5 years old in central Iowa. The standing stock of belowground biomass to a 90 cm soil depth at Milan was 1784 ± 120 g m^{-2} and was somewhat greater than measurements of root biomass (to 90 cm) reported by Ma et al. (2000) for 7-year old stands of Kanlow (1086 g m^{-2}), Alamo (1280 g m^{-2}) and Cave-in-Rock (1689 g m^{-2}) varieties grown in central Alabama, but similar to deep belowground biomass (1682 g m^{-2} to a 125 cm soil depth) in central Iowa (Tufekcioglu et al., 2003). Similar to results from west Tennessee, dead fine roots were also a small fraction of total belowground biomass beneath switchgrass fields in central Iowa (Tufekcioglu et al., 2003).

Both the vertical distribution and tissue chemistry of switchgrass root biomass have important implications for promoting soil C sequestration. Perennial grasses have the potential to store a significant amount of C deep in the soil profile (Fisher et al., 1994) where there are slower rates of organic matter decomposition (Van Dam et al., 1997; Accoe et al., 2002). Consistent with other studies of switchgrass root biomass (Ma et al., 2000; Tufekcioglu et al., 2003; Sanderson, 2008), most (approximately 70–80%) of the root biomass at Milan (to 90 cm soil depth) was contained in the surface 30 cm of mineral soil. However, at Milan approximately 25% of the live root biomass was found below 30 cm. Moreover, C/N ratios in coarse and fine live switchgrass roots at Milan increased with soil depth (Table 3) and, because root decomposition rates generally decline with increasing C/N ratio (Silver and Miya, 2001), less decomposition of dead root inputs to the subsoil is expected to promote deep soil C storage over the life of the plantation. In laboratory experiments, switchgrass root decomposition proceeds according to a double exponential decay model with approximately 30% mass loss at a rate of 3.2% d^{-1} and most material decomposing at a slower rate of 0.05% d^{-1} (Johnson et al., 2007). This relatively slow decomposition rate suggests that switchgrass root debris persists long enough to allow for physical protection against microbial attack through soil aggregate formation.

4.3. Remarkably large C stocks are present in surface litter

The amount of surface litter beneath switchgrass field trials at Milan was relatively large (approximately 1 kg m^{-2}) and exhibited negligible mass loss over the growing season (Table 1). Tufekcioglu et al. (2003) also reported surface litter amounts on the order of 1–1.6 kg m^{-2} under 7-year old switchgrass stands in central Iowa. Thus, ours is the second study to find remarkably high C stocks in surface litter (approximately half of the C present in annual aboveground switchgrass production). The fate of C residing in surface litter is unknown. Because surface litter is not plowed into the soil under conditions of no-till switchgrass agriculture, it may not be a large contributor to mineral soil C stocks.

Our data, and those of Tufekcioglu et al. (2003), indicate that residue collection under switchgrass may also constitute a potential resource as a bio-feedstock for production of cellulosic ethanol because surface litter beneath switchgrass, on a g m^{-2} basis, is more than double the amount of stover remaining in cornfields. Corn stover, as well as surface litter from other crops (including switchgrass), may be viable bioenergy feedstock in some agricultural regions of the U.S.A. (Graham et al., 2007). The dynamics of surface litter accumulation and decomposition over the life of a switchgrass plantation, as well as the reasons for surface litter accumulation, merit additional research to assess its feedstock potential.

4.4. The importance of N balance to sustainable management of switchgrass

Harvest management, possibly in combination with residue removal, has important implications for switchgrass N balance and sustainability of bio-feedstock production. One-cut systems remove approximately half as much soil N as two-cut systems (Reynolds et al., 2000). Moreover, N stocks in surface litter at the end of the growing season at Milan were nearly equal to N stocks in standing aboveground biomass. Thus, surface litter was an important N reservoir in our system, and the impact of its annual removal on field N balance is unknown but potentially significant. Nitrogen removal in one-cut switchgrass systems across the eastern U.S. varies from 4 to 13 g N m^{-2} with an average removal of 7.5 g N m^{-2} year^{-1} (Fike et al., 2006). End of the season N stocks in aboveground biomass at Milan (6.3 g N m^{-2}) compared favorably with other data for switchgrass grown in east Tennessee where the average N removal by one-cut systems 3- to 5-year following establishment averaged 5.7 g N m^{-2} (range 5.0–6.3 g N m^{-2}) (Reynolds et al., 2000).

Nitrogen reserves, both external and internal to the crop, may require management to maintain sustainable switchgrass production at some sites (Lemus et al., 2008). In our study, internal N cycling was important to maintaining N balance in the switchgrass field trials at Milan, Tennessee. Nitrogen stocks in belowground biomass declined by 1.4 g N m^{-2} from April to July due to acropetal N translocation during a period of rapidly growing aboveground biomass. Nitrogen stocks in belowground biomass subsequently increased by 3.2 g N m^{-2} from July to October due to basipetal N translocation during a period of increased root production, diminished aboveground growth, and senescence of leaves and stems. With continuous annual removal of N in aboveground biomass (and possibly surface litter), soil N availability must be high enough to repeatedly meet crop N demands over the long-term to ensure sustainability of switchgrass plantations as a bioenergy feedstock. Diminished biomass production as a result of

N limitation, if it were to occur, would also negatively impact belowground soil C sequestration by reducing root C inputs to the mineral soil. At our study site, the N budget for switchgrass production during the 2007-growing season was well balanced (i.e., N removal by plant harvest nearly equaled annual fertilizer N inputs). Although there was some uncertainty associated with estimates of soil N availability from only two points in time, the isotope dilution study indicated that soil N made available through decomposition of soil organic matter over the growing season was significant relative to annual fertilizer N applications and was of sufficient amount to account for a small deficit ($1.5\ g\ N\ m^{-2}$) between the starting and ending switchgrass N budget.

5. Conclusions

The timing of intra-annual changes in root biomass, C, and N dynamics was more important than differences among cultivars for the management of belowground C inputs beneath 4-year old switchgrass field trials in west Tennessee. Most aboveground production occurred during the first half of the growing season (April to July) while net root production occurred during the last half of the growing season (July to October). Although most of the root biomass resided in the surface 30 cm of soil, differences in tissue chemistry over the soil profile suggest that deeper roots (to 90 cm) could have a slower decomposition rate than surface roots because root decomposition rates are inversely related to C/N ratios (Silver and Miya, 2001). Thus, root debris at depth could be an important contributor to organic matter formation and soil C sequestration under switchgrass. The observed seasonal pattern in root production indicated that a single harvest at the end of the growing season was best suited for maximizing belowground C inputs and optimizing system N balance.

Acknowledgments

Research was sponsored by the U.S. Department of Energy's Office of Science, Biological and Environmental Research funding to the Consortium for Research on Enhancing Carbon Sequestration in Terrestrial Ecosystems (CSiTE) under contract DE-AC05-00OR22725 with Oak Ridge National Laboratory (ORNL), managed by UT-Battelle, LLC. We wish to thank Blake Brown at the Research and Education Center at Milan, Tennessee, and Janet Gibson, Ernest Merriweather, and Bobby Henderson at the University of Tennessee's West Tennessee Research and Education Center for their helpful support of laboratory and field activities.

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