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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 \pm SE) declined significantly ($P \le 0.05$) at the highest fertilizer nitrogen treatment ($2.16 \pm 0.08, 2.02 \pm 0.18$, 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.

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1. Introduction

Switchgrass is a perennial, warm-season grass that is wideranging over North America and a potential biofuel feedstock for

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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 ($35^{\circ}55'31''$ N latitude; $88^{\circ}42'57''$ 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" switch-grass (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 ($4.6 \text{ m} \times 7.3 \text{ m}$) 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 $14.7 \,^{\circ}$ 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-m² 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 m² 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 \times 6 depth increments \times 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 CyclotecTM 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 \leq 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

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