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Displacing Inorganic Nitrogen in Lignocellulosic Feedstock Production Systems

Amanda J. Ashworth,* Pat D. Keyser, Fred L. Allen, Donald D. Tyler, Adam M. Taylor, and Charles P. West

ABSTRACT

Second-generation feedstocks such as switchgrass (*Panicum virgatum* L.) have been proposed as sustainable alternatives to fossil fuels, although they still require nonrenewable inputs, notably, inorganic N. Therefore, our objectives were to determine (i) the effects of biochar (1 and 2 Mg ha^{-1}), three intercropped legumes [red clover (*Trifolium pratense* L.), partridgepea (*Chamaecrista fasciculata* [Michx.] Greene), and sunn hemp (*Crotalaria juncea* L.)] vs. inorganic N [67 kg ha^{-1} and 0 kg ha^{-1} (control)] on desired feedstock characteristics, yield, and soil characteristics; and (ii) feedstock alterations and tissue-nutrient levels for postsenescence (November) and overwintering (February) harvests in a two-factor randomized block design. Overwintering harvests increased P and K remobilization, ethanol yield, fermentable sugars, and in-field dry-down ($P \leq 0.05$), although yield losses occurred (22%). November harvests had greater tissue N and fermentable substrates, leading to greater soil nutrient removals. Consequently, harvests manipulated the desired feedstock traits, whereas soil amendments had little effect on feedstock characteristics. Therefore, the results suggest that legume intercrops (partridgepea) and biochar may supply analogous N to synthetic fertilizers ($P \leq 0.05$), thereby displacing inorganic N without altering feedstock quality. However, for inorganic N alternatives to be competitive on a break-even cost basis, greater biomass yields need to be obtained under these management practices.

Determining alternative organic N fertilizer sources and levels over a range of soils that are C-neutral is fundamental to producing a sustainable bioenergy feedstock. Specifically, inorganic N fertilizers have prices linked to petroleum markets, require fossil energy for their production, and can degrade surface and ground water (Pimentel et al., 2008; Ashworth et al., 2015). One likely replacement of synthetic N fertilizer is the use of legumes in biofuel production systems (Snapp et al., 1998). An additional inorganic N alternative is biochar or the by-product of switchgrass and other lignocellulosic biomass from thermochemical conversion processes such as pyrolysis (Lehmann and Joseph, 2009). Biochar applications to soil may increase C sequestration and plant nutrient retention, thereby decreasing chemical fertilizer inputs (Mullen et al., 2010). However, in order for legumes and biochar to be employed for feedstock production, a protocol for the most economical and nutrient efficient system is needed.

Soil fertility and crop yield can be increased by leguminous green manure intercrops through increases in soil organic C, N, and P compared with weedy fallows and nonleguminous cover crops (Tonitto et al., 2006). However, the extent of this in warm temperate intercropping systems is unknown, especially for switchgrass production. Smithson and Giller (2002) found that biologically fixed N from legumes can reach levels up to 450 kg N ha^{-1} per crop. Tonitto et al. (2006) concluded in a meta-analysis that legumes could supply up to 350 kg N ha^{-1} yr^{-1} in a fairly continuous distribution, with 50% of the studies having 50 to 150 kg N ha^{-1} yr crop^{-1}. At this level of N, legume intercrop systems can meet N requirements for switchgrass production. These results demonstrate the potential for N_2–fixing crops to support crop yields while reducing reactive mononitrogen oxide emissions and decreasing nitrate leaching by up to 40% (Tonitto et al., 2006).

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A.J. Ashworth and F.L. Allen, Dep. of Plant Sciences, Univ. of Tennessee, Knoxville, TN 37996; P.D. Keyser, Dep. of Forestry, Wildlife and Fisheries, Univ. of Tennessee, Knoxville, TN 37996; D.D. Tyler, Dep. of Biosystems Engineering and Soil Science, Univ. of Tennessee, Knoxville, TN 37996; A.M. Taylor, Center for Renewable Carbon, Dep. of Forestry Wildlife and Fisheries, Univ. of Tennessee, Knoxville, TN 37996; and C.P. West, Dep. of Plant and Soil Science, Texas Tech Univ., Lubbock, TX 79410. *Corresponding author (aashwor2@utk.edu).

Abbreviations: ETREC, East Tennessee Research and Education Center; GREC, Greenville Research and Education Center; CEC, cation exchange capacity; PLS, pure live seed; NDF, neutral detergent fiber; dNDF, digestible neutral detergent fiber; IVTD, in vitro true digestibility.

Biochar is assumed to be chemically and biologically recalcitrant (Lehmann, 2007). Consequently, biochar applications may contribute to soil C storage with a long turnover time, thereby potentially improving soil quality thanks to the vital role C plays in N cycling. Biochar may also improve soil characteristics either directly or indirectly, as biochar is highly adsorbent because of its high surface area and positive charge density, thereby increasing soil water-holding capacity and mineral nutrient retention (Chang and Zhihong, 2009; Clough and Condron 2010; Lehmann and Joseph, 2009). A wide range of N concentrations have been reported for biochar (1.8–56.4 g kg^{-1}), with some nutrients not being labile and therefore having a high C/N ratio (Sadaka et al., 2014; Mullen et al., 2010; Lehmann, 2007). In addition, biochar nutrient concentrations depend on feedstock sources as well as thermochemical operating conditions; therefore, not all chars are equivalent and some may have no crop yield impact (Chang and Zhihong, 2009; Ashworth et al., 2014). Heating feedstocks causes volatilization of some elements, such as N, whereas most minerals become sequestered in the remaining activated C. Anex et al. (2007) determined that when switchgrass coproducts are recycled into production systems, a N recovery rate of 111 kg ha^{-1} yr^{-1}can occur. This represents ~78% of the N fertilizer input required, assuming 142 kg N ha^{-1} yr^{-1} [i.e., 10.5 kg N ha^{-1} Mg^{-1} switchgrass removed (Anex et al., 2007)]. Therefore, enhanced nutrient cycling and favorable energy balances are prospective benefits of coupling agricultural and bioenergy systems.

A considerable knowledge gap still exists in understanding the mechanisms and relative nutrient contributions from biochar and legume intercrop systems. Peer-reviewed reports on biochar in switchgrass are still sparse (Clough and Condron, 2010) and data are needed to test biochar under dynamic field conditions. Similarly, a data gap exists for legume-based intercropping systems for their relative yield contributions in switchgrass feedstock systems. Furthermore, research is needed to reduce or eliminate the conventional reliance on fossil fuel-based N fertilizer to enhance the net energy balance of herbaceous cellulosic feedstock production, which is currently the primary input requirement in these systems (Pimentel et al., 2008). Information is also lacking on feedstock characterization (i.e., potential ethanol yield, cell wall constituents, nutrient levels, and ash) as affected by harvest timing and soil amendments. In addition, information on biomass water content during post-senescence harvest periods is needed to maximize in-field drying and minimize the economic and energy costs (Lindsey et al., 2013).

Therefore, our goal was to develop an economically and ecologically sustainable cellulosic energy production model for reducing inorganic N for the southeastern United States. In addition, the harvest date may influence feedstock sustainability through its impact on nutrient retention and remobilization after senescence. The result may be a higher quality feedstock for conversion, [i.e., lower mineral, moisture, and ash levels and greater level of digestible sugars (biological) and lignin (thermo-chemical)] and reduced nutrient requirements in subsequent years. This research will contribute to the understanding of N mineralization promotion from the two organic N sources (legumes and biochar) as they relate to sustainable bioenergy production. The specific objectives were to: (i) determine effects of two biochar rates, three legume intercrops vs. the inorganic N rate on feedstock characteristics, economic feasibility, yield, and soil nutrient additions; and (ii) compare biomass characteristics and nutrient tissue levels between fall postsenescence (15 November) and winter (mid-February) harvests in humid temperate areas.

MATERIALS AND METHODS

Site Descriptions

The experiment was conducted over a range of soils, with two locations in Tennessee. Sites included the East Tennessee Research and Education Center, located near Knoxville (ETREC; 35.53° N, −83.57° W) on a soil mapped as Huntington silt loam (fine-silty, mixed, active, mesic Fluventic Hapludolls) and at the Research and Education Center at Greeneville (GREC, 36.10° N −82.84° W) on a Dunmore silty clay loam (fine, kaolinitic, mesic Typic Paleudults). The mean annual precipitation during the duration of the study (2011–2014) at ETREC was 1450 mm, with a mean annual temperature of 15.6°C (NOAA, 2013). This site was under orchardgrass (*Dactylis glomerata* L.) hay production for 4-yr before implementation of this experiment. The mean precipitation at GREC was 1190 mm, with a mean annual temperature of 14.7°C (NOAA, 2013); the site was previously under fescue (*Schedonorus arundinaceus* Roem. & Schult.) hay production.

Experimental Design

This experiment used a factorially arranged randomized complete block design with three blocks per location. The first factor, biofuel harvest treatment, included a single post-senescence (mid-November) and a single winter (early February) harvest date from 2012 to 2014. For all locations, soil amendment comparisons (the second factor) of biochar at two application levels (1 and 2 Mg ha^{-1}) and three legume intercrops (sunn hemp, cultivar Tropic Sunn; red clover, cultivar Cinnamon Plus; partridgepea, cultivar Lark), all vs. inorganic N (67 kg ha^{-1}) and a zero-application control were made for yield, soil characteristics, and feedstock composition. Recent research conducted by Warwick (2011) in Tennessee found that red clover and partridgepea could be successfully intercropped with 'Alamo' switchgrass. Established Alamo switchgrass plots (1.5 by 7.6 m), planted at 9 kg pure live seed (PLS) ha^{-1} with 18-cm row spacing in the spring of 2007 at ETREC and in the spring of 2008 at GREC were used in this study. Broadleaf weeds were controlled during the establishment year by 2,4-dichlorophenoxyacetic acid at 0.42 a.i. ha^{-1}. Legumes were no-till drilled annually into switchgrass stubble at ETREC using a five-row plot drill (1000, Hege, Colwich, KS) and a five-row, 606 No-Till plot drill (Great Plains, Salina, KS) at GREC. Red clover was planted on 13 and 28 Feb. 2012 and 28 Feb. and 13 March 2013 at ETREC and GREC, respectively, at a rate of 12 kg PLS ha^{-1}. Partridgepea and sunn hemp were seeded on 12 Apr. and 6 May 2012 and 5 and 16 May 2013 at a rate of 18 and 24 kg PLS ha^{-1} at ETREC and GREC, respectively. Planting depth ranged from 0.6 to 1.3 cm, depending on seed size. Partridgepea and sunn hemp were inoculated with the cowpea [*Vigna unguiculata* (L.) Walp.] group of *Bradyrhizobium* spp. and clover group inoculum of *Rhizobium leguminosarum* biovar *trifolii*.

Table 1. Soil chemical characterization baseline (Year 1, 2012) and final year (Year 3, 2014), averaged on a per-plot basis within blocks.

Year	Soil amendment	pH	P†	K	Ca	Mg	NO_3^-	CEC¶
			kg ha^{-1}					cmol$_c$ kg^{-1} soil
1	B-High‡	5.7 a	39.3 a	69.5 a§	1491 a	287 a	0.5 b	8.9 a
	B-Low	5.6 a	52.5 a	61.0 a	1569 a	289 a	1.2 a	9.3 a
	N-0	5.7 a	34.4 a	73.9 a	1466 a	290 a	0.3 b	8.7 a
	N-67	5.9 a	33.0 a	77.2 a	1527 a	305 a	0.5 b	8.9a
	PP	5.8 a	33.3 a	71.2 a	1485 a	295 a	0.4 b	8.8 a
	RC	5.8 a	32.7 a	73.5 a	1503 a	305 a	0.5 b	9.0 a
	SH	5.7 a	37.3 a	82.6 a	1491 a	308 a	0.6 b	9.0 a
3	B-High	6.5 a	29.5 a	60.1 a	1391 a	345 a	0.9 a	7.9 a
	B-Low	6.6 a	31.0 a	54.5 a	1390 a	312 a	0.7 a	7.9 a
	N-0	6.5 a	32.4 a	62.3 a	1369 a	337 a	0.6 a	7.9 a
	N-67	6.5 a	28.1 a	72.8 a	1397 a	341 a	0.7 a	8.0 a
	PP	6.5 a	31.1 a	65.3 a	1459 a	354 a	0.6 a	8.2 a
	RC	6.6 a	31.4 a	55.9 a	1381 a	339 a	0.5 a	7.8 a
	SH	6.5 a	32.3 a	63.6 a	1429 a	351 a	0.5 a	8.2 a

† Soil test results were obtained with Mehlich-1 extractant.
‡ Biochar rates were applied each spring: high (B-high), 2 Mg ha^{-1}; low (B-low), 1 Mg ha^{-1}.
§ Different letters indicate a significant difference by the LSD procedure within a given analyte and experimental year at $P \leq 0.05$.
¶ CEC, cation exchange capacity; N-0, 0 kg N ha^{-1}; N-67, 67 kg N ha^{-1}; PP, partridgepea; RC, red clover; SH, sunn hemp.

Switchgrass biochar was used in both study years and locations; however, the high biochar rate (2 Mg ha^{-1}) was only applied on the post-senescence biofuel harvest (November) because of the limited availability of biochar. Material applied in Year 1 was carbonized at 400°C under a residence time of 2 h, as described in our earlier study (Sadaka et al., 2014). For material used in Year 2, a continuous, externally-heated auger system heated at 400°C at a constant residence time of 8 min was used for biochar production (Sadaka et al., 2014). The amorphous structure, nutrient ranges, and cell wall composition of the materials applied during each experimental year were similar (Ashworth et al., 2014). Biochars applied had P, K, and Ca concentrations of 5200, 9900, and 12,900 mg kg^{-1} and 56 and 0.76% C and N, respectively, with a cation exchange capacity (CEC) of 141 mmol$_c$ kg^{-1} (Ashworth et al., 2014; Sadaka et al., 2014).

Data Collection

Soil Characterization

Pretreatment soil tests were conducted on a per-plot basis to 0- to 15-cm depths in the spring of 2010 to determine initial pH, CEC (cmol$_c$ kg^{-1}), and concentrations of nitrate N, P, K, Mg, and Ca. Soil sampling was repeated after the final winter harvest (spring 2013) to track elemental fluxes based on treatment applications (Table 1). Samples were ground to pass through a 1-mm sieve on a Wiley soil crusher (Thomas Scientific, Swedesboro, NJ), and Mehlich-1 extractable nutrients were measured by inductively coupled plasma using a 7300 ICP–OES DV spectrometer (PerkinElmer, Waltham, MA). The pH was determined on a 1:1 soil/water ratio using an AS3010D Dual pH Analyzer (Labfit, Burswood, WA, Australia).

Influence of Soil Amendments on Switchgrass Yield

Legume stand densities were estimated in late spring annually following green-up using a 0.75 by 0.75 m Vogel grid (Vogel and Masters, 2001). Four frequency counts (100 cells in total) were made in each legume treatment plot. The count was multiplied by 0.4 according to Vogel and Masters (2001), based on a likelihood of one plant per cell to estimate plant density per m^2 and averaged over three blocks at each location.

Two harvests, fall (postsenescence) and winter, were implemented annually in 2012–2013 (Year 1) and 2013–2014 (Year 2). Plots were harvested with a forage harvester (Carter, Brookston, IN) with a 91-cm cutting width. For both harvests and all locations, plots were cut to a 20.3-cm stubble height. Grab samples of biomass (1–2 kg) were collected from all plots at harvest, weighed, dried at 55°C in a batch oven (Wisconsin Oven Corporation, East Troy, WI) for 48 to 72 h, and reweighed to determine moisture content. Samples were then ground through a 2-mm sieve on a Wiley mill (Thomas Scientific).

Influence of Nitrogen Amendments and Harvest Date on Switchgrass Biofeedstock Characteristics

The effects of soil amendments (i.e., legume intercrops, biochar, and inorganic N) and harvest date on feedstock characteristics were based on plant total N, total minerals (P, K, Ca, and Mg), and cell wall constituents. Switchgrass uptake and removal of P and K were calculated as biomass yield multiplied by the nutrient concentration. Ground grass tissue (separated from legumes) was analyzed with near-infrared spectroscopy (NIRS) using a LabSpec Pro Spectrometer (Analytical Spectral Devices, Boulder, CO). Five scans were taken at a scan range of 1003 to 2500 nm. Analysis of feedstocks included acid detergent fiber, neutral detergent fiber (NDF), NDF digestibility (NDFD), in vitro true digestibility (IVTD), lignin, cellulose, hemicellulose, sugars, fructans, moisture content, and ash content per amendment and per harvest time. Equations were standardized by wet chemistry and checked for accuracy with the Grass Hay equation developed by the NIRS Forage and Feed Consortium (NIRSC), Hillsboro, WI (Vogel et al., 2011).

Rapid simultaneous saccharification and fermentation ethanol yields are highly correlative to NIRS forage quality, as cell wall digestibility and fermentation concepts in rumen and in vitro apply (Lorenz et al., 2009b). Consequently, cell wall digestibility measurements were used to determine the convertibility and availability of structural cell wall carbohydrates, including NDF and dNDF. The former includes insoluble composites from NDF and contains the total cell wall concentration (i.e., lignin, hemicellulose, and cellulose). The latter is determined by combining IVTD and NDF, represents fermentability of cell wall carbohydrates, and is an important determinant for feedstock convertibility (Lorenz et al., 2009a). Therefore, the resulting NIR values (dNDF and NDF) were fitted to a regression model (dNDF + NDF – 0.114 + 0.00229dNDF + 0.00117NDF) to determine estimated the ethanol yield (L kg^{-1} dry mass), based on Lorenz et al. (2009b), in that these two digestibility measurements are very strong (R^2 = 0.93) predictors of actual in vitro ethanol yield.

Economic Implications of Alternative Soil Amendments

Higher yielding and lower input systems will lower break-even prices. Therefore, we determined the break-even prices of soil amendments in switchgrass production for Tennessee. The analysis for legume intercropping was based on forage budgets constructed by University of Tennessee, Department of Agricultural & Resource Economics developed by the University of Tennessee's Center for Native Grasslands Management (Doxon et al., 2011). Establishment costs were assumed to be equivalent for all treatments with the model assumptions including the following:

1. Glyphosate [*N*-(phosphonomethyl)glycine] was applied at fall burn-down, spring burn-down, and postemergence at a rate of 0.31, 0.31, and 0.14 L product ha^{-1}, respectively. The cost of glyphosate was estimated to be \$1.05 L^{-1}.
2. Switchgrass was established with no-till methods at 9 kg PLS ha^{-1}.
3. No N or lime was applied during establishment; P and K were applied at 45 and 89 kg ha^{-1}, respectively.
4. Interest (6%) on the operating capital was calculated at 6 mo.
5. Fixed expenses for establishment included depreciation, interest, equipment housing, and insurance.
6. Labor expenses were calculated at \$8.50 h^{-1}.
7. Establishment costs were amortized over 15 yr.
8. Yield was assumed to be no different from that under the current recommended inorganic N rate (i.e., 5.7 Mg ha^{-1} annually).

Once prorated establishment costs were determined, management costs for each treatment were calculated. All treatments included labor, fixed (prorated establishment costs, land costs, depreciation, interest, housing, and insurance), and variable expenses. Variable expenses included machinery costs (fuel, oil, and filters), interest on operating costs, and the costs of applying a particular treatment. The assumptions of each model were:

- Nitrogen treatment: N was applied at 67 kg ha^{-1} at US\$352 Mg^{-1} (April 2013 price).
- Legume treatments: Sunn hemp was planted at 34 kg PLS ha^{-1} at US\$1.11 PLS kg. Partridgepea was planted at 20 kg PLS ha^{-1} at US\$6.89 PLS kg. Red clover was planted at 13 kg PLS ha^{-1} at US\$5.66 PLS kg.
- Biochar treatment: The high application rate was applied at 2 Mg ha^{-1} at US\$0.46 kg.

Statistical Methods

Switchgrass yield, feedstock quality, nutrient uptake and removal (P and K), potential ethanol yield, fluctuations in soil characteristics (both annual and the change between Year 1 nad Year 3), and legume density were initially analyzed in a global model (combined across harvest treatments, locations, and years). ANOVA tests were performed using the Mixed procedure (SAS version 9.3, SAS Institute., Cary, NC) with block, location, and year considered to be random effects (SAS Institute, 2007). Legume species, biochar and inorganic-N levels, and harvest date were considered fixed effects. Mean separations were performed by the SAS macro 'pdmix800' (Saxton, 1998) with Fisher's LSD with a Type I error rate of 5%. On the basis of the random effects probability level, further pooled models were compared where appropriate. For all models, the Shapiro–Wilks test was used to test for normally distributed residuals and Levene's *F*-test was used to test the homogeneity of variance.

RESULTS AND DISCUSSION

Soil Characterization

Nondetectable transformations in soil chemical characteristics (Δ = Year 3 – Year 1) and terminal test levels were observed when combined across locations ($P \geq 0.05$; Table 1). Specifically, neither the change in soil pH, N, P, Ca, Mg, nor CEC was impacted by soil amendment treatments ($P \geq 0.05$, data not shown). Only the change in K was impacted by soil amendments ($P = 0.05$), with the low biochar rate, sunn hemp, and partridgepea treatments resulting in K increases. Measurable fluctuations often require several years for inherent biochemophysical alterations. Initial macronutrient and secondary nutrient (P, K, Ca, and Mg) levels at GREC were all considered high or medium, with the exclusion of a few plots testing low for K and sufficient for Mg. Conversely, at ETREC, all plots tested low in K, sufficient in Mg, and a variety of ratings (low to high) for P (data not shown).

Influence of Soil Amendments and Harvest Timing on Switchgrass Yield

Under the global yield model (analyzed across years and locations), harvest treatments differed ($P = 0.02$), with overwintering harvests yielding 22% less biomass, probably because of greater leaf loss and weathering of plant tissue. Similarly, Adler et al. (2006) reported that switchgrass yield in Pennsylvania decreased by almost 40% when harvest was delayed until spring (with above average precipitation), with the water content decreasing from about 35 to 7% of fresh weight. Under our global model, soil amendments did not influence yield, but the first harvest × soil amendment interaction did impact yield ($P \leq 0.05$). Furthermore, location ($P = 0.24$), year ($P = 0.19$), and location × year interactions ($P = 0.07$) did not impact switchgrass biomass yield.

Consequently, yield was analyzed by harvest system in a simplified model with locations and years pooled. Soil amendments impacted post-senescence biofuel harvests ($P = 0.01$), with 0 kg N ha^{-1} and sunn hemp intercrops yielding the lowest, neither of which differed from high biochar, partridgepea, or red clover (Table 2). Conversely, low biochar was

Table 2. Switchgrass biomass and estimated ethanol yield, and P and K removal from fall (mid-November) vs. late winter (mid-February-early March) from soil amendment treatments combined across locations (Tennessee Research and Education Centers at Knoxville and Greeneville) and years (2012–2013 and 2013–2014).

Harvest treatment	Soil amendment	Biomass yield	Potassium removal	Phosphorus removal	Estimated ethanol concentration
		Mg ha^{-1}	———— kg ha^{-1} ————		L kg^{-1} DM
Fall	B-High†	13.2 abc‡	54.9 a	20.3 abc	113 a
	B-Low	14.9 ab	47.8 a	21.4 ab	116 a
	N-0	10.7 c	44.5 ab	16.2 c	115 a
	N-67	15.8 a	54.2 a	22.2 a	116 a
	PP	13.3 abc	46.5 a	19.1 abc	112 a
	RC	12.7 bc	54.1 a	19.5 abc	115 a
	SH	12.1 c	49.1 a	17.9 abc	114 a
Late winter	B-High§	12.5 a	22.6 b	12.7 ab	121 a
	N-0	10.2 a	21.9 bc	10.0 b	120 a
	N-67	11.8 a	26.3 a	11.7 b	120 a
	PP	9.3 a	19.1 c	7.8 b	121 a
	RC	10.5 a	22.3 b	10.9 b	121 a
	SH	10.3 a	28.1 a	11.5 b	121 a

† Switchgrass biochar rates [high (B-High), 2 Mg ha^{-1}; low (B-Low), 1 Mg ha^{-1}] were applied each spring, excluding the low rate for the second experiment because of a shortage of material.
‡ Different letters indicate a significant difference within harvest regime and biomass characterization metric at the $P \leq 0.05$ level from the LSD test.
§ N-0, 0 kg N ha^{-1}; N-67, 67 kg N ha^{-1}; PP, partridgepea; RC, red clover; SH, sunn hemp.

not different from the 67 kg N ha^{-1} treatment, nor was this inorganic treatment different from partridgepea or the high biochar rate. This suggests that the lower biochar rate is preferred, perhaps because higher biochar applications covered the switchgrass stubble. Such contrasting benefits of biochar were observed in a meta-analysis in which approximately half the studies observed crop yield increases, whereas the other half resulted in no or even negative yield responses (Spokas et al., 2012). In addition, partridgepea may have more favorable yield impacts than the other two legume intercrops tested in this study.

The over-wintering harvests (mid-February) were not influenced by soil amendments ($P \geq 0.05$; Table 2), probably because biomass losses overrode any yield differences that may have occurred as a result of soil amendments. Similar to post-senescence cuts, yields from the overwintering harvest regime were not influenced by location, year, or location × year interactions ($P \geq 0.05$).

Legume density (based on our global model, combined across locations and years) varied among legumes ($P < 0.0001$; Fig. 1); however, neither harvest, legume × harvest, year, location, nor location × year affected plant frequency ($P \geq 0.05$). Trends for each harvest regime were similar ($P \geq 0.05$; Fig. 1), suggesting that harvest date did not impact legume self-reseeding or persistence. When compared across harvest dates, red clover frequency was the greatest (20.1 m^{-2}), followed by partridgepea (11.9 m^{-2}), with sunn hemp averaging only 4.8 m^{-2}, even with annual reseeding. Consequently, sunn hemp is not considered compatible with switchgrass growth in temperate environments, probably because of the overlap in these species breaking dormancy, which induces seedling competition with established switchgrass.

Influence of N Amendments and Harvest Date on Switchgrass Feedstock Characteristics

When combined across locations and years, plant nutrient uptake and removal for P and K varied on the basis of harvest timing ($P < 0.0001$) but not for soil amendments, soil amendments × harvest interactions, location, year, or location × year interactions ($P \leq 0.05$; Table 2). Potassium removal was greater for November harvests than overwintering harvests (19.1 vs. 11.0 kg ha^{-1}, respectively; Table 2). Phosphorus followed similar trends, with more removed by November harvests than overwintering harvests (50.2 vs. 25.0 kg ha^{-1}). Control plots generally had the lowest P and K removal during fall harvests, with all other soil amendments being greater ($P \geq 0.05$; Table 2). The removal rates (postsenescence and

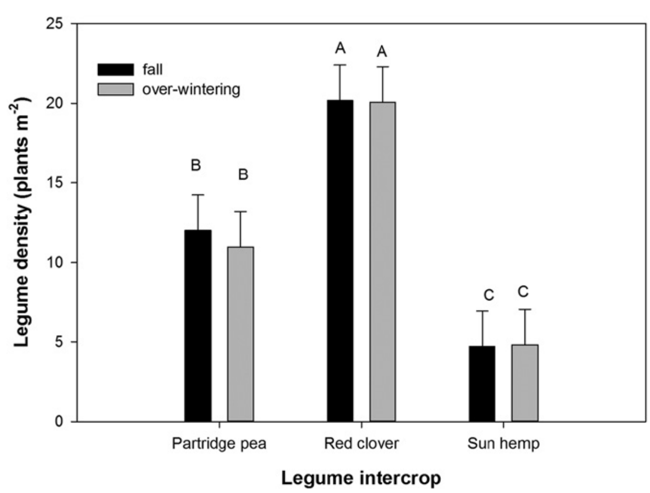

Fig. 1. Legume density (plants m^{-2}) combined across locations (Tennessee Research and Education Centers at Knoxville and Greeneville) and years (2012–2013 and 2013–2014). Different letters indicate a significant difference with the LSD procedure across both harvests [fall (post-senescence, mid-November) and overwintering (mid-February)] at $P \leq 0.05$.

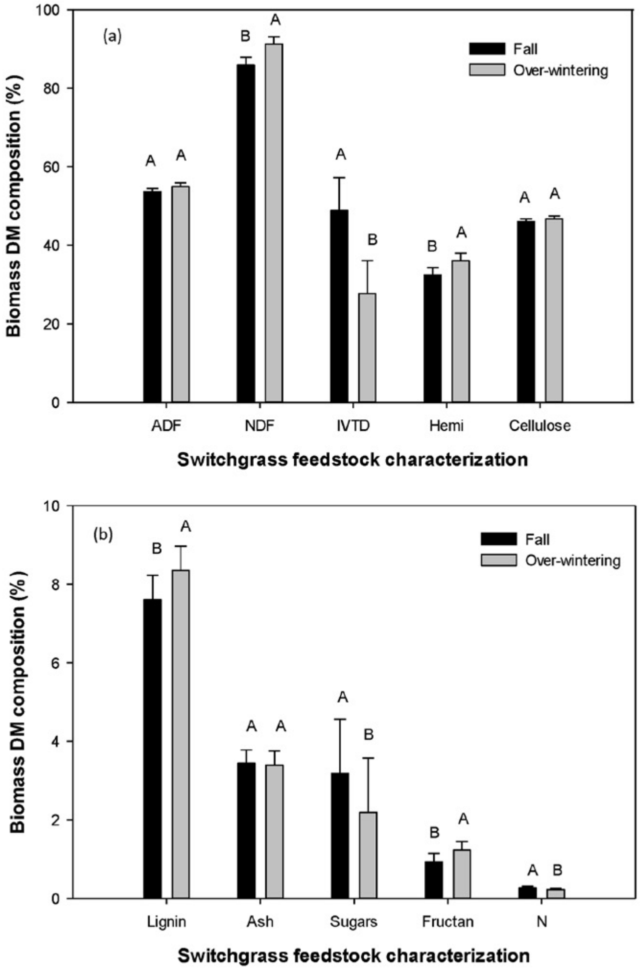

Fig. 2. Feedstock characterization of switchgrass dry matter (DM) composition (a, b) based on harvest [fall (post-senescence, mid-November) and overwintering (mid-February to early March)] averaged across soil amendment treatments, locations (the East Tennessee Research and Education Center and Research and Education Center at Greeneville), and years (2012–2013 and 2013–2014). Different letters indicate a significant difference with the LSD procedure at the $P \leq 0.05$ level.

overwintering) reported here were similar to those reported by Kering et al. (2013) for switchgrass harvested in December for K (11.7–16.4 kg ha^{-1}) and P (32.0–42.0 kg ha^{-1}). These results suggest that P and K declined through winter, probably because nutrient translocation was complete. The higher mass of P and K sequestered in senesced material has implications for feedstock usage in thermochemical conversion because of the high slagging potential of these nutrients in combustion chambers (Boateng et al., 2007), as well as higher nutrient input requirements long-term (Cahill et al., 2014).

Potential ethanol yield was affected by harvest timing ($P <$ 0.0001; Table 2), as overwintering biomass resulted in greater potential per-unit ethanol yield (1210 vs. 1150 L ethanol kg^{-1} dry matter, respectively). The increased ethanol yield for winter-harvested biomass may have been caused by the greater presence of digestible fibers and fermentable sugars (5 and 6 C) and because of in-field breakdown of the waxy cuticles that inhibit sugar availability (Adler et al., 2006). However, when harvestable biomass per harvest regime was taken into account, overwintering ethanol potential was lower than that of postsenescence harvest periods (12,700 vs. 15,520 L ethanol ha^{-1}). Soil amendments did not impact the potential ethanol yield of harvested biomass, nor did any soil amendment × harvest interaction ($P \geq 0.05$), indicating a greater influence on feedstock attributes by harvest period than by soil amendments.

When combined across locations and years, soil amendment treatments had no impact on feedstock characteristics ($P \geq$ 0.05), probably because of the vast genetic diversity of this species. Consequently, nonhomogenous plant cell constituents across populations may override any detectable chemical or physical characteristics. Physical differences observed for harvest date (Fig. 2) may have been caused by several factors, including (i) remobilization of nutrients to plant storage organs, (ii) leaching of soluble nutrients from the standing biomass, and (iii) weathering of senesced leaves, resulting in deposition on the soil surface. Specifically, November harvests had more N and sugars and consequently higher IVTD ($P \leq 0.05$). Conversely, overwintering harvests had greater NDF and lignin concentrations ($P < 0.0001$), indicating losses in the fermentability of plant material, probably because of lower leaf loss and greater stem constituents, which are greater in recalcitrant phenolic chains, rather than digestible sugars. The only tested feedstock traits not impacted by harvest timing were cellulose and acid detergent fiber ($P \geq 0.05$). Similar results were reported by Adler et al. (2006) with delayed (spring) harvests resulting in higher ethanol yields resulting from greater glucose, lignin, hemicellulose, and carbohydrate yields. Consequently, harvest management could be manipulated to obtain the desired feedstock traits depending on the conversion process (i.e., greater lignocellulosic and sugar yields favor fall harvests for biological conversion, whereas the reduced mineral concentrations during spring may favor thermochemical conversion).

Switchgrass moisture content was influenced by harvest date ($P < 0.0001$), with overwintered material having much lower (11%) moisture levels than that of November-harvested tissue (25%). Switchgrass tissue moisture content for both harvest dates decreased to low enough levels during in-field dry-down for direct storage (≤200 g kg^{-1}, wet weight basis). However, the 11% moisture content during overwintering harvests would require little additional dry-down, if any, for biological or thermochemical conversion (Boateng et al., 2007; Adler et al., 2006).

Economic Investment Potential of Soil Amendment Alternatives

Assuming consistent switchgrass establishment, the currently recommended N rate (67 kg N ha^{-1}) resulted in the lowest break-even price under our assumptions (Table 3). The second lowest break-even price was observed for sunn hemp (\$137.81 Mg^{-1}) and was influenced by the low costs associated with annual management compared to other legumes; however, it would not be recommended in temperate environments because of establishment failures and a lack of observed yield increases. Therefore, based on break-even price, red clover was the preferred legume because of lower seed costs, although its break-even price was greater (by \$59.63 Mg^{-1}) than the inorganic N scenario. Further, biochar was the most costly N alternative, with the break-even costs being twofold greater

Table 3. Total treatment costs (prorated establishment plus annual management costs per hectare and the break-even point for switchgrass for each treatment.

Treatment†	Total cost‡	Break-even cost§
	US$ ha^{-1}	US$ Mg^{-1}
Nitrogen only	117.31	106.40
Sunn hemp	151.94	137.81
Red clover	183.05	166.03
Partridgepea	243.32	212.53
Biochar (2 Mg ha^{-1} rate)	305.63	277.21

† Assumes the same establishment practices for all treatments.

‡ Establishment costs amortized over 15 yr.

§Yield is assumed to be equivalent to the average yield (i.e. 5.7 Mg ha^{-1} annually) from the recommended inorganic N rate of 67 kg ha^{-1}.

than those of inorganic N. Consequently, for inorganic input alternatives to be competitive on a break-even cost basis, greater biomass yields would be needed under these management practices or else some secondary benefits would have to be realized (e.g., C credits for using alternatives to inorganic N).

CONCLUSIONS

Terminal soil P and K were lowest and yield was greatest at ETREC (vis-à-vis GREC) despite no differences in nutrient removal being apparent. This suggests that uptake and removal were not limited by nutrient availability and that enhanced switchgrass nutrient translocation to perenniating plant parts may allow for continued plant uptake or removal in the short term. Further monitoring of nutrient removal is needed to determine if nutrient mining can occur, given that many elements shifted from adequate to low levels in Year 1 to Year 3. However, measurable soil fluctuations are often more attenuated and require more years for inherent biochemical alterations.

Soil amendment treatments impacted yield for November harvests but not overwintering harvests because biomass losses overrode any yield differences. Switchgrass yield during fall harvests was lowest for the 0 kg N ha^{-1} rate and sunn hemp intercrops. Conversely, the low rate of biochar was not different from the 67 kg N ha^{-1} rate, nor was the inorganic rate different from partridgepea intercrops and the high biochar rate. Consequently, sunn hemp is not considered compatible with temperate switchgrass growth, owing to the high competition from switchgrass during sunn hemp's germination and seedling growth. On the other hand, partridgepea and red clover can successfully be interseeded into established switchgrass swards, and may, in some cases, result in comparable post-senescence biomass yields to that of the current recommended inorganic N rate. Furthermore, use of biochar has the potential to provide a 'closed loop' system, considering that the feedstock coproduct can be applied to the bioenergy crop the following season. However, further research determining the proper application rates and improved economic viability are needed to make this soil amendment practice a viable option.

A sustainable management practice for switchgrass growers could be to delay harvests until after maturity during the subsequent winter months to stagger the work load and transport to a refinery, provide an out-of-season wildlife habitat, and allow further moisture loss in the standing crop, which confers optimum moisture conditions for safe storage. Nutrient (P and K) translocation, ethanol yield, hemicellulose, and in-field dry-down were maximized by delaying harvests. Such harvest delays may allow for greater presence of digestible fibers and fermentable sugars (5 and 6 C), which are caused by the in-field breakdown of the waxy cuticles that inhibit sugar availability. On the other hand, biomass yield losses (22%) were observed from delaying harvests. Consequently, the desired feedstock characteristics can be manipulated by harvest management; however, a trade-off with yield reductions must be considered. Therefore, the greater lignocellulosic and sugar yields that occurred during fall harvests favored biological conversion, whereas the reduced mineral concentrations for spring favored thermochemical conversion. The systems tested here could, in part, close the N loop, reduce inputs, and promote the diversification and long-term sustainability of perennial graminaceous feedstock systems.

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