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Chemical composition and bioethanol potential of different plant species found in Pacific Northwest conservation buffers

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Lands producing mixed lignocellulosic ethanol feedstocks may be able to produce more biomass with fewer resources than conventional monoculture crops, but lignocellulosic ethanol production processes and efficiencies can be highly dependent on feedstock composition. In this study, plants were collected from areas planted to simulate conservation buffers alongside stream channels within three common resource areas the interior Pacific Northwest. Two grasses (tall wheatgrass and alfalfa) and seven forb species (fiddleneck tarweed, dog fennel, kochia, downey brome, tall annual willowherb, prickly lettuce, and tumble mustard) commonly found in these buffers were examined to determine their chemical composition, potential bioethanol yields, and difficulties that may arise if they were to be harvested and processed in a single facility. Potential ethanol yields calculated on the basis of sugar monomer composition in the biomass ranged from 181.5 to 316.5 l/dry ton of biomass. Significant differences were noted in terms of structural sugars (cellulose 19%–33% w/w; hemicellulose 14%–26% w/w), lignin (10%–18% w/w), extractives (20%–40% w/w), and ash content (4.0%–13.8% w/w). These composition variations could vary the processing efficiency in terms of sugar recovery and eventual ethanol production yield. © *2012 American Institute of Physics*.
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I. INTRODUCTION

Advantages of bioethanol produced from renewable feedstocks such as cereal crops, sugarcane, and lignocellulosic crops have been demonstrated by many investigators. Many studies have also concluded that the fossil energy use and net greenhouse gas emissions during life cycle of bioethanol are below those of conventional petroleum fuels.[1–3] Presently, most of the bioethanol is produced from sugar or starchy crops such as sugarcane, cassava, and corn.[4] However, a significant research effort has been directed at the production of ethanol from lignocellulosic biomass (e.g., agricultural and forestry residue, energy crops, paper waste, etc.) since the use of these feedstocks for expanding ethanol production addresses some of the major issues associated with first generation ethanol such as food vs. fuel issues.[5–9]

Land use for production of cellulosic ethanol is a significant concern.[9] With typical yields of 275–360 l/ton of biomass,[10] it would be reasonable to expect that millions of hectares of land would be needed to cause any significant reduction in the use of petroleum based transportation fuels. This poses two challenges: It is very difficult, if not impossible, to implement such large scale conversion of land to dedicated monocultures for biomass production. Such massive

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changes in land use patterns can have global consequences.[11–13] Second, production of monocultures at such large scales increases the risk of catastrophic failures of the crops, reduces biodiversity, and introduces new risks due to the introduction of non-native species.[9,14] In this context, native grass land perennials containing several mixed species have been investigated for their potential for biofuel production.[15–17] Native grass land perennials not only require lower agrochemical inputs but can also produce higher bioenergy yields in the long term compared to conventional crops with lower overall greenhouse gas emissions compared to corn ethanol and soybean biodiesel.[18]

The United States Government Accountability Office estimates that half the native grasslands have been developed for other uses, predominately agriculture.[19] In the small-grain producing areas of interior Pacific Northwest, grassland conversion has been closer to 97%.[20] Consequently, the largest source of mechanically harvestable perennial grasses in this region is former cropland that has been enrolled in federal conservation programs, such as the Conservation Reserve Program (CRP) or National Conservation Buffer Initiative (NCBI). Within the NCBI, filter strips or riparian forest buffers have the most potential for biomass productivity and providing ecosystem services. These buffers were in all likelihood riparian areas adjacent to streams (ephemeral, intermittent, or perennial) and, although only comprising approximately 2% of the landscape,[21] the most productive areas in this semiarid landscape.[22] These buffers capture runoff and eroded soil from adjoining cropland, provide terrestrial habitat for wildlife, and enhance aquatic habitat through the slow release of water filtered through the buffers. Establishment of healthy stands of perennial grasses, however, can require substantial input from the producers, and in the drier areas of this region become dominated by invasive annual plant species.[23] Management of these sites might be enhanced by harvesting standing biomass after it is no longer needed for avian nesting cover. The goal would be to improve the competiveness of the desired perennial grass and reduce a substantial seed source of weeds that can be spread to the adjoin cropland, thus creating better conditions for crop management, improved grassland habitat, and a source of biofuel feedstocks. Although the end result of buffer installation is an increase in biodiversity over monoculture crop production, the species composition available for biofuel feedstocks is unlikely to be homogeneous.

However, from a biomass processing perspective, single biomass feedstock facilitates processing and optimization of the processing techniques to achieve optimal yields. Therefore, it has been the focus of most process optimization studies to date.[24–28] Biomass composition is an important determinant of potential ethanol production, as ethanol yield is directly correlated to carbohydrate content of the biomass and choice of processing technology is dependent on biomass composition. Therefore, it is critical to determine the variation in the composition of various mixed species and determine the enzymatic hydrolysis yields to assess their ethanol yield potential.

Biomass is a recalcitrant matrix of cellulose, hemicellulose, lignin, and extractives.[10,15,29–33] Production of cellulosic ethanol via biological conversion consists of four critical steps: (1) pretreatment (thermal and/or chemical treatment of biomass to decrease the structural recalcitrance), (2) enzymatic hydrolysis (conversion of sugar polymers to sugar monomers), (3) fermentation (conversion of sugar monomers to ethanol) and (4) ethanol recovery (using distillation and molecular sieves). Enzymatic hydrolysis of structural carbohydrates (cellulose and hemicellulose) provides the substrate for subsequent/simultaneous fermentation by yeast. However, the recalcitrant structure of cellulose along with the presence of lignin and hemicellulose decrease the hydrolysis efficiency. Various pretreatment processes (physical, chemical, physico-chemical, and biological) have been developed to facilitate the enzymatic hydrolysis process by increasing substrate accessibility.[30–34] Choice of pretreatment process is highly dependent upon the chemical composition and structural properties of biomass. Amount of ethanol production from a biomass is directly related to carbohydrate content of biomass. Therefore, chemical composition of biomass is an important factor that determines the choice of conversion technologies and determines ethanol yields.[29,35]

Past studies have examined the feedstock potential of unmanaged, early and late succession old fields as well as mixed species prairie plots.[36,37] These studies identified several C3 and C4

grasses as being highly digestible feedstocks, but also noted a high range of sugar yields based on specific species and the portions of plant anatomy considered. The suitability of species harvested from conservation buffers was not explored in these studies and could prove to be a valuable cellulosic feedstock with added erosion control and conversation benefits. Additionally, buffer zones may have a different degree of species diversity not captured in prior studies.

The NCBI effort has been implemented through partnerships of State and Federal agencies with private and nongovernmental organizations, with a goal of establishing 80 938 ha (200 000 acres) of stream channel buffers in Oregon and Washington. There were approximately 9973 ha (24 643 acres) enrolled in conservation buffers in central and eastern Oregon in 2006 and Washington in 2008.[38,39] Altogether, this value represents 12% of the NCBI goal, 80% and 66% of the total buffers established have been in central and eastern Oregon and Washington, respectively. Biomass harvested from these lands could contribute to the total available biomass for bioethanol industry, while providing for a more active management program than is now available to producers. However, as discussed above, it is important to determine the species abundance, composition and possible yields to estimate the biomass that could be harvested sustainably from these lands.

The objective of this study was to analyze the chemical composition of two planted and seven volunteer plant species found in Pacific Northwestern riparian conservation buffers to determine their potential as biomass feedstock. We collected samples from three common resource areas (CRA) corresponding to the three agronomically important precipitation zones of the semiarid inland of the region.

II. MATERIAL AND METHODS

A. Biomass

Biomass samples were collected from plots established to simulate riparian conservation buffers in dryland wheat fields in one of three CRA (Table I[40–42]). A CRA is defined as a geographical area where resource concerns, problems, or treatment needs are similar, and is a subdivision of a major land resource area in which landscape conditions, soil, climate, human considerations, and other natural resource information are used to determine the geographic boundaries of a common resource area (from USDA-NRCS General Manual, Title 450, Part 401, Subpart C, 401.21).

A mixture of tall wheat grass (*Thinopyrum ponticum*, THPO) and alfalfa (*Medicago sativa*, MEDIC) was seeded at 10 kg/ha and 6 kg/ha, respectively, in 3.7 m × 50.3 m plots at two locations adjacent to ephemeral or intermittent stream channels within each CRA, with two replications per location. Locations within CRAs were separated by 1 km or greater, and the plot positioned randomly within a population of potential sites at each location. Plots in CRA 7.2 and 8.2 were established within or adjacent to privately owned crop production fields using light tillage and herbicide applications of glyphosate and 2,4-dichorophenoxyacetic acid (2,4-D) to establish a clean seedbed. The plots were seeded, without fertilizer, in February and March of 2009. Typically, 1 yr is required for conservation plantings to establish and begin to compete with weeds. Thus, plots were mowed in the first year to control weed production and allow establishment of the planted species. Our intent was to evaluate the production potential of these sites without further active management other than an annual harvest. Plots in CRA 9.2 were located within federal and university research farms, where seedbed preparation was similar to those in the drier CRAs with light tillage, appropriate herbicide applications, and no fertilizer application. However, herbicide applications were made for additional weed control after germination in CRA 9.2.

Samples were collected from the center of each plot using 0.5 m^2 quadrats positioned at 8.4 m, 25.1 m, and 41.9 m from the stream channels. Samples were collected in June and July of 2011 and 2012, returned to the laboratory, oven dried at 40 °C, and weighed. Species composition in each CRA was determined by clipped biomass composition. Species were selected for biochemical composition analysis based on their stature (indicator of ability to be harvested) and abundance. Samples of individual species from each year of sampling and all three sites were

TABLE I. Conservation buffer research site descriptions.[40–42]

CRA	Streamside buffer area[a] (ha)	Precipitation zone	Longitude/ latitude	Soil	Elevation (m)	MAP[b] (m)	MAT[c] (°C)	FFD[d]
7.2 Columbia Basin—Silty Missoula flood deposits	1812	Low (<0.30 m)	45°40′16″, 119°07′29″	Kimberly fine sandy loam (coarse-loamy, mixed, mesic Torrifluventic Haploxerolls)	268	0.20–0.36	9.4–11.7	150–180
8.2 Columbia Plateau—Loess Islands	31 694	Intermediate (0.30–0.45 m)	45°51′14″, 118°39′31″	Onyx silt loam (coarse-silty, mixed, mesic Cumulic Haploxerolls	530	0.30–0.41	9.4–12.2	140–170
9.2 Palouse and Nez Perce Prairies—Palouse Hills	21 997	High (0.45–0.60 m)	46°45′41″, 117°11′38″	Palouse silt loam (fine-silty, mixed, superactive, mesic Ultic Haploxerolls	766	0.23–0.29	8.3–10.6	130–150
			46°47′07″, 117°04′31″	Thatuna silt loam (fine-silty, mixed, mesic Xeric Argialbolls)	794	0.46–0.58	7.2–8.9	110–130

[a]Based on 2% of total area of CRA.
[b]Mean annual precipitation.
[c]Mean annual temperature.
[d]Frost free days.

combined and mixed for this biochemical analysis. *Bassia scoparia* (BASC) was included in this analysis because it can compose up to 21% cover in streamside conservation buffers in CRA 8.2 (Ref. 22) and is a persistent problem in rainfed croplands and adjacent upland CRP. A representative sample of BASC was taken from immediately outside of the plots located in CRA 8.2.

B. Composition analysis

1. Sample preparation

Biomass samples were ground in Wiley knife mill until the entire sample passed through a 2 mm screen. The milled samples were sieved using sonic sifter (Allen-Bradley Sonic sifter) to obtain the particle size profile. A total of five sieves ranging from sieve no. 20 (850 μm) to no. 100 (149 μm) were used in the sonic sifter and the biomass retained between sieve 20 and 80 was used for further analysis of composition.[43]

2. Moisture and ash Contents

Moisture and ash contents in the feedstocks were determined using gravimetric analysis with the gravimetric hot air oven method.[44] Moisture content was measured by drying the samples in the oven at $105 \pm 3\,^{\circ}$C for 24 h. Total ash content was measured by accounting weight loss after combustion of the biomass at 575 °C in a muffle furnace for about 24 h.[45] Average of three replications was taken.

3. Extractives

Water soluble and ethanol soluble extractives were determined by the procedure described in NREL laboratory analytical procedure (LAP) technical report number NREL/TP-510-42619. Soxhlet apparatus was used for the two-step extraction process. Major water soluble extractive portion may contain inorganic material, non-structural sugars, and nitrogenous material; whereas ethanol soluble material may include chlorophyll, waxes, or other minor components. These are the non-structural components of biomass and must be removed from the biomass to avoid interference and improve the precision of downstream carbohydrate and lignin content analysis. For each experiment, 5 g of sample was placed in a cellulose thimble and extraction was performed for 24 h using de-ionized water. This was immediately followed by extraction with 95% ethanol for 24 h. Extractives were transferred to a 200 ml volumetric flask after each extraction. A 50 ml sample was withdrawn and transferred to a pre-weighed oven dried and cooled round bottom flask. This sample was dried in rotary vacuum evaporator at 45 °C followed by further drying in vacuum oven at 40 °C for 24 h. Change in weight recorded to the nearest 0.1 mg was used to calculate the amount of extractives. Average of three replications for each sample was taken.

4. Protein content

Nitrogen content in the biomass was measured using an elemental analyzer (Costech Analytical Technologies, Inc.) to calculate the amount of protein in the feedstock. Crude protein concentration was estimated as $N \times 6.25$.[46] Average protein content was measured using five replicates for each sample.

5. Structural carbohydrates and lignin

Carbohydrates and lignin fractions of the biomass were determined using a two-step hydrolysis process. Cellulose and hemicellulose are converted to sugar monomers using sulfuric acid as described in NREL biomass analytical procedure.[47] Briefly, biomass samples were hydrolyzed using 72% (w/w) sulfuric acid at 30 °C for 1 h, followed by acid dilution (to 4% by adding deionized water) and hydrolysis at 121 °C (in autoclave) for 1 h. During this process, cellulose and hemicellulose are broken down to sugar monomers: glucose, xylose, galactose, arabinose, and mannose. Concentrations of these sugars were determined by high performance liquid chromatography (HPLC) (Agilent Technologies) equipped with refractive index detector. Bio-Rad Aminex HPX-87P column

and Bio-Rad cation and anion Deashing Cartridge micro-Guard columns were used for sugar analysis using 0.2 -μm filtered de-ionized water as the mobile phase at a flow rate of 0.6 ml/min at 80 °C. Bio-Rad Aminex HPX-87H column was used for the determination of acetic acid using 0.005M H_2SO_4 as mobile phase at a flow rate of 0.6 ml/min at 65 °C. Pure sugars and acetic acid standards (Sigma-Aldrich, St. Louis, Mo.) were used for calibration. Acid insoluble lignin was determined using gravimetric analysis at 105 °C and 575 °C as per the NREL protocol. The acid-soluble lignin (ASL) was estimated by measuring absorbance of the hydrolysate at 320 nm in UV-vis spectroscope using water as blank. All analyses were performed in triplicate.

C. Ethanol yield

The ethanol yield of a biomass is dependent on its sugar content, hydrolysis efficiency, and fermentation efficiency. The theoretical potential ethanol yields were calculated using the following equations:

$$\text{Ethanol from cellulose} = \text{Biomass} * \text{glucans} * \text{hydrolytic gain\_C6} * \eta_{\text{hydro\_cellulose}} * 0.511 * \eta_{\text{ferment\_C6}}. \quad (1)$$

$$\begin{aligned}\text{Ethanol from hemicellulose} = {} & (\text{Biomass} * (\text{Xylans} + \text{Arabinans}) * \text{hydrolytic gain\_C5} \\ & * \eta_{\text{hydro\_hemicellulose}} * 0.511 * \eta_{\text{ferment\_C5}}) \\ & + (\text{Biomass} * (\text{Galactans} + \text{Mannans}) * \text{hydrolytic gain\_C6} \\ & * \eta_{\text{hydro\_hemicellulose}} * 0.511 * \eta_{\text{ferment\_C6}}).\end{aligned} \quad (2)$$

$$\text{Total Ethanol} = \text{Ethanol from cellulose} + \text{Ethanol from hemicellulose}, \quad (3)$$

where $\eta_{\text{hydro\_cellulose}}$ and $\eta_{\text{hydro\_hemicellulose}}$ are hydrolysis efficiencies of cellulose and hemicellulose (assumed 95% and 85%, respectively); hydrolytic gain_C6 and hydrolytic gain_C5 are hydrolytic gains during hydrolysis of hexose and pentose sugars, respectively (1.11 and 1.136, respectively). $\eta_{\text{ferment\_C6}}$ and $\eta_{\text{ferment\_C5}}$ are fermentation efficiencies of hexose and pentose sugars assumed to be 98% and 60%, respectively.

D. Statistical analysis

Statistical analysis (one-way ANOVA) was conducted on ash, protein, lignin (acid soluble and acid insoluble), extractives (water and ethanol), glucan, xylan, mannan, galactan, arabinan, and acetic acid content with a 95% confidence interval. Fisher's method with a Fisher's individual error rate of 5% was used to find the significant differences among the grasses individually. All statistical analysis was performed using MINITAB 16.[48]

III. RESULTS AND DISCUSSION

A. Biomass yields and species composition

Successful establishment of perennial species in conservation buffers can be hindered by abundant reserves of weed seeds in former cropland, weed seed washed into the area by intermittent flooding, and management restrictions.[23,49,50] In the spring of 2011, weed pressure was crowding out the planted species, and herbicide applications made in an attempt to salvage the crop. We identified 30 plant species in the three CRAs that were not planted or desired. Most of these species were small in stature and not harvestable, and thus not important to this analysis, and only served to compete for resources with the desired species. However, the seven species analyzed for biochemical composition were all of great enough stature to be harvested with the potential of contaminating the feedstock composition of the desired species.

Establishment of a MEDIC/THPO mixed species buffer in CRA 7.2 was unsuccessful (species abbreviations Table II), with *Bromus tectorum* (BRTE) dominating the site by fall 2011 (Table III). The successful establishment of perennial plants in these dry areas requires a unique

TABLE II. Nine plant species with substantial contribution to conservation buffers sampled in the dryland cropping area of the interior Pacific Northwest.

Code	Scientific name	Common name	Class
Planted species			
MEDIC[a]	*Medicago sativa*	Alfalfa	Perennial forb
THPO[a]	*Thinopyrum ponticum*	Tall wheatgrass	Perennial graminoid
Volunteered species			
AMLY	*Amsinckia lycopsoides*	Fiddleneck tarweed	Annual forb
ANCO	*Anthemis cotula*	Dog fennel	Annual forb
BASC	*Bassia scoparia*	Kochia	Annual forb
BRTE	*Bromus tectorum*	Downey brome	Annual graminoid
EPBR	*Epilobium brachycarpum*	Tall annual willowherb	Annual forb
LASE	*Lactuca serriola*	Prickly lettuce	Annual/biennial Forb
SIAL	*Sisymbrium altissimum*	Tumble mustard	Annual/biennial Forb

[a]Plants purposefully planted in conservation buffer.

combination of well-timed precipitation, temperatures, and limited competition from opportunistic annuals to become successfully established. Three summers after the site was planted, the plant species recorded in this CRA appear the same as the surrounding area not in crop, indicating the presence of an abundant seed source for competitive annuals. It is unlikely that CRAs with similar low annual precipitation and preexisting plant community structure can provide reliable supplies of perennial species for biofuel feedstocks.

Establishment of the desired species in CRA 8.2 and CRA 9.2 was more successful, with the combination of THPO and MEDIC accounting for 93% and 89% of the harvested biomass at each site (Table II). These sites were much more productive (Table III) than one might expect for these soils; the CRA 8.2 site was 1.7 time and the CRA 9.2 site 4.7 time more productive than the published values for dryland forage of 11.5 Mg/ha and 3.4 Mg/ha under favorable conditions, respectively.[40] This degree of success is likely the result of exceptionally high spring precipitation in 2010 and 2011; fewer competitive weeds in the surrounding and productive croplands, and abundant stream flow contributed to improved soil water conditions.

As a measure of successful plant species establishment, results from sampling conservation buffers in the second and third summers following seeding must be treated with caution. Williams *et al.*[23] sampled an early CREP project in CRA 8.2 and found that species diversity increased 8 yr after the project was initiated. Unfortunately, the diversity was gained through a loss of planted species that were replaced by many of the same invasive annuals that we have analyzed here. Maintenance of the desired species in these systems, and consequently the usefulness of harvested biofuel feedstocks, will require new and innovative management practices such as harvesting, burning, tilling, nitrogen manipulation, or herbicide use.[51] A primary objective for any conservation planting is to provide avian nesting habitat through spring and summer months. Two advantages of annually harvesting these perennial species in the fall are maximization of lignocellulosic biomass and opening the site up for more efficient herbicide applications to control annual weeds. A fall application of herbicide to control fall annuals reduces competition for resources needed for fall and winter growth of the desired perennials, allowing them to close canopy and outcompete annuals that, in turn, germinate in the spring. Because one goal of using these feedstocks is to obtain them from low-input systems, additional research is required to find the optimal minimum use of herbicide for these systems.

B. Biomass composition

The particle size profile of the biomass after passing through a 2 mm screen in a Wiley knife mill was obtained with the sieve analysis and is illustrated in Figure 1. Major fractions of all feedstocks were retained between sieve nos. 20 and 60 (passed through sieve size 850 μm

TABLE III. Ash, protein, lignin, and extractive contents of nine feedstocks (% dry basis) from combined and mixed samples collected in 2nd and 3rd years of establishment (2010 and 2011) from three common resource areas in the Pacific Northwest. All different letters in each column represent significantly different values of the corresponding parameter ($p < 0.05$). Standard errors are based on standard deviation of 3 replicates.

Sample Name	Weight mean contribution ± se (Mg/ha)			Ash content	Protein content	Acid insoluble lignin (AIL)	ASL	Extractives	
	CRA 7.2	CRA 8.2	CRA9.2					Water	Ethanol
					Planted species				
MEDIC	<0.01 ± 0.00	3.07 ± 2.48	2.16 ± 1.77	10.32 ± 0.29^{b}	7.55 ± 1.54^{a}	11.60 ± 0.06^{c}	060 ± 0.02^{f}	26.63 ± 0.98^{d}	3.36 ± 0.05^{c}
THPO	0.22 ± 0.17	15.89 ± 11.80	13.45 ± 5.58	8.92 ± 0.13^{c}	2.06 ± 0.28^{e}	13.47 ± 0.28^{b}	0.79 ± 0.00^{c}	20.40 ± 0.37^{g}	1.94 ± 0.16^{d}
					Volunteered species				
AMLY	0.18 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	13.80 ± 0.92^{a}	4.67 ± 1.05^{c,d}	16.59 ± 1.10^{a}	0.59 ± 0.03^{f}	25.16 ± 0.35^{e}	2.56 ± 0.50^{d}
ANCO	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.02	8.68 ± 0.18^{c}	3.78 ± 0.41^{d}	9.11 ± 0.03^{e}	0.73 ± 0.02^{d}	33.71 ± 0.46^{b}	5.06 ± 0.39^{a}
BASC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.93 ± 0.17^{d}	5.93 ± 1.12^{b}	11.80 ± 0.57^{c}	0.98 ± 0.01^{a}	23.30 ± 0.70^{f}	2.86 ± 2.49^{a,b}
BRTE	2.13 ± 0.47	0.00 ± 0.00	0.04 ± 0.03	6.24 ± 0.11^{e}	3.38 ± 1.14^{d,e}	11.67 ± 0.92^{c}	0.85 ± 0.02^{b}	17.53 ± 1.00^{h}	2.37 ± 0.25^{d}
EPBR	0.01 ± 0.01	0.00 ± 0.00	0.14 ± 0.11	5.54 ± 0.18^{f}	5.62 ± 0.51^{b,c}	11.49 ± 0.31^{c}	0.82 ± 0.03^{b,c}	35.51 ± 0.97^{a}	4.41 ± 0.30^{b}
LASE	0.00 ± 0.00	0.25 ± 0.19	0.07 ± 0.06	8.51 ± 0.20^{c}	5.35 ± 0.62^{b,c}	10.17 ± 0.21^{d}	0.83 ± 0.01^{b}	28.06 ± 1.14^{c}	4.74 ± 0.28^{a,b}
SIAL	0.52 ± 0.52	0.45 ± 0.23	0.00 ± 0.00	4.04 ± 0.08^{g}	6.15 ± 1.07^{b}	17.26 ± 0.05^{a}	0.68 ± 0.01^{e}	16.84 ± 0.86^{h}	4.24 ± 0.50^{b}

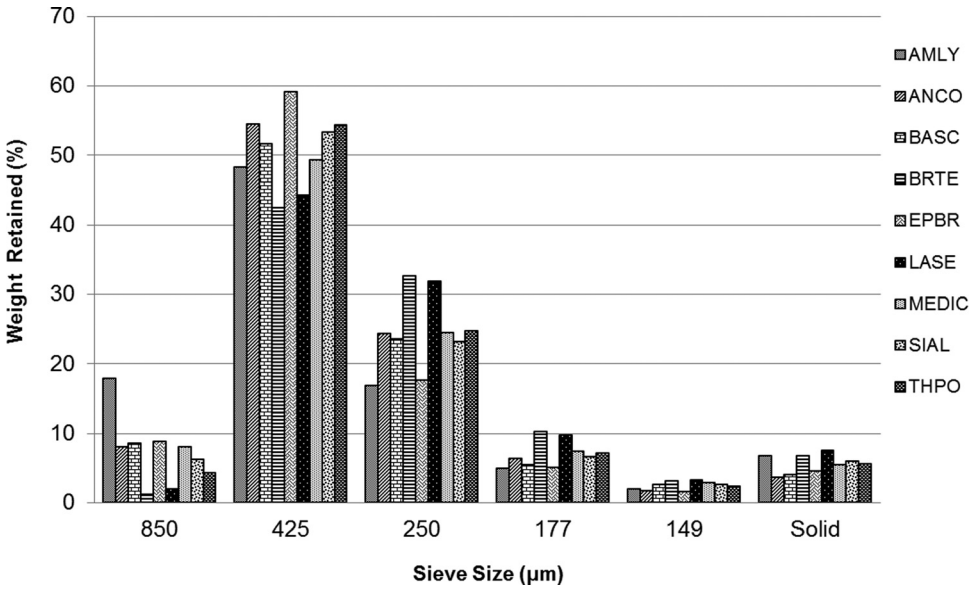

FIG. 1. Particle size profile of nine feedstocks.

and retained on 250 μm). In case of *Amsinckia lycopsoides* (AMLY), a significant fraction ($\sim$18%) was retained on the top sieve (850 μm), which indicates its high resistance to grinding compared to other feedstocks. Larger particle size could require longer reactor residence time to achieve equivalent hydrolysis.[52] Considerable amount of fines (3.7%–7.6%) that passed through sieve size 149 μm was present in all the feedstocks.

Ash content, which represents inorganic compounds in the grasses, ranged from 4.04% to 13.84%. A portion of this ash could come from dirt or sand, as the samples were not washed after collection. *Anthemis cotula* (ANCO), THPO, and *Lactuca serriola* (LASE) were found to have significantly similar ash content, which was different from all others. AMLY had maximum ash, which may be due to higher fines fraction (Figure 1) and *Sisymbrium altissimum* (SIAL) had the lowest fraction of ash. A significant fraction of the biomass was found to be water soluble extractives (17%–36%), however, ethanol soluble extractives were not as high

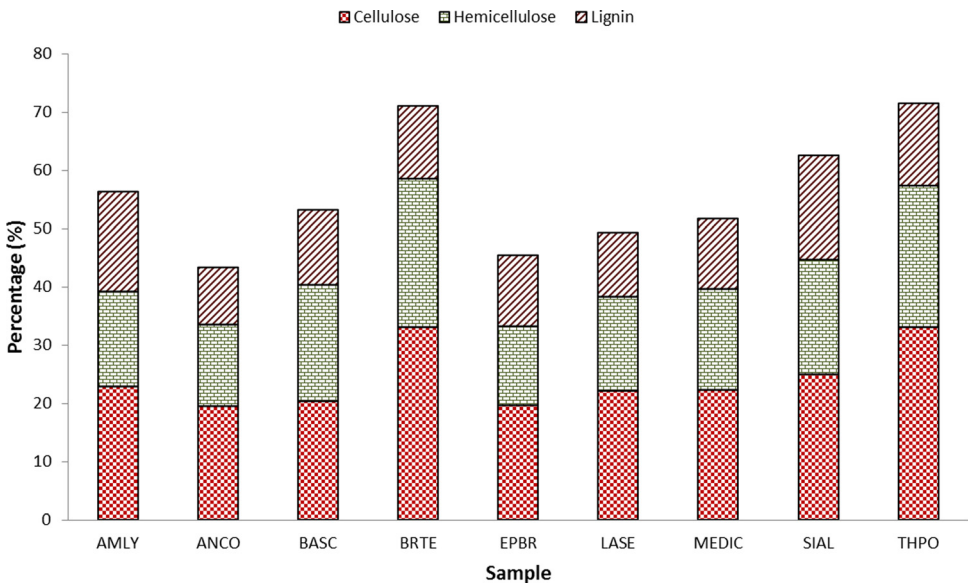

FIG. 2. Cellulose, hemicellulose, and lignin contents of nine feedstocks.

TABLE IV. Five carbon and six carbon sugar content of nine feedstocks (% dry basis). All different letters in each column represent significantly different values of the corresponding parameter. Standard errors are based on standard deviation of 3 replicates.

Sample	Weight mean contribution ± se (Mg/ha)								
Planted species	CRA 7.2	CRA 8.2	CRA9.2	Glucan	Xylan	Galactan	Arabinan	Mannan	Acetic acid
MEDIC	<0.01 ± 0.00	3.07 ± 2.48	2.16 ± 1.77	22.19 ± 0.48^{c,d}	7.51 ± 0.13^{f}	3.00 ± 0.04^{a}	2.81 ± 0.03^{b}	1.91 ± 0.03^{a}	2.09 ± 0.03^{a}
THPO	0.22 ± 0.17	15.89 ± 11.80	13.45 ± 5.58	32.95 ± 0.13^{a}	20.12 ± 0.07^{a}	0.98 ± 0.08^{h}	2.21 ± 0.10^{c}	0.49 ± 0.05^{f}	0.53 ± 0.05^{f}
				Volunteered Species					
AMLY	0.18 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	22.88 ± 0.22^{b,c}	9.28 ± 0.05^{d}	2.38 ± 0.05^{c,d}	1.31 ± 0.04^{f}	1.57 ± 0.09^{b,c}	1.72 ± 0.10^{b,c}
ANCO	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.02	19.39 ± 0.27^{e}	7.03 ± 0.08^{g}	2.36 ± 0.05^{d,e}	1.55 ± 0.12^{e}	1.52 ± 0.09^{c}	1.66 ± 0.10^{c}
BASC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.30 ± 0.29^{d,e}	9.79 ± 0.24^{c}	2.66 ± 0.07^{b}	4.76 ± 0.13^{a}	1.34 ± 0.04^{d}	1.46 ± 0.04^{d}
BRTE	2.13 ± 0.47	0.00 ± 0.00	0.04 ± 0.03	33.06 ± 0.17^{a}	20.31 ± 0.12^{a}	1.19 ± 0.03^{g}	2.69 ± 0.07^{b}	0.59 ± 0.02^{f}	0.65 ± 0.02^{f}
EPBR	0.01 ± 0.01	0.00 ± 0.00	0.14 ± 0.11	19.53 ± 4.05^{e}	7.64 ± 0.31^{f}	2.25 ± 0.07^{e}	1.39 ± 0.01^{f}	1.09 ± 0.06^{e}	1.19 ± 0.07^{e}
LASE	0.00 ± 0.00	0.25 ± 0.19	0.07 ± 0.06	22.19 ± 0.17^{c,d}	8.48 ± 0.19^{e}	2.48 ± 0.13^{c}	1.65 ± 0.11^{e}	1.64 ± 0.11^{b}	1.79 ± 0.11^{b}
SIAL	0.52 ± 0.52	0.45 ± 0.23	0.00 ± 0.00	24.96 ± 0.39^{b}	12.41 ± 0.18^{b}	2.08 ± 0.02^{f}	1.82 ± 0.04^{d}	1.61 ± 0.04^{b,c}	1.76 ± 0.04^{b,c}

(2%–5%). Water-soluble extractives were found to be significantly different in all the samples except BRTE and SIAL, which were not statistically different. Maximum water soluble extractives were present in *Epilobium brachycarpum* (EPBR) and maximum ethanol soluble extractives were found in ANCO.

The cellulose, hemicellulose, and lignin contents of all feedstocks are illustrated in Figure 2. Cellulose, a polymer of glucose, ranged from 19% to 33%, with the highest being present in BRTE. The glucose content was significantly different in all individual grasses (Table III). The xylan, galactan, arabinan, mannan, and acetic acid fractions (Table III), considered as part of hemicellulose, accounted for about 14% to 26% of the total biomass. Xylan constituted a major proportion of hemicellulose (up to 83% in THPO). The total structural sugars were found to be highest in THPO (57.3%) and BRTE (58.5%). Total lignin content (acid soluble and acid insoluble lignin), a major factor that affects the hydrolysis efficiency, was found to be in the range of 10% to 18% of total biomass.

All components of grasses were observed to be significantly different from each other with a p-value of 0.05 and R^2 value greater than 98%. Individual differences among feedstocks for each component are mentioned in Tables III and IV. For all analyzed samples, the total mass closure after all analyses ranged from 92.11% to 104.86%. Mass closure can serve as an indicator of analysis accuracy. The reported mass closure values for feedstocks are within the range of reported mass closure values of other feedstocks.[53]

C. Ethanol yield

Potential ethanol yields from all feedstocks, calculated on the basis of sugar content, are illustrated in Figure 3. The yields ranged from 181.5 to 316.5 l/dry ton of biomass. High sugar content grasses, THPO, and BRTE, were estimated to produce maximum ethanol yield, i.e., 311.5 and 316.5 l/dry ton of biomass, respectively. Ethanol yields were also calculated on the basis of six carbon sugars only. These estimates were in the range of 129.6 to 221 l/dry ton of biomass. THPO and BRTE had maximum glucose content, which leads to maximum theoretical ethanol yields of 220 and 221 l/dry ton biomass. The actual ethanol yield could be different from the theoretical yield, depending on processing techniques (pretreatment and type of hydrolysis and fermentation[33]), but the purpose of this study was to estimate potential ethanol production from riparian conservation buffers and hence ethanol yields were estimated based on reasonable assumptions. Based on the contribution of the feedstocks to CRA sites, the average

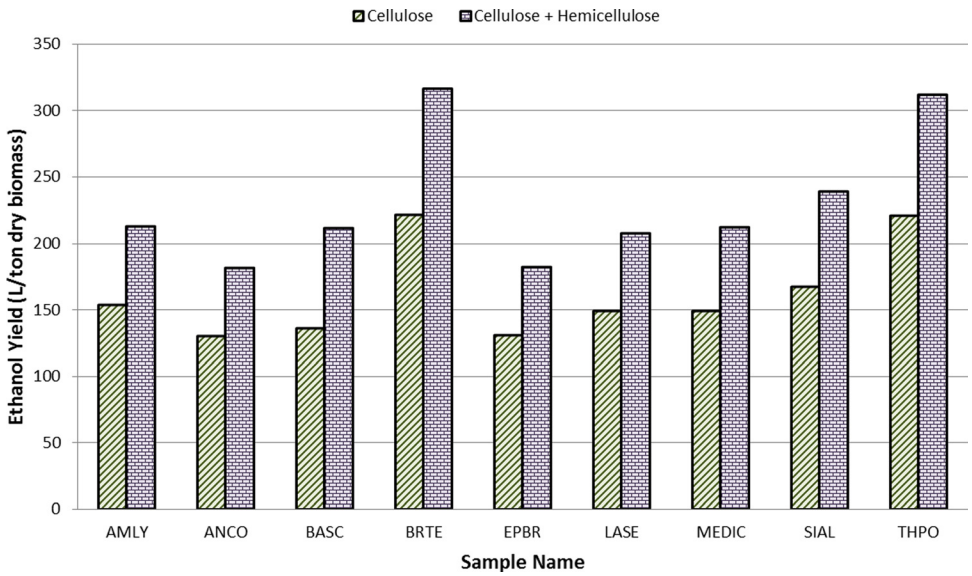

FIG. 3. Potential ethanol yields from nine feedstocks.

TABLE V. Total ethanol production potential from each CRA.

Species	EtOH potential (l/ton)	% contribution		
		CRA 7.2	CRA 8.2	CRA9.2
	Planted species			
MEDIC	212.1	0.1	15.1	12.5
THPO	311.6	7	78.2	77.4
	Volunteered species			
AMLY	212.8	5.4	0	0
ANCO	181.5	0	0	0.4
BRTE	316.5	66.2	0	0.2
EPBR	181.9	0.3	0	0.8
LASE	207.3	0	1.2	0.4
SIAL	238.7	16.3	2.1	0
Average EtOH yield (l/ton)		282.5	283.2	271.3
Area (ha)		1812.0	31694.0	21997.0
Yield (Mg/ha)		3.1	19.7	15.9
Total EtOH (l)		1 571 455	176 447 121	95 070 965
ETOH (MM l)		1.6	176.4	95.1

ethanol production per CRA was found to be 282.5, 283.2, and 271.3 l/ton for CRA 7.2, 8.2, and 9.2, respectively. Considering the areas under each CRA, total ethanol potential from CRA sites was estimated as 1.6, 176.5, and 95.1 million liter from CRA 7.2, 8.2, and 9.2, respectively (Table V).

D. Additional applications

This work focused on the compositional analysis of a newly developed source of biomass for cellulosic ethanol production; however, there is potential to use this biomass in a wide range of applications that are well suited to distributed feedstock production as found in the CRP lands. Potential feedstock applications include pyrolysis, gasification, anaerobic digestion, pelletization, and use as forage for livestock production. Each application has its own set of feedstock requirements some of which can be addressed by this data set. For example, low ash content is important to pyrolysis, gasification, and pelletization applications as to avoid slag formation.[54] Ash is comparatively less important in anaerobic digestion and forage applications where low lignin content improves digestibility.[55,56]

IV. CONCLUSION

The establishment and productivity of mixed species conservation buffers were largely successful in CRAs 8.2 and 9.2, although the same goal in CRA 7.2 was unsuccessful. New and innovative management techniques are required to insure the long term productiveness and usefulness of these sites if they are to provide biofuel feedstocks. The aim of this study was to determine the variability of composition among samples to determine the ease with which multiple feedstocks could be processed in a single facility running a single process. A significant composition differences for total glucan (19.39 ± 0.27 to 33.06 ± 0.17) and xylan (7.03 ± 0.08 to 20.31 ± 0.12) content were found among the nine feedstocks. Potential maximum ethanol yields ranged from 181.5 to 316.5 l/dry ton of biomass for different plant species. Such large differences in composition could lead to processing difficulties and make it difficult to recover high sugar yields from mixed feedstocks. Ecologically, it may be favorable to establish mixed species conservation buffers, but when bioethanol production processes are considered homogeneous stands of grasses with high glucose content are more

preferable with our current processing technology. Grasses with higher glucose concentrations will produce more ethanol and having a diverse and balanced ecosystem from which the biomass is harvested will make the land as a whole more productive. Future studies should focus on developing optimized processing conditions and management options for mixed species.

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