

Natural Variation for Nutrient Use and Remobilization Efficiencies in Switchgrass

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Abstract Nutrient management in biomass production systems serves to maximize yield and minimize production costs and environmental impact. Loss of soil nutrients with harvested biomass can be reduced by the judicious choice of genotype and harvest time. Sustainable production of switchgrass for biofuel will depend, in part, on breeding of varieties that are conservative in their use of soil nutrients to produce biomass. To aid such breeding programs, we assessed the natural variation in nutrient-use and remobilization efficiencies of 31 accessions of *Panicum virgatum* by measuring the concentration of 20 elements (N, P, K, Li, B, Na, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, and Cd) in shoots of field-grown plants harvested at two different stages of development. Significant differences between accessions were found for elemental composition at maturity and after senescence. The concentration of several elements (N, P, K, and Rb) decreased in the shoots of all accessions during senescence, although the efficiency

of remobilization ranged from 20% to 61% for N, 31% to 65% for P, 25% for 84% for K, and 33% to 84% for Rb. The accessions/cultivars with the greatest nutrient-use efficiency (smallest loss of nutrient per unit biomass) were BN-14668-65, Kanlow, Caddo from the point of view of N content, and Kanlow, Cave-in-Rock, and Blackwell from the point of view of P content in senescent shoots. Finally, differences in elemental composition between upland and lowland ecotypes were also found. The information presented here will help to guide future breeding programs and nutrient management practices.

Keywords Switchgrass · Nutrient remobilization · Accessions · Ecotypes

Abbreviations

ha Hectare, 10,000 m²
PCA Principle component analysis
PCC Pearson correlation coefficient
RE Remobilization efficiency

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Introduction

The current use of fossil fuels for energy is unsustainable, not only because fossil fuel reserves are finite but also because of its environmental impact [7, 19]. Biofuel derived from biomass/photosynthesis is an alternative to fossil fuel that is economically feasible, environmentally benign, and socially acceptable [19].

Since the mid-1980s, there has been increasing interest in the use of perennial grasses as bioenergy crops in the USA and Europe [17]. Perennial grasses are more promising than annual crops because of their higher biomass

production, higher lignin and cellulose contents, lower annualized establishment and maintenance costs, and reduced soil erosion [1]. Switchgrass (*Panicum virgatum* L.) is a perennial grass native to the North American tall grass prairies and was selected for development as a bioenergy crop by the US Department of Energy after decades of research.

Switchgrass comprises two ecotype classes: “upland” and “lowland,” which refer to latitude not altitude. Upland ecotypes are mostly octaploid ($2n=8x=72$), although hexaploid and tetraploid ecotypes exist. Lowland ecotypes are mostly tetraploid ($2n=4x=36$) [10, 12]. According to Moser and Vogel [22], the top switchgrass candidates for high biomass yield were two lowland accessions, “Alamo” for the deep South and “Kanlow” for mid-latitudes, and one upland accession, “Cave-in-rock” for the central and northern states of the USA. Their annual yields were shown to be about 16 to 22 Mg dry matter per ha [17]. Although switchgrass remobilizes some nutrients from shoots to roots each year during senescence, substantial amounts of nutritive elements are removed with harvested biomass. For example, the total N removed with biomass in a one-cut fall harvest system varied from 31 to 63 kg N ha⁻¹ year⁻¹, and from 90 to 144 kg ha⁻¹ year⁻¹ for a two-cut system, over 5 years of measurements [26]. Such nutrient withdrawal rates inevitably result in N depletion from the soil and necessitate the addition of fertilizer N to maintain switchgrass productivity. Synthesis and application of fertilizer N is energy intensive and economically and environmentally costly. High N content in harvested biomass can be an additional liability because it yields NO_x compounds upon oxidation, which are potent atmospheric pollutants [15]. High concentrations of other macronutrients such as P, K, and S in harvested biomass can lead to significant depletion of these in the soil, necessitating fertilizer amendments to maintain soil fertility. Finally, the presence of certain elements in biomass, especially alkali metals, can negatively affect biomass digestion, fermentation, or combustion [21]. This will also increase the cost of bioenergy production.

Development of cultivars with high yield potential and high nutrient-use efficiency will help to establish switchgrass as a sustainable source of biomass for biofuel. Although switchgrass is largely self-incompatible [30], plants of the same ploidy level can usually be crossed regardless of their ecotype [18]. Moreover, natural diversity present in the hundreds of accessions now available will facilitate breeding of new cultivars with desired traits through modern breeding programs. However, basic information about nutrient-use efficiency and remobilization in different switchgrass accessions is still lacking. The aim of this study was to assess the natural diversity in these traits in field-grown plants. This involved measuring the elemen-

tal composition of shoots of 31 different switchgrass accessions before and after senescence. The results of this work are presented below.

Materials and Methods

Plant Material and Cultivation

The 31 switchgrass accessions used in this study (Supplemental Table 1) were obtained from the Germplasm Resources Information Network. Eight of these accessions (ID nos. 2, 8, 11, 12, 14, 15, 16, and 31) were classified as lowland ecotypes and the remaining 23 accessions as upland types [24]. Ten genotypes from each accession were clonally multiplied into four replicates. Field planting in July 2007 was carried out following an R-36 honeycomb design with 1.5 m plant spacing. All plants are being grown in a field of the Samuel Roberts Noble Foundation, Ardmore, OK, USA under routine field management. The soil texture is Normangee clay loam type with pH 5.7. The field was fertilized with N at 112 kg/ha and supplemented with agricultural lime at 5,600 kg/ha in April 2007. The crop canopy was harvested once during 2007 in November.

Sample Harvesting and Processing

Tiller samples were harvested at two stages of plant development: first, in August 2008 when switchgrass plants were mature but still green (maturity stage, Fig. 1a) and second in late December after senescence (yellowing and drying) of above-ground tissues (post-senescence stage, Fig. 1b). For each accession, five plants were selected as biological replicates, and one representative tiller was collected from each plant at each stage.

The harvested tillers were dried for at least 48 h at 60°C to constant weight, chopped into 1–2-cm pieces, and then ground into a fine powder using a SPEX SamplePrep 6870 Freezer/Mill (Metuchen, NJ, USA).

Nutrient Analysis

The total nitrogen content (%) of each sample was analyzed by Ward Laboratories, Inc. (Kearney, NE, USA) using a combustion method [11]. The contents of other macro- and micro-nutrients (Li, B, Na, Mg, P, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, and Cd) were analyzed using inductively coupled plasma–mass spectrometry (ICP–MS) at the Purdue University, IN, USA [29]. About 2 to 8 mg of dried powdered tissue was placed into 100×16 mm Pyrex tubes and digested with 0.70 ml of conc. HNO₃ (Mallinckrodt, AR Select grade) at 110°C for 4 h. Each sample was diluted to 6.0 ml with 18 MΩ water and analyzed on a

Table 1 Concentrations of mineral macronutrients and sodium in tillers of 31 switchgrass accessions at maturity

Plant ID	N (%)		P (ppm)		K (ppm)		Ca (ppm)		Mg (ppm)		Na (ppm)	
1	0.89	ab	1,206	abc	8,335	ae	5,547	a	3,547	abe	15	a
2	0.78	a	1,351	abcd	12,927	b	4,561	ac	3,615	ae	1,741	b
3	0.80	a	1,107	ab	7,730	ace	7,168	abd	4,275	ae	102	a
4	0.92	ab	1,277	abcd	5,035	acd	8,910	bd	3,106	abc	18	a
5	0.85	a	1,301	abcd	8,524	ae	6,228	ab	2,883	abcd	105	a
6	1.06	bc	1,234	abc	7,106	ace	7,941	bd	4,063	ae	16	a
7	0.83	a	1,079	ab	6,619	acde	6,243	ab	4,030	ae	49	a
8	1.05	bc	1,247	abc	13,144	b	2,464	c	2,154	bcd	1,216	b
9	0.90	ab	1,166	abc	9,628	be	5,203	a	2,993	abcd	50	a
10	1.06	bc	1,126	ab	9,759	be	4,722	ac	2,651	bcd	10	a
11	1.00	bc	1,213	abc	9,875	be	4,788	ac	2,523	bcd	387	a
12	1.00	bc	1,401	abcd	10,128	be	3,999	c	2,006	cd	2,763	c
13	0.88	a	1,467	acd	4,423	cd	7,228	abd	4,120	ae	38	a
14	1.05	bc	1,407	abcd	10,306	be	3,284	c	2,976	abcd	1,535	b
15	1.10	c	1,336	abcd	10,813	b	3,036	c	1,630	d	1,169	b
16	0.88	a	1,044	b	7,768	ace	7,220	abd	3,139	abc	192	a
17	0.93	abc	1,376	abcd	3,274	d	9,532	d	4,802	e	37	a
18	0.95	bc	1,287	abcd	4,541	cd	6,058	a	3,600	ae	24	a
19	1.04	bc	1,409	abcd	7,057	acde	6,051	a	3,719	ae	17	a
20	0.91	ab	1,036	b	6,750	acde	5,367	a	3,480	abe	45	a
21	0.91	ab	1,392	abcd	8,908	e	4,539	ac	2,136	bcd	151	a
22	0.89	a	1,216	abc	6,389	acd	6,731	ab	4,214	ae	149	a
23	0.96	bc	1,333	abcd	8,332	ae	3,504	c	2,377	bcd	47	a
24	0.86	a	1,202	abc	9,637	be	4,579	ac	3,231	abc	49	a
25	0.92	ab	1,561	cd	9,323	e	4,633	ac	3,132	abc	65	a
26	0.78	a	1,230	abc	7,120	ace	7,207	abd	4,462	e	45	a
27	1.02	bc	1,675	d	8,437	ae	7,014	abd	3,247	abc	23	a
28	0.81	a	1,209	abc	5,241	acd	7,039	abd	3,715	ae	28	a
29	1.02	bc	1,415	abcd	6,189	acd	5,572	a	2,913	abcd	48	a
30	0.85	a	1,200	abc	6,625	acde	5,052	ac	3,359	abc	76	a
31	0.90	ab	1,101	ab	7,910	ace	5,701	a	4,593	e	1,787	b
LSD _{0.05}	0.17		410		3,811		2,716		1,431		894	

Values are averages of five biological replicates for each accession. Values within columns followed by the same letter are not significantly different at the 0.05 level according to the LSD test

PerkinElmer Elan DRCe ICP–MS. Indium (EM Science) was used as an internal standard. National Institute of Standards and Technology traceable calibration standards (ULTRAScientific) were used for the calibration. Sample weights were calculated from the weights of a subset of samples and the signal intensities of the most stable elements.

Data Analysis

Based on element contents at maturity (*M*) and post-senescence (*S*) stages, the difference between contents at *M* and *S* stages divided by the content at *M* stage $[(M-S)/M]$ was calculated for each element for all 31 accessions. This value represents the efficiency of nutrient remobilization during senescence. Fisher’s least significant difference (LSD) test ($P<0.05$) was performed to compare data from different

accessions. To reveal upland and lowland ecotype differences, the 31 accessions were grouped into the two ecotype classes, and the Student *t* test was conducted to compare the means of each class. Pearson correlation coefficients (PCC) were calculated for pairs of element contents at certain developmental stage across 31 accessions. Principle component analysis (PCA [13]) of contents and remobilization efficiencies of 20 elements from all accessions were calculated to identify similarities and differences between accessions and ecotypes.

Results

The two methods employed here measured all but one (S) of the six mineral macronutrients (in decreasing order of abundance in plant tissues: N, K, Ca, Mg, P, and S) and all

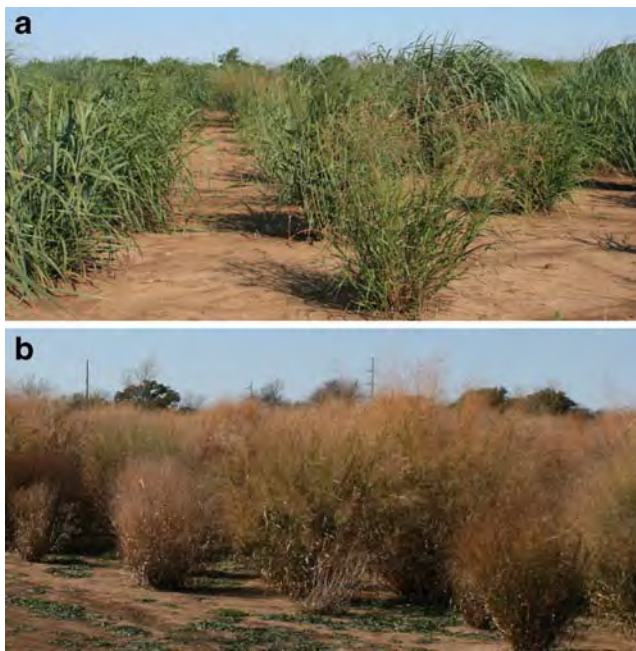


Fig. 1 **a** Field-grown switchgrass plants at maturity in August 2008. **b** Field-grown switchgrass plants, post-senescence, in late December 2008

but one (Cl) of the eight mineral micronutrients (in decreasing order of abundance: Cl, Fe, B, Mn, Zn, Cu, Mo, and Ni). ICP–MS analysis also quantified the levels of Li, Na, Co, As, Se, Rb, Sr, and Cd. Therefore, a total of 20 elements were analyzed in this study.

The relative abundance of macro- and micronutrient elements in switchgrass tillers was similar to that of other plant species, as outlined above [5]. However, significant differences in the concentrations of elements were found both within accessions at different stages of development and between accessions at the same stage of development (Tables 1 and 2 and Supplementary Table 2).

Elemental Composition of Mature Green Tillers

For tillers harvested in August at plant maturity, N content ranged from 0.78% (for accession no. 2) to 1.10% (accession no. 15), P ranged from 1,036 (accession no. 20) to 1,675 ppm (accession no. 27), K ranged from 3,274 (accession no. 17) to 13,144 ppm (accession no. 8), Ca ranged from 2,464 (accession no. 8) to 9,532 ppm (accession no. 17), and Mg ranged from 1,630 (accession no. 15) to 4,802 ppm (accession no. 17; Table 1). Interestingly, accession no. 17 had the lowest level of K but the highest levels of Ca and Mg, while accession no. 8 had the highest level of K but the lowest level of Ca and one of the lowest levels of Mg. Further analysis of all accessions combined showed an inverse relationship between K and other cations, including Sr (PCC=−0.63),

Ca (PCC=−0.62), Mg (PCC=−0.56), Fe (PCC=−0.47), Cu (PCC=−0.42), Mn (PCC=−0.42), Co (PCC=−0.41), and Li (PCC=−0.39). These inverse relationships indicate a certain degree of cation homeostasis in switchgrass, although the total contents of the 15 cations measured were not constant across the 31 accessions.

Although not a nutrient, Na concentrations approached those of some of the macronutrients, at least in a few accessions. However, Na content varied radically between accessions, ranging from 10 ppm in accession no. 10 to 2,763 ppm in accession no. 12. Interestingly, Na levels were generally higher in lowland than in upland ecotypes (Table 1).

Elemental Composition of Senescent Yellow Tillers

For tillers harvested in late December following shoot senescence, N content ranged between 0.40% and 0.77% (accession nos. 2 and 27, respectively), P between 445 and 847 ppm (accession nos. 8 and 28), K between 774 and 8,129 ppm (accession nos. 17 and 8), Ca between 5,009 and 8,684 ppm (accession nos. 14 and 25), and Mg between 2,346 and 4,449 ppm (accession nos. 23 and 31; Table 2). Again, Na content exhibited the largest range from 33 ppm (accession no. 10) to 2,024 ppm (accession no. 31), with the highest levels in lowland ecotypes (Table 2).

Compared to their contents at maturity, there was a general decline in N, P, K, and Rb contents in all 31 accessions. The contents of Ca, Mn, and Sr were higher, while the contents of Ni, Cu, and Zn were lower after senescence than at maturity in most accessions, with few exceptions (Tables 1 and 2).

The simple equation $RE = (M - S) / M$, where M and S represent nutrient content at the mature and senescent stages, respectively, was used to calculate the nutrient remobilization efficiency (RE) during senescence. REs of five macronutrients and Na for all accessions are shown in Table 3. RE for N ranged from 20% (accession no. 3) to 61% (accession no. 8), for P from 31% (accession no. 28) to 65% (accession no. 19), and for K from 25% (accession no. 31) to 84% (accession no. 2). Thus, N, P, and K were remobilized and exported from tillers of all accessions during senescence, albeit to varying degrees. In contrast, Ca, Mg, and Na accumulated in more than half of the accessions as indicated by negative values of RE (Table 3).

Comparison of Upland and Lowland Ecotypes

Significant differences in macronutrient content were found between the eight lowland and 23 upland ecotypes/accessions (Table 4). Although there was no significant difference between upland and lowland ecotypes in average N content of tillers at maturity, the average N content of

Table 2 Concentrations of mineral macronutrients and sodium in tillers of 31 switchgrass accessions after senescence

Plant ID	N (%)		P (ppm)		K (ppm)		Ca (ppm)		Mg (ppm)		Na (ppm)	
1	0.53	ab	513	a	2,748	ac	7,001	abc	3,211	abcd	56	a
2	0.40	a	494	a	1,891	ac	5,702	ac	3,897	ace	780	ab
3	0.63	bc	546	a	1,748	ac	6,994	abc	3,497	abce	65	a
4	0.68	cd	641	abc	2,116	ac	8,440	b	2,487	bd	51	a
5	0.57	bc	553	a	3,271	ac	8,032	ab	3,038	abd	87	a
6	0.52	ab	456	a	1,762	ac	6,893	abc	3,062	abcd	41	a
7	0.45	a	459	a	1,982	ac	7,196	abc	3,562	abce	56	a
8	0.41	a	446	a	8,129	b	6,160	abc	3,505	abce	1,206	bc
9	0.48	a	469	a	1,900	ac	6,633	abc	3,037	abd	59	a
10	0.66	cd	507	a	2,611	ac	6,986	abc	3,139	abcd	33	a
11	0.56	bc	510	a	3,208	ac	8,549	b	3,437	abcde	256	a
12	0.57	bc	551	a	3,371	ac	6,384	abc	2,681	bd	1,659	c
13	0.57	bc	582	a	1,426	ac	6,834	abc	3,014	abd	70	a
14	0.45	a	491	a	4,599	a	5,009	c	4,159	ce	800	ab
15	0.49	a	461	a	2,466	ac	8,531	b	3,993	ace	1,455	bc
16	0.57	bc	460	a	1,410	ac	7,386	abc	2,534	bd	105	a
17	0.56	bc	558	a	774	c	7,285	abc	2,983	abd	49	a
18	0.62	bc	621	ab	1,028	c	6,934	abc	2,829	bd	38	a
19	0.57	bc	481	a	1,299	ac	7,503	abc	3,415	abcde	35	a
20	0.57	bc	598	a	2,781	ac	7,139	abc	3,398	abcde	62	a
21	0.59	bc	563	a	2,095	ac	7,668	ab	2,469	bd	66	a
22	0.59	bc	572	a	2,345	ac	7,732	ab	4,094	ace	118	a
23	0.60	bc	595	a	2,816	ac	5,410	ac	2,346	d	74	a
24	0.49	a	466	a	2,033	ac	6,810	abc	3,341	abcde	50	a
25	0.48	a	549	a	2,914	ac	8,684	b	3,984	ace	96	a
26	0.43	a	470	a	1,991	ac	7,248	abc	3,807	ace	62	a
27	0.77	d	836	bc	3,966	ac	6,663	abc	2,419	d	111	a
28	0.60	bc	847	c	3,607	ac	7,861	ab	3,479	abce	52	a
29	0.56	bc	774	bc	2,519	ac	6,855	abc	3,070	abcd	85	a
30	0.47	a	496	a	1,404	ac	6,036	ac	3,591	ace	191	a
31	0.44	a	528	a	2,249	ac	7,438	abc	4,449	e	2,024	c
LSD _{0.05}	0.13		221		3,505		2,626		1,116		839	

Values are averages of five biological replicates for each cultivar. Values within columns followed by the same letter are not significantly different at the 0.05 level according to the LSD test

lowland ecotypes was significantly lower than that of the upland ecotypes after senescence, reflecting a greater average RE of the lowland ecotypes (Table 4). Likewise, although there was no significant difference between upland and lowland ecotypes in average P content at maturity, the average P content of lowland ecotypes was significantly lower than that of upland ecotypes after senescence (Table 4). The average content of K was higher in lowland ecotypes at both maturity and senescent stages, although RE of the two ecotype classes was similar (Table 4). The average contents of Ca and Mg in upland ecotypes were significantly higher than those of lowland ecotypes for tillers harvested at maturity, but not for tillers harvested after senescence (Table 4). Accumulation of more Ca and Mg in lowland ecotypes between the mature and senescent stages accounted for these results. A most

remarkable difference between lowland and upland ecotypes was found for Na content, which was an order of magnitude higher in lowland ecotypes than in upland types at both stages of tiller development (Table 4).

PCA of elemental contents at maturity of all 31 accessions distinguished six of the eight lowland ecotypes (accession nos. 2, 8, 11, 12, 14, and 15) from the upland types (Fig. 2a). The six elements that contributed most to the first principal component (PC1) were Sr, Ca, As, Fe, K, and Rb, while Ni, B, Mg, P, Mo, and Co contributed most to the second principal component (PC2). Most of the same lowland types (accession nos. 2, 8, 12, 14, 15, and 31) remained distinct based on the elemental contents of senescent tillers (Fig. 2b), and the top six elements contributing to PC1 were Fe, Co, Na, Li, As, and K, while Cd, Zn, Mo, Li, Na, and As contributed most to PC2. Based

Table 3 Remobilization efficiencies (%) of mineral macronutrients and sodium for tillers of 31 accessions

Plant ID	N	P	K	Ca	Mg	Na						
1	40.1	bc	57.1	abc	63.4	abc	-33.1	a	7.3	a	-347.3	a
2	48.8	bcd	61.3	ab	84.5	a	-33.0	a	-13.0	a	31.4	a
3	19.5	a	50.6	abc	74.6	a	-2.5	a	15.7	a	11.6	a
4	27.1	a	44.8	acd	51.0	abc	6.9	a	16.7	a	-210.1	a
5	32.0	a	54.1	abc	57.0	abc	-74.3	a	-31.6	ab	-15.8	a
6	51.3	cd	62.2	b	73.5	ab	12.7	a	24.5	a	-327.5	a
7	46.0	bcd	57.2	abc	63.0	abc	-85.0	a	23.8	a	-25.8	a
8	61.0	d	64.2	b	36.0	bc	-245.2	b	-96.8	b	9.0	a
9	45.6	bcd	58.9	ab	78.8	a	-58.5	a	-19.9	ab	-68.5	a
10	38.6	bc	55.1	abc	72.7	ab	-64.1	a	-27.6	ab	-271.9	a
11	43.3	bc	55.6	abc	66.5	abc	-134.0	b	-70.9	b	17.7	a
12	41.9	bc	59.0	ab	64.5	abc	-133.5	b	-68.3	b	33.2	a
13	34.1	ab	56.8	abc	65.2	abc	8.0	a	18.3	a	-183.8	a
14	56.5	d	64.2	b	50.6	abc	-73.1	a	-56.5	b	42.2	a
15	54.4	d	65.4	b	77.1	a	-249.5	b	-187.0	c	-59.1	a
16	34.7	ab	54.5	abc	80.4	a	-3.6	a	14.2	a	36.7	a
17	38.8	bc	58.3	ab	75.9	a	19.0	a	36.9	a	-55.9	a
18	35.2	b	51.6	abc	64.3	abc	-20.9	a	9.4	a	-143.7	a
19	45.2	bc	65.4	b	80.0	a	-36.7	a	7.3	a	-188.0	a
20	37.4	bc	40.8	cd	55.2	abc	-32.9	a	2.2	a	-55.4	a
21	34.7	ab	57.9	abc	78.5	a	-70.6	a	-16.4	a	22.6	a
22	33.8	ab	50.6	abc	61.9	abc	-22.6	a	-5.5	a	-25.9	a
23	33.0	a	53.7	abc	64.0	abc	-89.0	a	9.2	a	-101.7	a
24	43.5	bc	59.8	ab	79.9	a	-58.8	a	-11.4	a	-4.2	a
25	46.0	bcd	58.5	ab	65.5	abc	-146.1	b	-55.2	b	-59.1	a
26	43.8	bc	61.6	ab	71.8	ab	-4.0	a	9.5	a	-139.9	a
27	22.6	a	49.4	abc	48.8	abc	14.4	a	21.1	a	-290.8	a
28	25.4	a	31.0	d	33.4	c	-14.0	a	5.3	a	-142.5	a
29	45.4	bc	47.7	acd	56.8	abc	-29.7	a	-7.9	a	-117.0	a
30	44.5	bc	59.5	ab	72.5	ab	-30.2	a	-20.5	ab	-117.5	a
31	50.6	cd	50.0	abc	63.1	abc	-24.4	a	0.9	a	-40.1	a
LSD _{0.05}	15.5		17.4		38.04		117.9		78.2		546.4	

Values are averages of five biological replicates for each accession. Values within columns followed by the same letter are not significantly different at the 0.05 level according to the LSD test

on PCA analysis of remobilization efficiencies of all 20 elements (Fig. 2c), six lowland ecotypes (accession nos. 8, 11, 12, 14, 15, and 31) stood out from the upland types, and the top six contributing elements to PC1 were Sr, Ca, Mg, Mn, Fe, and N, while K, Rb, P, Cd, Se, and B contributed most to PC2.

Discussion

Nutrient management is a key component of sustainable agriculture. Nutrient uptake from the soil is not only primarily a function of plant biomass but it is also influenced by plant genotype and environment interactions, especially soil properties, weather, and management practices [27]. One reason that switchgrass was selected as a

promising species for biofuel production is that it can be grown on marginal soils, which would minimize competition with food crops for prime arable land [6, 25]. Nonetheless, sustainable management of soil nutrients and fertilizers will be important if switchgrass is to become part of a long-term solution to the looming energy crisis. Nutrients are always removed from the soil when biomass is harvested and taken away from the site of production. However, the amount of each element removed depends on the plant species, genotype, time of harvest, and other factors. Time of harvest is particularly important for perennial plants, such as switchgrass, which remobilize some nutrients during shoot senescence and store them in the root for subsequent re-use during shoot growth in the next season [15, 25]. Diversity exists within and among natural populations of plants both in the timing of

Table 4 Mean contents of mineral macronutrients and sodium in tillers at maturity (*M*) and post-senescence (*S*) and remobilization efficiencies (RE) of N, P, and K in upland and lowland ecotypes

Parameters	Growth stage	Upland		Lowland		
		Mean	SE	Mean	SE	
N content (%)	<i>M</i>	0.915	± 0.017	0.970	± 0.038	NS
	<i>S</i>	0.564	± 0.016	0.486	± 0.026	S
N RE (%)		37.5	± 1.7	48.9	± 3.0	S
P content (ppm)	<i>M</i>	1,282	± 32	1,263	± 48	NS
	<i>S</i>	572	± 23	493	± 13	S
P RE (%)		54.0	± 1.6	59.3	± 2.0	NS
K content (ppm)	<i>M</i>	7,173	± 380	10,359	± 702	S
	<i>S</i>	2,223	± 167	3,415	± 759	S
K RE (%)		65.5	± 2.4	65.3	± 5.7	NS
Ca content (ppm)	<i>M</i>	6,177	± 306	4,382	± 548	S
	<i>S</i>	7,167	± 150	6,895	± 458	NS
Mg content (ppm)	<i>M</i>	3,481	± 141	2,830	± 341	S
	<i>S</i>	3,186	± 100	3,582	± 242	NS
Na content (ppm)	<i>M</i>	52	± 8	1,349	± 290	S
	<i>S</i>	70	± 7	1,035	± 238	S

NS not significant, S a significant difference between upland and lowland ecotypes at the 0.05 level

senescence and in the extent of nutrient remobilization during senescence. The aim of this project was to quantify natural variation among switchgrass accessions for nutrient-use efficiency during the growth phase and remobilization efficiency during the senescence phase, with a view to identify accessions with the lowest levels of macronutrients in harvested shoot material for possible use in breeding programs.

Although N, P, K, and other elements are important nutrients for optimal plant growth and biomass yield [16], N is generally considered the most limiting nutrient for switchgrass growth [6, 20, 25]. Nitrogen fertilization has been shown to increase switchgrass production [23], although not in all contexts [20]. Therefore, it is important to identify switchgrass accessions that use N as efficiently as possible to produce biomass and that leave as much N as possible in the root–soil system after shoot harvest. On average, significantly less N was lost with tillers of lowland ecotypes harvested after senescence than was lost in upland tillers, although the average level of N was higher in the mature green tillers of lowland types than of upland types (Table 4). In other words, lowland ecotypes were generally more efficient than upland types at remobilizing N out of tillers during senescence. Likewise, P content was significantly lower in senescent tillers of lowland ecotypes than of upland types. This presumably reflects more complete degradation and/or more efficient export of the breakdown products of proteins, nucleic acids, phospholipids, and other organic macromolecules during shoot senescence in lowland ecotypes. These processes are known to be activated during shoot senescence to promote recycling of nutrients between organs of other plant species [9].

Despite the fact that lowland ecotypes were, on average, more efficient in their use of N and P to produce biomass, the three upland ecotypes with the lowest levels of N in senescent tillers [accession no. 26 (Caddo), 0.43%; accession no. 7 (Blackwell), 0.45%; and accession no. 30 (Ankara), 0.47%] were comparable to those the best three lowland types [accession no. 2 (BN-14668-65), 0.40%; accession no. 8 (Kanlow), 0.41%; and accession no. 31 (Alamo), 0.44%]. Be that as it may, our analysis revealed substantial diversity in N- and P-use efficiency for biomass production in both lowland and upland accessions, which could be utilized to reduce losses of these two nutrients from the soil in future cropping systems. For example, the amount of N lost with a hypothetical 20 tonne/ha late autumn harvest of accession no. 27 (Dacotah), the least efficient user of N, would be 154 kg compared to a loss of just 80 kg/ha to produce the same mass of accession no. 2 (BN-14668-65), the most efficient user of N. In view of the cost of N fertilizer, the value of capturing such natural diversity in breeding programs should be clear. A similar argument can be made for enhancing P-use efficiency through the use of natural diversity in breeding programs. It is interesting to note, in this regard, that N and P contents of senescent tillers are roughly correlated in different accessions (PCC=0.46), possibly because of coordination between N and P remobilization during senescence, which means that breeding for improvements in N-use efficiency may have the beneficial side effect of improving P-use efficiency.

Previously, three switchgrass accessions were identified as promising material for biomass production because of their high yield: upland Cave-in-rock (accession no. 6) and

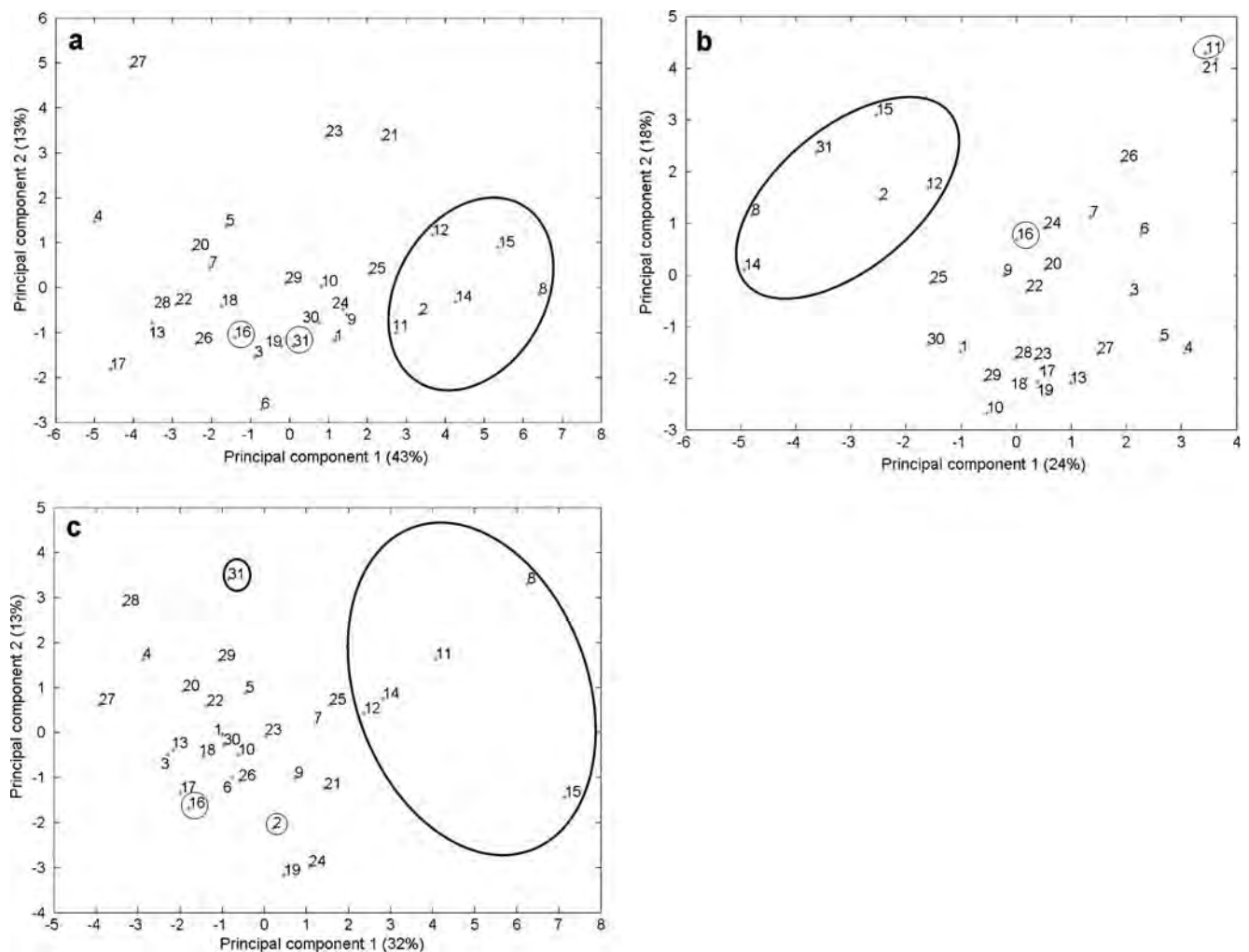


Fig. 2 Principal component analysis of elemental composition of tillers from 31 switchgrass accessions at maturity (a), after senescence (b), and of element remobilization efficiencies (c). The numbers in the

figure are accession IDs. Lowland accessions are indicated by encircled numbers. Percentages in parentheses indicate the contribution of each principal component to the total variance

lowland Kanlow (accession no. 8) and Alamo (accession no. 31) [22]. Interestingly, Kanlow and Alamo were among the best three lowland types with respect to low residual N in senescent tillers, indicating little room for improvement of this trait in these two accessions (Table 2). Kanlow also had the lowest P content in senescent tillers (446 ppm) of all the 31 accessions tested, while Alamo had a slightly higher than average level of residual P (528 ppm) compared to other lowland ecotypes (Tables 2 and 4). Clearly, there is room for improvement of this trait in Alamo. Cave-in-rock had lower than average residual P (446 ppm) but about average N content for an upland ecotype, indicating that improvements in the latter could be achieved via breeding with a more N-efficient upland accession, such as accession nos. 26, 7, or 30. Furthermore, consistent with other studies, the dry biomass yield per plant of most lowland accessions (except nos. 11 and 16) was significantly higher than that of upland accessions (Supplemental Table 2).

Integrating the biomass yield and nutrient content data from senescent material, it was estimated that the amount of nitrogen removed with harvested biomass per plant was 3.41 g for Cave-in-rock, 6.92 g for Kanlow, and 5.01 g for Alamo. While the use of natural variation in classical breeding programs is one sure way to reduce nutrient losses with harvested biomass, biotechnology and precise gene transfer are alternative approaches that may extend what is possible using natural variation alone [8, 19].

At least two systems for switchgrass harvesting have been tested: one-cut in late fall/early winter and two-cut in both mid-summer and late fall. Although the one- and two-cut systems often produce similar yields [17], the two-cut system was shown to remove more nutrients from soil than the one-cut system [14–16]. Consistent with such results, the concentration of N, P, and K in mature tillers harvested in August were higher than those of senescence tillers harvested in December for all 31 accessions in our study

(Tables 1 and 2). Thus, a single harvest in late fall/early winter would conserve more soil nutrients for subsequent biomass production.

Morphological and physiological differences between upland and lowland ecotypes in the field have been reported in the past [3, 4, 31]. We found significant differences between the two ecotypes classes in Ca and Mg contents at the maturity stage, in N and P contents at the senescent stage, and K and Na at both developmental stages (Table 4). PCA of elemental composition of mature and senescent tillers and of remobilization efficiencies also distinguished most lowland accessions from upland accessions (Fig. 2). These results indicated that there are elemental differences between upland and lowland ecotypes grown in the field. In view of the fact that K and Na homeostasis are closely related to plant salt tolerance [28, 32], it will be interesting to test if the higher levels of K and Na in lowland ecotypes make them more or less susceptible to salt stress. Except for a mention that lowland Alamo has moderate tolerance to salinity [2], no other information about salt tolerance of switchgrass ecotypes is available in the literature, to our knowledge. Given the likelihood that switchgrass will be planted on marginal soils, including saline soils, this is an area of research well worth pursuing.

In summary, we found significant natural variation for elemental contents in switchgrass ecotypes/accessions that could be harnessed in future breeding programs to limit losses of macronutrients such as N, P, and K from soils. In addition, we found that harvesting shoots after senescence substantially reduced losses of nutrients from the production system. Therefore, judicious choice of accessions for breeding programs and of harvest dates should help to put switchgrass production onto a sustainable path in the future.

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References

- Adler PR, Sanderson MA, Boateng AA, Weimer PJ, Jung H-JG (2006) Biomass yield and biofuel quality of switchgrass harvested in fall or spring. *Agron J* 98:1518–1525
- Alderson J, Sharp WC (1995) Grass varieties in the United States. CRC Press, Boca Raton, pp 194–199
- Brunken JN, Estes JR (1975) Cytological and morphological variation in *Panicum virgatum* L. *The Southwest naturalist* 19:379–385
- Casler MD, Vogel KP, Taliaferro CM, Wynia RL (2004) Latitudinal adaptation of switchgrass populations. *Crop Sci* 44:293–303
- Epstein E (1965) Mineral metabolism. In: Bonner J, Varner JE (eds) Plant biochemistry. Academic Press, New York, pp 438–466
- Ghimire S, Charlton N, Craven K (2009) The mycorrhizal fungus, *sebacina vermifera*, enhances seed germination and biomass production in switchgrass (*Panicum virgatum* L). *BioEnergy Res* 2:51–58
- Goldemberg J (2007) Ethanol for a sustainable energy future. *Science* 315:808–810
- Good AG, Shrawat AK, Muench DG (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends Plant Sci* 9:597–605
- Gregersen PL, Holm PB, Krupinska K (2008) Leaf senescence and nutrient remobilisation in barley and wheat. *Plant Biol* 10:37–49
- Hopkins AA, Taliaferro CM, Murphy CD, Christian DA (1996) Chromosome number and nuclear DNA content of several switchgrass populations. *Crop Sci* 36:1192–1195
- Horneck DA, Miller RO (1998) Determination of total nitrogen in plant tissue. In: Kalra YP (ed) Handbook of reference methods for plant analysis. CRC Press, Boca Raton, pp 75–83
- Hultquist SJ, Vogel KP, Lee DJ, Arumuganathan K, Kaeppler S (1996) Chloroplast DNA and nuclear DNA content variations among Cultivars of switchgrass, *Panicum virgatum* L. *Crop Sci* 36:1049–1052
- Jolliffe IT (1986) Principal component analysis. Springer-Verlag, New York
- Landström S, Lomakka L, Andersson S (1996) Harvest in spring improves yield and quality of reed canary grass as a bioenergy crop. *Biomass Bioenergy* 11:333–341
- Lemus R, Parrish D, Abaye O (2008) Nitrogen-use dynamics in switchgrass grown for biomass. *BioEnergy Res* 1:153–162
- Lemus R, Parrish D, Wolf D (2009) Nutrient uptake by ‘Alamo’ switchgrass used as an energy crop. *BioEnergy Res* 2:37–50
- Lewandowski I, Scurlock JMO, Lindvall E, Christou M (2003) The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass Bioenergy* 25:335–361
- Martinez-Reyna JM, Vogel KP, Caha C, Lee DJ (2001) Meiotic stability, chloroplast DNA polymorphisms, and morphological traits of upland lowland switchgrass reciprocal hybrids. *Crop Sci* 41:1579–1583
- McLaren JS (2005) Crop biotechnology provides an opportunity to develop a sustainable future. *Trends Biotech* 23:339–342
- McLaughlin S, Button J, Bransby D, Conger R, Ocumpaugh W, Parrish D, et al (1997) Evaluating and improving switchgrass as a bioenergy crop. In: Proceedings of the 3rd Biomass Conference of the Americas. August 24–29, Montreal, Quebec, Canada. 1: 137–143
- Miles TR, Miles TR, Baxter LL, Bryers RW, Jenkins BM, Oden LL (1996) Boiler deposits from firing biomass fuels. *Biomass Bioenergy* 10:125–138
- Moser LE, Vogel KP (1995) Switchgrass, big bluestem, and indianagrass. In: Barnes RF, Miller DA, Nelson CJ (eds) Forages, an introduction to grassland agriculture, 5th edn. University Press, Ames, pp 409–420
- Muir JP, Sanderson MA, Ocumpaugh WR, Jones RM, Reed RL (2001) Biomass production of ‘Alamo’ switchgrass in response to nitrogen, phosphorus, and row spacing. *Agron J* 93:896–901
- Narasimhamoorthy B, Saha M, Swaller T, Bouton J (2008) Genetic diversity in switchgrass collections assessed by EST-SSR markers. *BioEnergy Res* 1:136–146
- Parrish DJ, Fike JH (2005) The biology and agronomy of switchgrass for biofuels. *Crit Rev Plant Sci* 24:423–459
- Reynolds JH, Walker CL, Kirchner MJ (2000) Nitrogen removal in switchgrass biomass under two harvest systems. *Biomass Bioenergy* 19:281–286

27. Robinson DL (1996) Fertilization and nutrient utilization in harvested forage systems: Southern forage crops. In Joost R.E. and Roberts C.A. (eds). Nutrient cycling in forage systems. Proc. Symp. Columbia, MO. Potash and phosphate Inst. Manhattan, KS, pp 65–92
28. Rus A, Yokoi S, Sharkhuu A, Reddy M, B-h L, Matsumoto TK et al (2001) AtHKT1 is a salt tolerance determinant that controls Na⁺ entry into plant roots. Proc Natl Acad Sci USA 98:14150–14155
29. Salt DE (2004) Update on plant ionomics. Plant Physiol 136:2451–2456
30. Talbert LE, Timothy DH, Burns JC, Rawlings JO, Moll RH (1983) Estimates of genetic parameters in switchgrass. Crop Sci 23:725–728
31. Warner DA, Ku MSB, Edwards GE (1987) Photosynthesis, leaf anatomy, and cellular constituents in the polyploid C4 Grass *Panicum virgatum*. Plant Physiol 84:461–466
32. Zhu J-K (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6:441–445