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Article *in* BioEnergy Research · December 2009

DOI: 10.1007/s12155-009-9055-9



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Bioenerg. Res. (2009) 2:257–266
DOI 10.1007/s12155-009-9055-9

# Natural Variation for Nutrient Use and Remobilization Efficiencies in Switchgrass

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Published online: 14 October 2009


**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 $m^2$ |
| PCA | Principle component analysis |
| PCC | Pearson correlation coefficient |
| RE | Remobilization efficiency |

**Electronic supplementary material** The online version of this article (doi:10.1007/s12155-009-9055-9) contains supplementary material, which is available to authorized users.

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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 NOx 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 elemental 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. $HNO_3$ (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

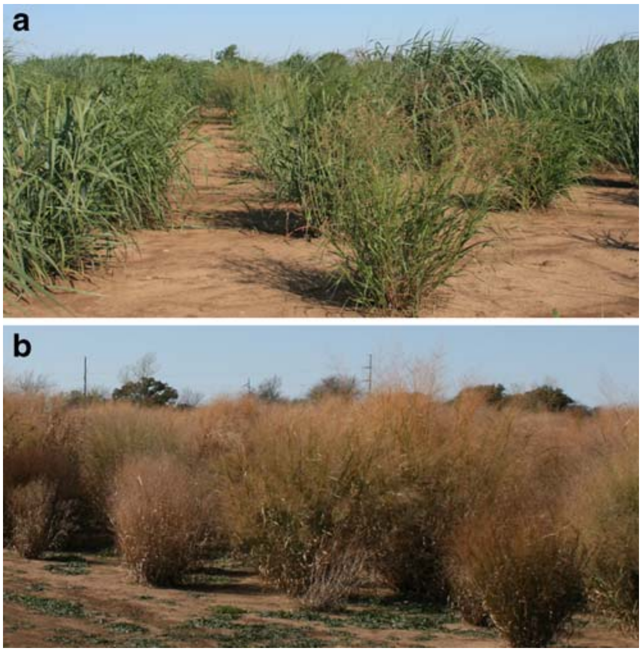

**Fig. 1** **a** Field-grown switchgrass plants at maturity in August 2008. **b** Field-grown switchgrass plants, post-senescence, in late December 2008

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

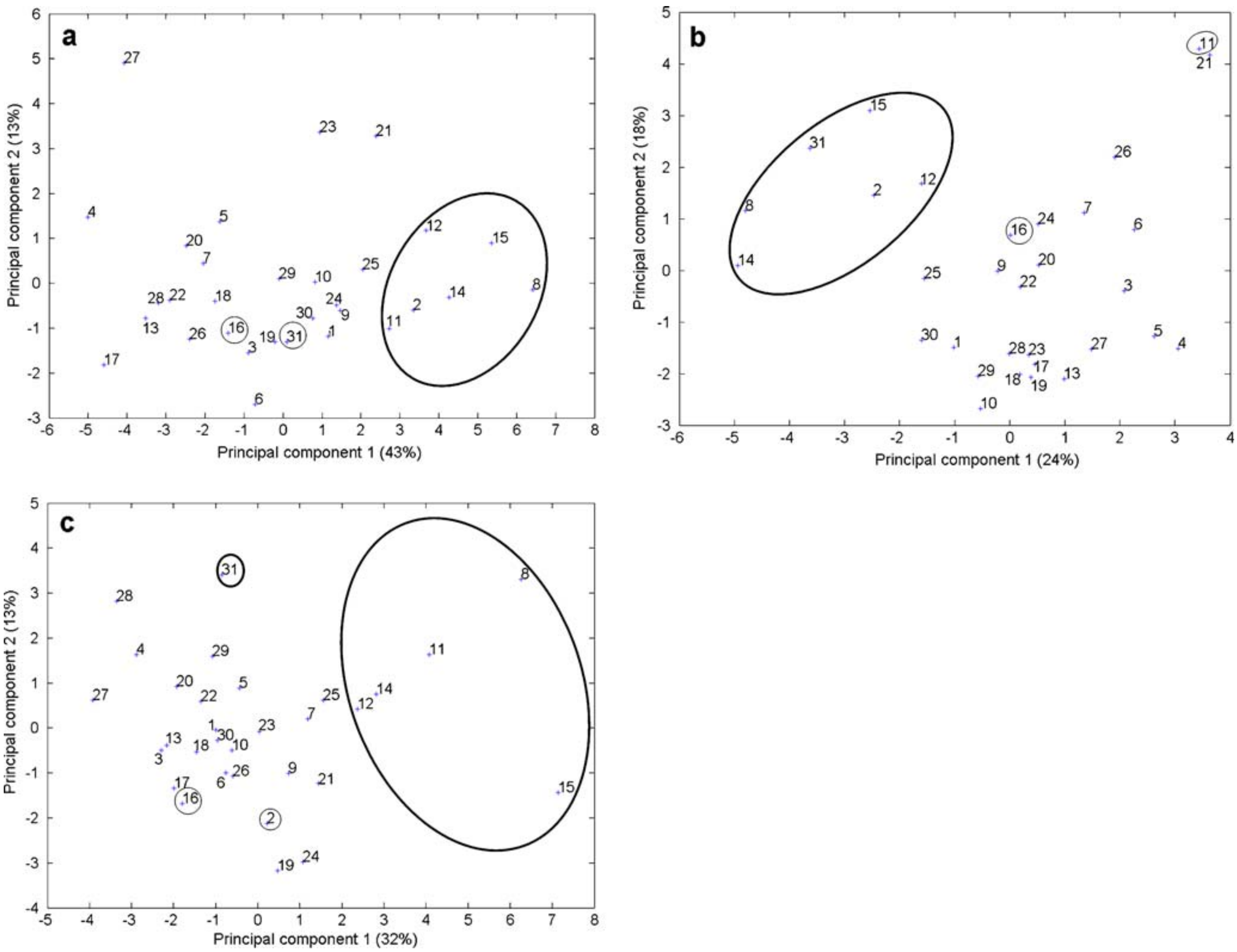


**Fig. 2** Principal component analysis of elemental composition of tillers from 31 switchgrass accessions at maturity (**a**), after senescence (**b**), and of element remobilization efficiencies (**c**). The *numbers in the figure* are accession IDs. Lowland accessions are indicated by *encircled numbers*. *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.

**Acknowledgments** This work was done under the auspices of the BioEnergy Science Center, a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science, and the US National Science Foundation, Plant Genome Research program (DBI-070119) to DES. We are grateful to Dr. Jiyi Zhang and Yi-Ching Lee for help in plant harvesting. The field nursery was established with funding from Ceres, Inc., Thousand Oak, CA, USA.

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