

All Washed Out? Foliar Nutrient Resorption and Leaching in Senescing Switchgrass

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Abstract Ideal bioenergy feedstocks are low in nutrients that act as anti-quality factors during conversion processes. Research has shown that delaying harvest of temperate perennial grasses until late winter reduces nutrient content, primarily due to end-season resorption, but also indicates a role for foliar nutrient leaching. While end-season resorption has been estimated, foliar nutrient leaching has not, and is a factor that could refine harvest recommendations. Additionally, establishing a baseline of mineral loss during switchgrass senescence will improve our understanding of leaf-level nutrient resorption. Therefore, we applied simulated rainfall to replicated ($n = 5$) plots within a previously established switchgrass stand to determine if heavy precipitation can induce nutrient leaching in senescing, unharvested foliage. Hour-long simulated rainfalls of ~120 mm were applied every 2 weeks from early September to a killing frost in 2014 and 2015. Leaf samples were taken from the upper and lower canopy before and after simulated rainfalls and from no-rain controls and analyzed for elemental N, P, K, S, Mg, and Ca. Nutrient resorption estimates ranged from 33 to 82% in control plots. Comparison of rainfall plots to controls indicated that lower canopy leaves, upon reaching $\geq 50\%$ senescence, were slightly susceptible to foliar nutrient leaching, with losses ranging from 0.3 to 2.8 g kg⁻¹

dry matter for K, P, and Mg. Nitrogen, Ca, and S were not susceptible to foliar leaching. Although statistically significant ($P \leq 0.05$), these values suggested that foliar leaching was not a strong driver of nutrient loss during senescence.

Keywords Biomass crop · *Panicum virgatum* L. · Translocation · Delayed harvest · Biomass quality · Throughfall

Introduction

Switchgrass as Biomass Feedstock

Perennial grasses bring both production and conservation benefits to diversified farming systems [1, 2]. When grown for bioenergy, these grasses can provide a high-yielding feedstock for solid and liquid fuel applications [3, 4]. A sustainability advantage of perennial grasses is their capacity to recycle and store mineral nutrients and carbohydrates over dormant periods (e.g., winter or dry seasons), thus making for a nutrient efficient, low-input, bioenergy crop [5–7]. These traits, in addition to broad geographic adaptation, make switchgrass (*Panicum virgatum* L.) a leading biomass crop [8, 9]. In the 1980s, the US Department of Energy identified switchgrass as a model bioenergy species and, since then, research has focused on increasing yield and improving its harvested biomass characteristics [7, 10]. A particular area of interest is reducing mineral nutrient content of the harvested biomass to improve crop sustainability and increase conversion efficiency [3, 11, 12].

Biomass Quality—Mineral Nutrients and Avoiding them

Bioenergy grass breeding programs around the world strive to maximize yield while minimizing residual mineral composition

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of the harvested biomass [10, 13, 14]. Biomass should be low in mineral nutrients because excess amounts can cause complications and inefficiencies in both biochemical and thermochemical conversion processes [15–17]. Many of the essential mineral nutrients required by plants, however, remain in the aboveground biomass after senescence, even in naturally efficient perennial C₄ grasses [18]. While genetic improvement approaches are underway to develop more efficient cultivars, the market for biomass crops is currently small and uncertain, which slows widespread adoption of new varieties [10]. Thus, it is prudent to develop alternative methods to reduce mineral nutrients in biomass, either through changes in agronomic management or pre-treatment of biomass prior to conversion [4, 11, 16].

Although it comes with tradeoffs, one of the seemingly easiest and most cost-effective solutions to reducing residual mineral nutrient content in perennial grasses grown in temperate regions is to delay harvest several months after senescence until late winter or early spring. This approach takes advantage of end-season nutrient resorption (often broadly referred to as translocation) to belowground tissues, as well as the somewhat unexplained, but empirically demonstrated, nutrient loss that occurs when biomass is left standing in temperate areas with a cold winter [6, 8, 16]. While delayed harvest can lead to yield losses from leaf drop and lodging, it typically provides cleaner feedstock, reduces nutrient removal from the field, and provides ecosystem services associated with winter cover [1, 6, 8].

Nutrient Movement and Loss Processes

Prior to complete end-season senescence, resorption (i.e., nutrient translocation to belowground storage tissues) is often indicated as the main driver of nutrient loss from aboveground perennial grass biomass [19–21]. Here, we use the term “end-season resorption” to connect bioenergy research with more recent ecology literature, thus advancing a more nuanced understanding of the differences between resorption and the broader term translocation [20, 22–24]. Further, we are concerned only with end-season senescence of aboveground tissues and do not address other forms of senescence like those that occur during programmed cell death or plant mortality [25]. A functioning phloem is required for the active process of resorption to occur [26, 27]. Therefore, once the aboveground biomass is fully senesced, only passive forms of nutrient loss can occur. Several delayed harvest studies in perennial grasses have found a reduction in biomass nitrogen (N), phosphorus (P), and potassium (K) concentrations, resulting in a decrease in the ash concentration of the harvested biomass [8, 16, 28]. It is currently unclear, however, whether magnesium (Mg), calcium (Ca), or sulfur (S) are similarly reduced by a delayed harvest.

In addition to resorption, a passive process that is assumed to contribute to this additional mineral nutrient loss over winter in delayed harvest studies is biomass nutrient leaching [7, 28, 29], also referred to as throughfall in studies of woody perennials and

field or biomass leaching in perennial grass studies. Biomass nutrient leaching is defined as the passive loss of organic and inorganic nutrients from any aboveground tissue upon extended wetness (such as a rainy day) and it increases in intensity with the age of the affected tissue [22, 30, 31]. This process is most notable in leaf tissue, which typically contains higher nutrient concentrations than stems in perennial energy grasses [6, 32, 33]. Senescence and its subsequent loss of membrane integrity within plant cells exacerbate biomass leaching [30]. While the exact mechanism for biomass nutrient leaching is unknown, it is likely similar to foliar nutrient uptake [34], as saturated cuticles and/or degraded membranes allow movement of otherwise precluded molecules to occur.

Objective 1: Develop Nutrient Concentration Baselines and Resorption Efficiency Estimates in Senescing Switchgrass Leaves

A nuanced understanding of the degree to which macronutrients are lost from aboveground biomass during annual, end-season senescence in temperate climates has not yet been developed, but is necessary to inform agronomic and harvest management decisions in perennial grasses grown for biomass purposes [8]. Leaf tissues contain higher concentrations of nutrients than stems and are a logical target for assessing nutrient loss potential in switchgrass. Before biomass nutrient leaching can be assessed, however, a baseline of leaf nutrient concentration changes during senescence (which may be largely attributed to resorption) must be developed. Therefore, our first objective was to develop such baselines and use them to calculate leaf-level resorption efficiency, a metric with bearing on whole-plant nutrient use efficiency. As recently highlighted by Vergutz et al. [20] and previously demonstrated by Van Heerwaarden et al. [23], calculations of nutrient resorption efficiency should be corrected for senescence-related losses in leaf area and mass by utilizing a correction factor in order to prevent underestimation. Based on current understanding, we hypothesized that phloem mobile macronutrients (N, P, S, K, and Mg) would initially decrease in actively senescing leaves and then appear to level off after complete leaf senescence. For a non-mobile mineral nutrient (such as Ca), we hypothesized concentrations would remain constant or increase (in relative terms) as other compounds are exported from the leaves during senescence.

Objective 2: Quantify Foliar Nutrient Leaching under Simulated Rainfall in Senescing Switchgrass Leaves

While biomass nutrient leaching has been demonstrated to be of importance in some species [35–37] and hinted to be important in senescing switchgrass [4, 6, 8], no studies have yet specifically investigated precipitation effects on undamaged (not cut or conditioned) perennial grass biomass grown in

temperate climates. Therefore, our second objective was to assess whether simulated rainfall would induce leaf-level biomass leaching (foliar nutrient leaching) in actively senescing switchgrass during the early fall season. We hypothesized that foliar nutrient leaching would occur with simulated rainfall and would increase as senescence progressed.

Materials and Methods

Rainfall Simulation Overview

In the fall of 2014 and 2015, simulated rainfall was applied to five plots in a mature field of switchgrass using an outdoor, portable simulator (Fig. 1). Five control plots did not receive simulated rain. Leaf samples were taken from all plots and analyzed for macronutrient mineral concentrations before and after “rainfall” or “no rainfall” treatments. Simulated rainfall events were spaced approximately 2 weeks apart and occurred over the course of 2 months, during which the switchgrass aboveground tissues completed senescence, as indicated by disappearance of green tissue prior to a killing freeze.

Study Site and Climate

The experiment was conducted at the Iowa State University’s (ISU) Woodruff Research Farm in Boone County, Iowa (41° 59′ 10.0″ N; 93° 41′ 26.8″ W). A 0.5-ha field of “Cave-In-

Rock” switchgrass was established in 2009, received broadcast applications of urea at 56 kg N ha⁻¹ every spring, and was harvested annually following senescence. Soils were a mixture of fine-loamy, mixed, superactive, calcareous, mesic, Typic Endoaqualls and fine-loamy mixed superactive, mesic, Typic, and Aquic Hapludolls (Canisteo and Nicollet series) [38]. The stand was healthy and produced an average annual yield of 5.8 Mg ha⁻¹. Weather information was obtained from the ISU Agronomy and Agricultural Engineering Farm approximately 10 km from the experimental site [39].

Experimental Design

The experiment was organized as a completely randomized design ($n = 5$) with repeated measures over five sampling dates and replicated for two growing seasons. Five plots received simulated rainfall with five additional plots serving as no-rain controls. All plots were exposed to natural rainfall that occurred throughout the season. Treatments were randomly assigned to the ten plots at the beginning of each season and new plots were chosen each year (i.e., 2014 plots were not reused in 2015). The plots were spaced 3 m apart and 1.5 m inward from the edge of the field. The plots were sized to accommodate the rainfall simulator at 2.5 by 3 m. Simulated rainfall treatments were applied on five dates between September and November of 2014 and 2015 (Fig. 2), coinciding with maximum biomass and the beginning of post-anthesis senescence of switchgrass. Date of anthesis (August 1) was determined as the date at which at least 50% of the switchgrass at the experimental site was in anthesis.

Rainfall Simulator

Rainfall was applied in situ to standing switchgrass using a portable simulator similar in function and design to that described by Miller [40] (Fig. 1). Briefly, the simulator was constructed of an aluminum frame with two solenoid-operated nozzles (Spraying Systems Inc. Model Number ½-HH-SS-30WSQ) centered 3 m above the soil surface to consistently deliver 120 mm of simulated rain in 1 h over the 6 m² sampling area within the 7.5 m² plots. Water was pumped to the simulator from external water tanks. Upwind tarps were used around the simulator on days with wind speeds exceeding 8 km h⁻¹ to minimize drift. Four rain gauges were placed above the crop canopy in two randomly assigned simulated rainfall plots to assess uniformity of the simulated rainfall. Water was obtained from the local rural water supply (Xenia Rural Water Supply, Ames, IA) and had a pH between 7 and 8. In order to better mimic slightly acidic Midwest rainfall, solid carbon dioxide (dry ice) was added to each water tank the day before rainfall simulations to achieve a pH between 5.5 and 6.5, which more closely resembles that of rainwater. In 2015, water samples before and after addition of dry ice were analyzed

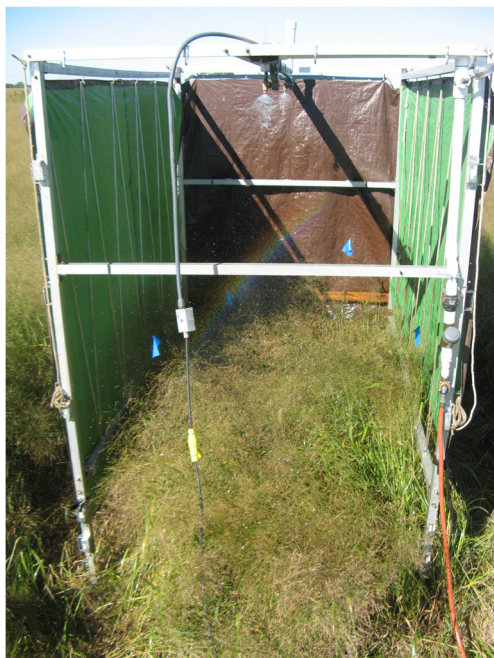


Fig. 1 In situ rainfall simulator in an experimental switchgrass plot in Boone County, IA. Note the tarps on three sides used to minimize drift and increase rainfall uniformity

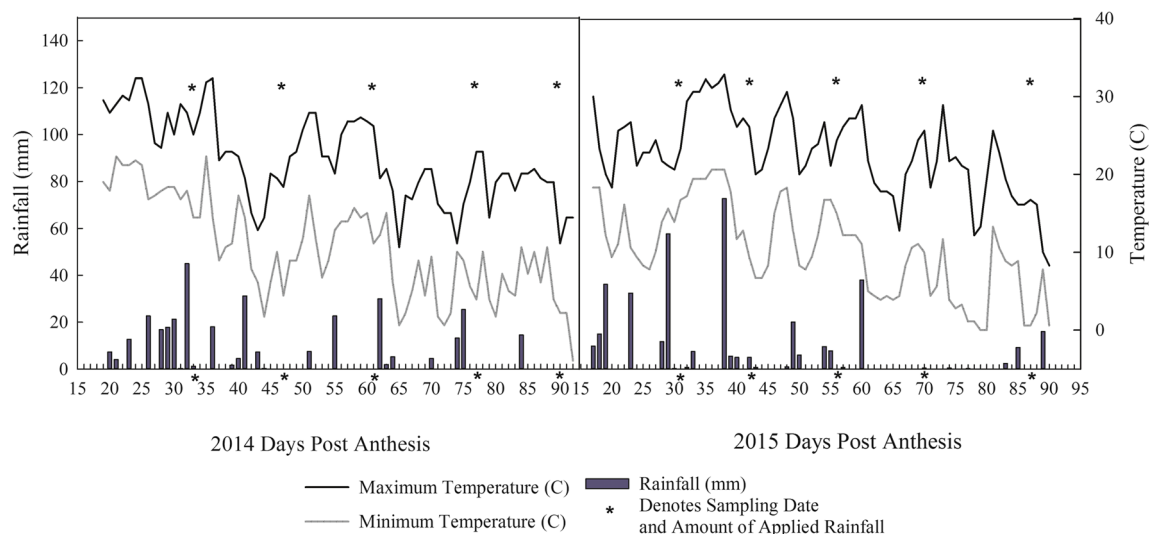


Fig. 2 Daily weather conditions during the fall season sampling periods in 2014 and 2015. Date of anthesis, August 1, is the date by which 50% or more of the switchgrass in the experimental site was in anthesis. Asterisks denote sampling dates and amount of simulated rainfall applied (120 mm) in each event

for mineral content in order to ensure that nutrients were not inadvertently being applied to the experimental plots.

Leaf Sample Collection and Analysis

Leaf samples were taken immediately before and after the 1-h simulated rainfalls or in the case of the control plots, before and after 1-h time lapse with no simulated rainfall. In both cases, samples were labeled “time 0” and “time 60.” Plots were divided into four equal quadrants, and five leaves (lamina only, excised at the ligule) were randomly collected from the upper and lower canopy positions in each quadrant for a total of 40 leaves collected at each time point, and 80 leaves collected from each plot, on every sampling date. Upper canopy position was defined as the topmost two leaves and lower canopy position was defined as anything lower than the topmost two leaves. All leaves were sampled from the interior of the plots at least 0.3 m from the edges.

Leaf samples were composited by canopy position and plot on each sampling date, producing four combined leaf samples per experimental unit (plot) per date: an upper and lower canopy sample taken at times 0 and 60. In order to estimate the progression of seasonal senescence, each combined leaf sample was visually assessed for percent green leaf area and assigned a level of 0, 25, 50, 75, or 100%. The samples were collected in paper envelopes and placed in a forced air dryer at 60 °C for 48 h. After drying, the leaves were ground in a cyclone sample mill (UDY Corp., Fort Collins, CO, USA) to pass through a 1-mm screen and analyzed for mineral nutrient content by Midwest Laboratories (Omaha, NE, USA). Nitrogen concentration was determined via combustion analysis in a LECO 6 analyzer (LECO Corp., St. Joseph, MI, USA) and P, K, S, Ca, and Mg concentrations were determined by inductively coupled plasma

atomic emission spectroscopy analysis following nitric acid digestion. For all analyses, data were reported as the percent macronutrient concentration per unit plant dry matter.

Data Analysis

All data were analyzed using linear models with either the PROC GLM or PROC MIXED procedures of SAS (SAS 9.4, SAS institute Inc., Cary, NC, USA). Canopy position and year were analyzed separately. Main effect significance was determined at $\alpha = 0.05$.

Objective 1: Baseline Estimates

Data from the control (non-rainfall) plots were used to develop nutrient concentration baselines. This was done using analysis of variance in PROC GLM to determine if leaf macronutrient concentrations changed significantly between times 0 and 60 (without rain). No difference was found for any date or macronutrient. Therefore, times 0 and 60 data were pooled for each plot on each date, and the averages used to develop temporal baselines of switchgrass macronutrient concentration changes from anthesis through senescence. To assess the change in baseline levels over time, these data were analyzed by year and canopy position using analysis of variance in PROC MIXED with date as a fixed effect and replicated plots as a random effect. An estimate of the difference between the macronutrient concentration on the initial sampling date and the final sampling date was calculated for each macronutrient and significance determined at $\alpha = 0.05$ using an estimate statement *t* test. Additionally, in order to determine whether a baseline trend was increasing, decreasing, or remaining the

same, modified orthogonal polynomial contrasts were used to assess whether a significant linear trend was visible over the 2-month sampling period for each mineral nutrient with significance determined at $\alpha = 0.05$. Leaf-level resorption efficiency estimates were calculated using the mass loss correction factor established by Vergutz et al. [20] in the following manner:

$$\text{NuR} = \left(1 - \frac{\text{NuS}}{\text{NuG}} 0.713 \right) \times 100$$

where NuR is nutrient resorption efficiency, NuS is nutrient concentration of senesced leaves (final sampling date), NuG is nutrient concentration of green leaves (initial sampling date), and 0.713 is the graminoid mass loss correction factor. A mass loss correction factor is calculated as the ratio of dry mass of senesced leaves to dry mass of green leaves. The factor used in this study and generated by Vergutz et al. [20] is an average value calculated from a review of multiple perennial grass studies. Therefore, leaf-level resorption efficiency can be interpreted as the degree to which a nutrient is resorbed from aboveground leaf tissues (and presumably shuttled to storage tissues) while simultaneously correcting for the mass and area loss that occurs in leaf tissue during senescence. Mass and area are lost during senescence because other leaf constituents are leaving the leaves at the same time as the nutrient in question.

Objective 2: Foliar Nutrient Leaching

Times 0 and 60 data from rainfall plots were compared with baseline data separately over treatment and date. Time 60 data was compared to determine if an hour of simulated rainfall induced detectable foliar nutrient leaching within a given sampling date (short-term effects). Time 0 data was compared to assess whether a cumulative effect of simulated rainfall on foliar macronutrient concentrations could be determined across the 2-month period. Data were analyzed by macronutrient, year, and canopy position with repeated measures. Treatment was considered categorical and consisted of either simulated rainfall or no rainfall (baseline values). Sampling date was treated as a fixed effect occurring in 2-week intervals starting at the beginning of September. Because the experimental units (plots) differed in both field placement and treatment each year, they were nested within treatment. Equal variance assumptions among sampling dates were tested and best-fit covariance models were chosen following Littell et al. [41]. Interaction effects, consisting of the difference between baseline and rainfall plot means on each sampling date, were estimated using the slice statement in PROC MIXED with significance determined at $\alpha = 0.05$.

Results

Weather

Total precipitation during the measurement period (235 mm in 2014; 219 mm in 2015) was greater than the 30-year average of 145 mm and was roughly a third of the 600 mm applied in the five 120-mm simulated rainfalls. Monthly temperatures were average and ranged from 18 to 12 °C in 2014 and 20 to 12 °C in 2015 (Fig. 2).

Objective 1: Baseline Estimates

Three distinct trends were noted in the baseline macronutrient concentrations of senescing switchgrass leaves over time (Figs. 3 and 4). Two trends were similar for both canopy positions: certain nutrient concentrations either (1) did not change over the five sampling dates or (2) they appeared to increase. For example, Mg concentrations did not change significantly across either year, while Ca concentrations appeared to increase in both canopy positions, although this was not consistent across years. Alternatively, (3) concentrations for N, P, K, and S declined over time, a trend that differed depending on canopy positions.

In the upper canopy leaves, N, K, P, and S concentrations continued to decline during the sampling period for both years (Figs. 3 and 4). Averaged over the 2 years, upper canopy concentrations for N decreased by 6.4 g kg⁻¹ (58%), K by 5.3 g kg⁻¹ (74%), P by 0.9 g kg⁻¹ (53%), and S by 0.3 g kg⁻¹ (43%) (Table 1). The mineral concentrations in the lower canopy leaves tended to follow similar trends as their upper canopy counterparts, but leveled off during the experimental period. While significant decreases were found between the first and final sampling date, N, K, P, and S concentrations leveled off rather than continuing to decline over the entire sampling period (Figs. 3 and 4). Averaged over the 2 years, lower canopy N concentrations decreased by 2.7 g kg⁻¹ (47%), while K decreased by 6.0 g kg⁻¹ (82%), P by 1.1 g kg⁻¹ (63%), and S by 0.2 g kg⁻¹ (43%) (Table 1).

For both upper and lower canopies, the initial sampling date in both years corresponded with the most green leaf tissue and the final sampling date corresponded with completely senesced leaf tissue. Therefore, for each year, canopy, and baseline plot, NuR estimates were calculated using the initial and final sampling date nutrient concentrations. After accounting for average leaf mass loss, estimates of resorption efficiency in the upper canopy leaves were 58% N, 74% K, -1% Ca, 53% P, 43% S, and 33% Mg during the experimental period in the absence of simulated rainfall (Table 1). Lower canopy leaf resorption efficiency estimates were 47% N, 82% K, 16% Ca, 63% P, 43% S, and 36% Mg from the initial leaf levels (Table 1).

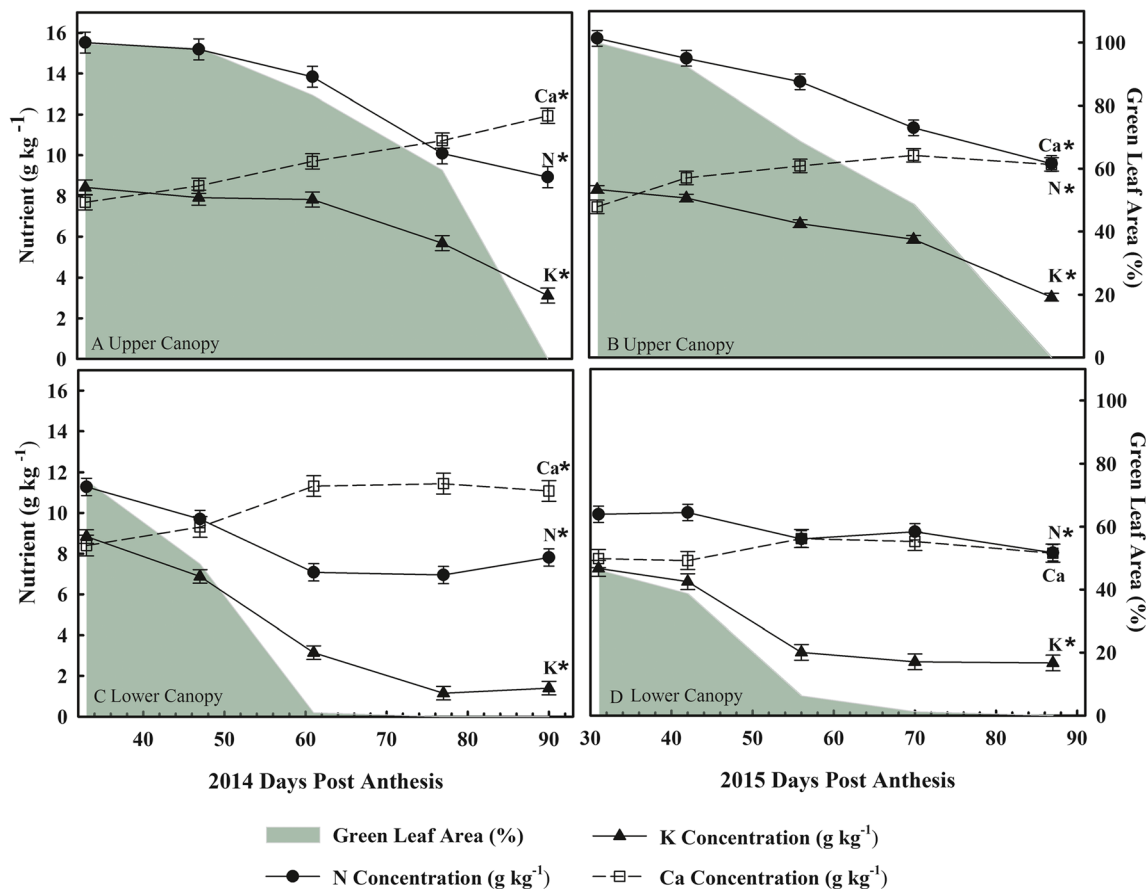


Fig. 3 Baseline leaf nutrient concentration (N, K, Ca) changes over time in post-anthesis switchgrass grown near Boone, IA. Upper canopy mineral nutrient concentrations for 2014 (a) and 2015 (b) sampling periods, respectively. Lower canopy concentrations for 2014 (c) and 2015 (d)

sampling periods, respectively. Asterisks next to each baseline denote the presence of a significant linear trend for that mineral nutrient. Shaded area in each graph represents percent green leaf area

Objective 2: Foliar Nutrient Leaching

The potential for foliar nutrient leaching was determined by assessing short-term and cumulative differences in leaf nutrient concentrations between the plots that received simulated rainfall and the no-rain control plots used to calculate baseline values. The presence of short-term effects was determined by assessing the significance of the differences between the baseline values and time 60 rainfall plot values on each date with significance determined at $\alpha = 0.05$ (Table 2). Significant short-term effects were only noted in some years and for some elements, and occasionally did not persist from one sampling date to the next. Fewer significant effects were found in upper canopy than in lower canopy leaves. In the upper canopy, short-term effects from the addition of simulated rainfall were only found towards the end of the sampling period in 2015. On the fourth sampling date, S concentration increased minimally, by 0.1 g kg^{-1} . On the fifth sampling date, K concentration decreased by 0.7 g kg^{-1} and Mg by

0.3 g kg^{-1} , while Ca concentration increased by 1.0 g kg^{-1} . Nitrogen concentration in the upper canopy did not differ significantly from the baseline averages for either year.

In the lower canopy, short-term effects of simulated rainfall were more common. Significant differences were found for K and Ca in both years, S and P in 2014, and Mg in 2015. On the second and third sampling date in 2014, K concentrations from lower canopy leaves receiving rainfall were an average of 2.8 and 1.3 g kg^{-1} lower than baseline values, and on the fourth and fifth sampling dates of 2015, they were 0.9 and 1.3 g kg^{-1} lower, respectively. On the second sampling date in 2014, P concentrations from lower canopy leaves in the rainfall plots were an average of 0.6 g kg^{-1} lower than baseline values. Magnesium concentrations in the rainfall plots on the fourth and fifth sampling dates of 2015 were 0.3 and 0.4 g kg^{-1} lower than the respective baseline averages for those days. Interestingly, the significant short-term effects found for S and Ca were negative, indicating that average concentrations were higher in the rainfall plots

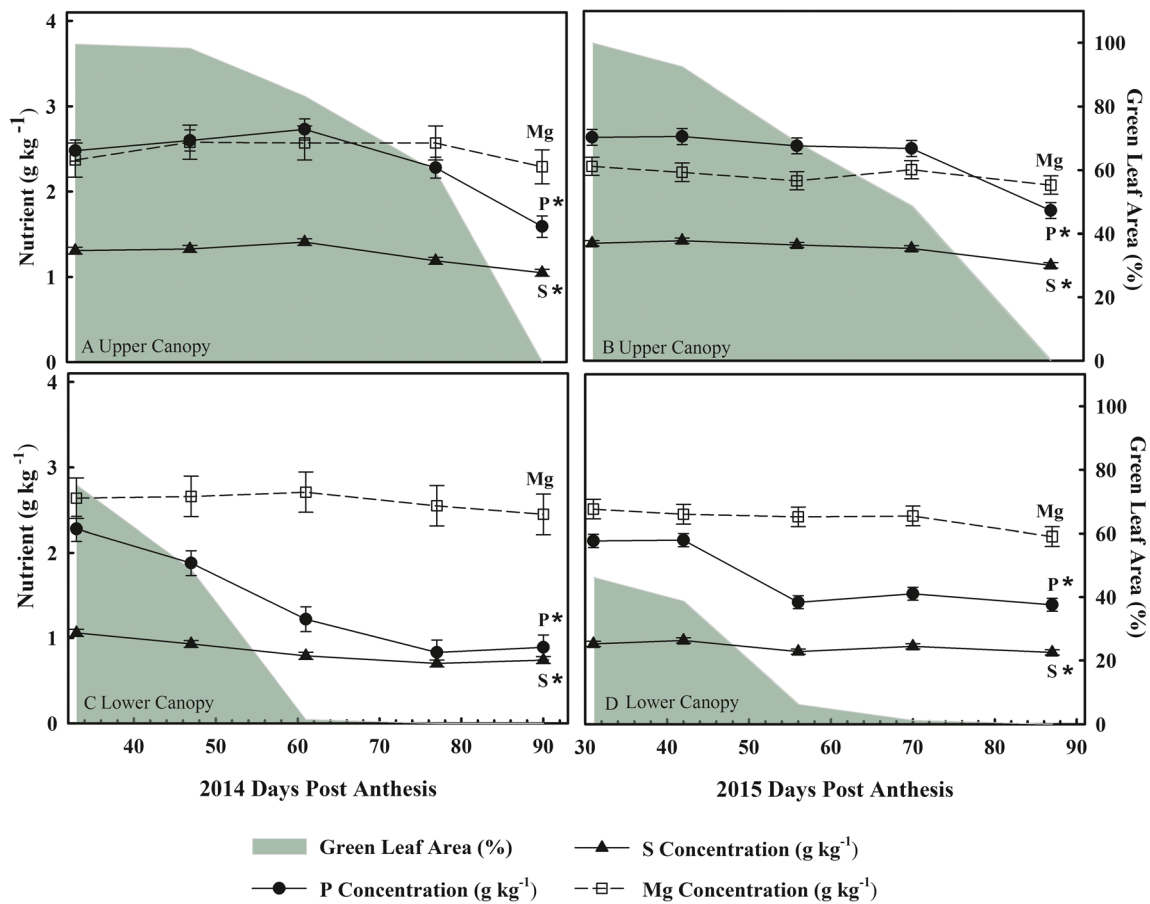


Fig. 4 Baseline leaf nutrient concentration (P, S, Mg) changes over time in post-anthesis switchgrass grown near Boone, IA. Upper canopy mineral nutrient concentrations for 2014 (a) and 2015 (b) sampling periods, respectively. Lower canopy concentrations for 2014 (c) and 2015 (d)

sampling periods, respectively. Asterisks next to each baseline denote the presence of a significant linear trend for that mineral nutrient. Shaded area in each graph represents percent green leaf area

than the baseline averages. On the fourth and fifth sampling dates of 2014, S concentrations in the rainfall plots were an average of 0.1 g kg^{-1} higher than the baseline averages for those days. On the second sampling date in 2014, Ca concentrations in the rainfall plots were an average of 1.7 g kg^{-1} higher than the respective baseline value and in 2015, Ca concentrations on the third, fourth, and fifth sampling dates were an average of 1.5, 1.3, and 2.1 g kg^{-1} higher than the respective baseline averages. Nitrogen concentrations in the lower canopy did not differ significantly from the baseline averages in either year.

Time 0 data from rainfall plots were compared to the baseline values to assess if there was a cumulative effect of simulated rainfall on foliar macronutrient concentrations (data not shown). In other words, potential cumulative effects were assessed by subtracting the rainfall plot time 0 means from baseline means on each date and testing the significance of this difference ($\alpha = 0.05$) (data not shown). Few and sporadic cumulative effects were seen and their magnitude was minimal compared to values found in the time 60 analysis.

Discussion

Objective 1: Baseline Estimates

To separate changes in nutrient concentration associated with foliar nutrient leaching from those associated with other end-season processes, notably nutrient resorption, we first had to develop a baseline of the temporal changes in leaf nutrient concentrations in the absence of simulated rainfall. Our observed macronutrient baseline changes during senescence can largely be explained by the potential phloem mobility of the nutrient in question.

In order for a mineral nutrient to be susceptible to resorption, it must be phloem mobile, although this does not guarantee that a nutrient will be resorbed during senescence [26, 27]. Additionally, the very nature of end-season senescence eventually inhibits active resorption due to phloem tissue breakdown [22, 25]. Phloem mobile nutrients include N, P, K, Mg, and S while Ca is considered immobile due to its significant structural role as a constituent of pectin polysaccharide bonds between cell walls

Table 1 Estimates of baseline concentration differences (N, K, Ca, P, S, Mg) between the final sampling date and initial sampling date and respective *P* values. Average differences as well as estimates of nutrient resorption efficiency (NuR) are reported for each canopy across both years

Graph	Estimate $\Delta \text{Conc}_{(\text{final} - \text{initial})}$ (g kg ⁻¹ ± SE)	<i>P</i> value (Den DF 20)	Average (g kg ⁻¹)	NuR (% from leaf)
N 2014 upper canopy	-6.6 ± 0.7	<0.0001	-6.4	58
N 2015 upper canopy	-6.1 ± 0.5	<0.0001		
N 2014 lower canopy	-3.5 ± 0.6	<0.0001	-2.7	47
N 2015 lower canopy	-1.9 ± 0.6	0.0035		
K 2014 upper canopy	-5.3 ± 0.5	<0.0001	-5.3	74
K 2015 upper canopy	-5.3 ± 0.3	<0.0001		
K 2014 lower canopy	-7.4 ± 0.5	<0.0001	-6.0	82
K 2015 lower canopy	-4.6 ± 0.5	<0.0001		
Ca 2014 upper canopy	4.3 ± 0.5	<0.0001	3.2	-1
Ca 2015 upper canopy	2.1 ± 0.5	0.0003		
Ca 2014 lower canopy	2.7 ± 0.7	0.0013	1.5	16
Ca 2015 lower canopy	0.3 ± 0.6	0.6717		
P 2014 upper canopy	-0.9 ± 0.2	<0.0001	-0.9	53
P 2015 upper canopy	-0.9 ± 0.1	<0.0001		
P 2014 lower canopy	-1.4 ± 0.2	<0.0001	-1.1	63
P 2015 lower canopy	-0.8 ± 0.1	<0.0001		
S 2014 upper canopy	-0.3 ± 0.1	0.0002	-0.3	43
S 2015 upper canopy	-0.3 ± 0.0	<0.0001		
S 2014 lower canopy	-0.3 ± 0.1	<0.0001	-0.2	43
S 2015 lower canopy	-0.1 ± 0.0	0.0319		
Mg 2014 upper canopy	-0.1 ± 0.3	0.7806	-0.2	33
Mg 2015 upper canopy	-0.2 ± 0.2	0.1634		
Mg 2014 lower canopy	-0.2 ± 0.3	0.5772	-0.3	36
Mg 2015 lower canopy	-0.3 ± 0.2	0.0634		

[26, 30]. With the exception of Mg, the baselines established here for N, P, K, S, and Ca supported our first hypothesis that phloem mobile nutrients would display a significant decrease in concentration over the senescence period and phloem immobile nutrient concentrations would either remain constant or potentially appear to increase as other cell constituents were exported (Figs. 3 and 4). Magnesium did not appear to change in concentration over the 2-month sampling period in either year. While this appears to contradict Mg's phloem mobility, similar results have been observed in mineral resorption studies conducted with other plant species [26, 42, 43]. White [26] suggested that an ample supply of Mg in the soil solution precludes a pressure flow gradient in the phloem to spur resorption in response to sink demands. It is also possible that due to its large, hydrated ionic form, Mg is an energetically expensive nutrient to move across increasingly weakening leaf cellular membranes at the end of the season [44]. Additionally, a portion of the Mg pool is permanently tied up within pectin between cell walls [45]. Most likely, all three factors jointly affected the relative end-season immobility of Mg.

Current cross-species estimates for nutrient resorption efficiencies average 60% for N and P and are more varied for other mineral nutrients [20, 23, 24]. Studies that specifically focus on total end-season resorption efficiency in perennial grasses are rare and have not reached consensus for the resorption of nutrients other than N. For instance, some studies

support the resorption of P [8, 21, 46] and others negate it [5, 47]. The same dichotomy of opinions has been found for K [20, 47–49]. One reason for this disagreement may be attributed to methodology, since leaf mass loss and area changes can affect how resorption efficiency is measured [23], which may cause estimates of resorption to be significantly lower than actual amounts. Vergutz et al. [20] quantified potential resorption efficiencies across several plant functional groups for five macronutrients and established a mass loss correction factor for each group that can be incorporated into future calculations of resorption. Their nutrient resorption efficiency estimates for N (74.6%), P (82.1%), K (84.9%), and Mg (39.8%) were similar to our calculated leaf-level estimates using the same correction factor whereas their estimate for Ca (32.5%) was much higher than ours (Table 1). Because we observed slight increases in baseline Ca concentration during both senescence seasons, our observed Ca resorption efficiency estimates were either negative (biologically unlikely) or very low. As previously explained, this was not unexpected due to Ca's phloem immobile nature. Additionally, the mass loss correction factor developed by Vergutz et al. [20] is a composite term from a broad range of grass species and may have under corrected for mass loss in this instance.

Another potential reason that dichotomies exist in the biomass literature regarding quantification of end-season

Table 2 Short-term effects. Analysis of variance and estimates of switchgrass leaf nutrient concentrations following 1 h (120 mm) of simulated rainfall on five dates. Model term probabilities include treatment (baseline or simulated rainfall), date, and the treatment by date interaction. Difference of the means was calculated by subtracting rainfall plot means from baseline plot means. Significant differences ($\alpha = 0.05$) between the baseline and rainfall plot means (baseline minus rainfall) are presented as the difference over all dates as well as the difference for each date. Non-significant differences are indicated by a dash sign

Upper canopy		N	2014	2015	P	2014	2015	K	2014	2015	Ca	2014	2015	Mg	2014	2015	S	2014	2015
Model term probabilities																			
Treatment			0.8904	0.4386	0.7812	0.5721	0.9057	0.196	0.6294	0.1776	0.5838	0.1499	0.585	0.2266					
Date			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001					
Treatment × date interaction			0.6763	0.5271	0.5736	0.8832	0.7948	0.4176	0.8168	0.4395	0.2521	0.3988	0.9533	0.0792					
Difference of the means (g kg ⁻¹)		N	2014	2015	P	2014	2015	K	2014	2015	Ca	2014	2015	Mg	2014	2015	S	2014	2015
Treatment (over all dates)			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Treatment (each date)																			
Sampling date 1			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sampling date 2			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sampling date 3			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sampling date 4			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sampling date 5			–	–	–	–	–	0.7	–	–1.0	–	–	–	–	–	0.3	–	–	–0.1
Lower canopy		N	2014	2015	P	2014	2015	K	2014	2015	Ca	2014	2015	Mg	2014	2015	S	2014	2015
Model term probabilities																			
Treatment			0.7932	0.3978	0.6485	0.6111	0.0014	0.0156	0.1389	0.0181	0.6406	0.1211	0.3377	0.21					
Date			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001					
Treatment × date interaction			0.2317	0.7062	0.0036	0.6379	0.0024	0.5718	0.4983	0.2291	0.15	0.1462	0.0734	0.9348					
Difference of the means (g kg ⁻¹)		N	2014	2015	P	2014	2015	K	2014	2015	Ca	2014	2015	Mg	2014	2015	S	2014	2015
Treatment (over all dates)			–	–	–	–	–	0.8	–	–1.2	–	–	–	–	–	–	–	–	–
Treatment (each date)																			
Sampling date 1			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sampling date 2			–	–	0.6	–	2.8	–	–1.7	–	–	–	–	–	–	–	–	–	–
Sampling date 3			–	–	–	–	1.3	–	–	–1.5	–	–	–	–	–	–	–	–	–
Sampling date 4			–	–	–	–	–	0.9	–	–1.3	–	–	–	–	–	0.3	–0.1	–	–
Sampling date 5			–	–	–	–	–	1.3	–	–2.1	–	–	–	–	–	0.4	–0.1	–	–

resorption is that some studies attempt to balance above-ground nutrient loss with belowground nutrient gain [29, 47, 50]. Resorption efficiency estimates are often based on leaf-level changes and do not indicate where the nutrients are going once they have exited the leaves [22]. Our focus here on leaf-level nutrient loss precluded quantification of total nutrient resorption via mass balance between above- and below-ground biomass for switchgrass, but the way our baseline leaf nutrient concentrations changed during senescence effectively supported resorption at some level. Future research in this area should focus on concomitant sampling of above and below-ground tissues in order to balance concentration changes of the macronutrients over time, while also refining a leaf mass loss correction factor for grasses in the calculations of resorption efficiency.

Objective 2: Foliar Nutrient Leaching

Varying significant differences were seen for short-term effects which indicate a real but small role for foliar nutrient leaching in senescing switchgrass leaves. Within these analyses, the majority of differences observed after exposure to five sequential 1-h, 120-mm simulated rainfalls were in lower canopy leaves (Table 2). This may be because lower canopy leaves were further along in the senescence process than upper canopy leaves and thus more vulnerable to leaching. Additionally, only a somewhat consistent trend was seen for K and Ca, which showed significant differences each year, while significant differences for P, Mg, and S were only detected in 1 year. Nitrogen was not observed to leach at all. While the differences found between the control plots and the rainfall plots were quite small, they were within detectable measurement ranges for the analysis techniques used.

The short-term differences seen between the rainfall plot and baseline concentrations for K, P, and Mg were consistent with the nature of their roles within plant cells. All three nutrients are transported to varying degrees throughout the plant in water-soluble forms, which may increase their vulnerability to leaching. Potassium is present in high concentrations in cytosol and phloem sap, is used for regulating osmotic potential, and remains in its ionic form throughout the plant [51]. Phosphorus is transported throughout a plant primarily as inorganic phosphate, a simple phosphate ester, or it attaches itself to another phosphate through high-energy pyrophosphate bonds [45]. Magnesium is transported in its ionic form and, while it does not appear to be highly resorbed, it may be mildly susceptible to foliar leaching due to increased solubility of ionic Mg. All significant differences observed for K, P, and Mg were positive differences, indicating that the baseline concentrations were higher than in rainfall plots. Furthermore, differences only occurred after greater than 50% visible senescence had occurred in both canopy positions in each year. This supported our hypothesis that leaching increased with senescence.

The lack of short-term effects observed for N and apparent short-term increases observed for S and Ca are also better understood with knowledge of their utilization within plants. Nitrogen is a highly internally conserved nutrient within perennial plants and it is possible that the only forms left in senescing leaves are those that are not susceptible to leaching, such as structural proteins. Sulfur is a component of larger-weight molecular compounds and is also a structurally incorporated nutrient. Neither of those traits lends itself to leaching vulnerability. As previously discussed, Ca is primarily incorporated between cell walls and is not likely vulnerable to leaching for this reason. Furthermore, studies have demonstrated that compounds other than mineral nutrients can leach from foliage due to extended wetness, including non-structural carbohydrates, amino acids, and other low-weight molecular compounds [30, 34, 37, 52]. Therefore, it is realistic to assume that concentrations of S and Ca appeared to “increase” in biomass exposed to simulated rainfall because the loss of other, non-measured, cell constituents was greater relative to the stability of S and Ca. Some N compounds may have been leaching, but at a rate concurrent with the loss of other cell constituents, which may have masked any significant difference between rainfall plots and baseline averages. Quantifying or identifying other leachable cell constituents, however, was outside the scope of this study.

Because only a few significant differences were observed for cumulative effects, it appeared that the simulated rainfalls did not have a lasting effect on the rainfall plots during the senescence seasons (data not shown). It is important to highlight that the significant short-term effects seen for Ca, S, P, Mg, and K occurred after at least 50% senescence of the leaves. Similar differences should have been seen in the cumulative effects towards the end of the senescence season, given that any upward remobilization of nutrients would have ceased after complete senescence of the phloem in the leaves. Such differences were not seen, although this could be due to the leaching effects of natural rainfall, which were not measured.

Other Factors Affecting End-Season Nutrient Loss

Given that leaf biomass was fully senesced by the last sampling date and only weak leaching effects were found, it seems unlikely that foliar nutrient leaching, and by extension biomass nutrient leaching, are significant drivers of nutrient loss during active senescence. Researchers that have cited biomass leaching as the cause for loss of mineral nutrients, however, usually sampled biomass shortly after a killing frost and then again, several months later or even in the spring [16, 49]. It may be that several freeze/thaw cycles are needed to appropriately lyse cells and cause enough internal damage to senesced tissue to leave mineral nutrients vulnerable to biomass leaching. Additionally, other passive nutrient loss processes were acting equally on control and rainfall plots, such as volatilization and microbial degradation of senesced tissue, both of which also increase with senescence but

are greatly reduced at cool temperatures [34, 53]. Finally, delayed harvest studies often cite nutrient changes on a total standing crop basis. It is perhaps more likely that overwinter physical changes such as leaf drop are influencing the change in mineral nutrient content from the fall to the spring [12, 54]. Attempts to quantify these nutrient loss processes are worthy avenues of future research.

Conclusions and Harvest Management Recommendations

From a practical point of view, the addition of simulated rainfall during active senescence did not appear to drive a significant loss of macronutrients from the leaves via foliar nutrient leaching. Instead, senescence-mediated macronutrient concentration changes in standing switchgrass biomass appeared to fit the narrative of phloem mobile and non-mobile nutrients. Changes in these concentrations over the course of senescence were likely due to resorption to stem or belowground storage tissues unless the mineral nutrient in question served a structural role, was bound within larger-weight molecular compounds, or was already present in high concentrations in the soil solution. In these cases, resorption may be delayed or inhibited altogether. Due to potential difficulties in harvesting biomass in late winter or early spring, and because foliar nutrient leaching is not likely to significantly alter the mineral nutrient concentration of the biomass, it may be best to harvest shortly after full senescence in order to avoid harvest difficulties due to wet ground and lodging of plant material. Future research should focus on determining what levels of mineral nutrients are acceptable in biomass for its target end use, and whether or not these levels are attained shortly after fall senescence. Additionally, switchgrass biomass should be studied over winter with an analysis of the different morphological parts to better understand how mineral content and mass are changing throughout the entire plant over multiple freeze/thaw cycles.

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