Quantifying senescence-mediated nutrient loss processes in switchgrass (Panicum virgatum L.) biomass

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Quantifying senescence-mediated nutrient loss processes in switchgrass (*Panicum virgatum* L.) biomass

by

**Ruth Helen Burke**

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE**

Major: Crop Production and Physiology

Program of Study Committee:
Emily Heaton, Major Professor
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Iowa State University
Ames, Iowa
2016

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DEDICATION

I would like to dedicate my thesis to my mother, Deanna. She has always believed in my academic ability and I love her more than anything in the world (yes, even more than Cheerios). I would also like to dedicate this thesis to my father, Jonathan, who probably would have gotten a real kick out of all of this.
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ABSTRACT

The annual senescence that occurs in many perennial grass species is not a terminal event, and multiple internal processes are occurring as a plant enters winter or dry-season dormancy. When grown as bioenergy feedstocks, end-season nutrient conservation and loss processes taking place in perennial grasses are of interest to producers attempting to maximize yield while reducing residual nutrient content in the harvestable biomass. The residual nutrient content in these bioenergy feedstocks can reduce conversion efficiency, damage biorefinery equipment, and even cause pollution. In order to better understand nutrient cycling in perennial grasses as well as improve harvest management decisions for bioenergy feedstock producers, I quantified three nutrient loss processes in the model perennial grass species *Panicum virgatum* L.: 1) end-season nutrient resorption, 2) biomass nutrient leaching, and 3) mass loss due to overwinter leaf drop (litterfall). Over two autumn seasons in 2014 and 2015, I established a baseline of macronutrient reduction in senescing, undamaged *in situ* switchgrass plots and compared those baselines to plots exposed to heavy simulated rainfall in order to quantify potential foliar nutrient leaching. I found that leaf-level resorption may drive the bulk of phloem mobile nutrient (i.e., N, P, K) reduction from aboveground biomass during senescence, but phloem immobile nutrients (i.e., Ca) tend to remain behind in the standing biomass. From a practical point of view, foliar leaching was not observed to be a significant driver of nutrient loss during senescence. During the winter of 2015 – 2016, *in situ* switchgrass biomass samples were harvested monthly from undamaged stands. The harvested material was analyzed by aboveground morphological component (stem, leaf, panicle) for mass and macronutrient content to quantify nutrient
losses and the passive process driving them. I observed that losses of non-leachable nutrients (i.e., N and Ca) were primarily due to overwinter leaf drop and losses of the more water-soluble nutrients (i.e., K and P) were primarily due to biomass leaching. These studies will improve knowledge of end-season nutrient cycling in perennial grasses and help inform management decisions for producers seeking to diversify their operations with switchgrass for bioenergy.
CHAPTER 1: GENERAL INTRODUCTION

Introduction

Perennial grasses have several important roles when included in diversified farming [1-3]. Not only do they contribute positively to ecosystem services such as soil protection, water regulation and habitat for wildlife, they also provide provisioning services like livestock forage and bioenergy feedstock [4,5]. Switchgrass (*Panicum virgatum* L.) is a perennial, warm season, C₄ grass species native to North America that has been developed for conservation, forage, and more recently, has become a leading biomass crop for bioenergy, biofuels, and bioproducts. Its broad geographic and environmental adaptation along with favorable agronomic traits, including good monoculture yields and a smooth, hard seed coat, made the US Dept. of Energy choose switchgrass as a model biomass crop in 1991 [6]. Since then switchgrass has been researched and commercialized with consistent public and private support. It is a target feedstock in the US Dept. of Agriculture (USDA) Regional Biomass Research Centers [7] and US Dept. of Energy Bioenergy Research Centers. Despite these efforts, however, key questions remain about the biology of switchgrass and related species. Of particular ecophysiological interest are nutrient dynamics, since they underpin both technoeconomic and environmental sustainability of perennial grasses grown in temperate environments.

In this thesis, I will describe work undertaken to elucidate active and passive nutrient movement processes in aboveground tissues of switchgrass, with broader implications for related species grown in temperate environments. In this introductory chapter, I will review nutrient movement literature to provide background and rationale for this research, as well as
present specific research questions and objectives to be addressed in the subsequent research chapters.

**Background**

An advantage of perennial grasses grown in temperate environments is their ability to move mineral nutrients and carbohydrates from above- to belowground tissues before winter, making them ideal, low-input, bioenergy crops when harvested after senescence [8-10]. Unfortunately, many of the essential mineral nutrients required by all plants to successfully grow and reproduce remain in aboveground tissues after senescence, even in perennial grasses [11]. This is problematic for two reasons: 1) it is important to keep nutrients in the field for next year’s growth, and 2) nutrients can cause complications and inefficiencies in the conversion of biomass to bioenergy [12-14]. Considerable work has therefore been done to reduce nutrients in harvested biomass, whether through developing varieties best suited for bioenergy production, changes to agronomic management, or pre-treatment of biomass prior to conversion [12,15,16]. A simple solution to reduce biomass nutrient content in temperate climates is to delay harvest [4,16]. Delaying harvest until late winter or early spring can lead to yield losses due to lodging and potential harvest difficulties depending on the year and latitude, but it also provides cleaner feedstock, reduces nutrient take-off from the field, and provides ecosystem services associated with winter cover [4,9]. The mechanism by which delayed harvest leads to cleaner biomass, however, is not entirely clear, but depends on a combination of active and passive processes that I will now briefly review.

**Senescence, nutrient movement, and nutrient loss**

Switchgrass is a perennial plant in which aboveground tissues senesce annually. Thomas [17] describes senescence as a developmental stage “absolutely dependent on cell
viability”; Thomas and Stoddart [18] describe leaf senescence in particular as “the series of events concerned with cellular disassembly in the leaf and the mobilization of materials released during this process”. In a global review of the topic, Aerts [19] explains that nutrient resorption (and reuse) is a major nutrient conservation mechanism with “important implications at both the population and ecosystem level”, but data is scant on grasses – most is on shrubs and trees. Further, most bioenergy crops will be grown in managed ecosystems, typically with fertilizer inputs. Therefore, is nutrient resorption important for bioenergy grass nutrient budgets? And further, why does delayed harvest result in biomass with lower residual nutrients? Do nutrients move in a meaningful way after tissues senesce?

Prior to aboveground senescence, downward nutrient translocation to belowground storage tissues (also known as end-season nutrient resorption) is often indicated as the main driver of nutrient loss from aboveground perennial grass biomass [20,21,9]. However, current literature disagrees on whether nutrients other than nitrogen (N) and simple photosynthates are shuttled to belowground tissues prior to winter or a dormant period [22-24]. Additionally, as pointed out by Thomas [17], functioning phloem is required for the active process of end-season resorption to occur [25,26]. Once the aboveground biomass is fully senesced, only passive forms of nutrient loss can occur. Delayed harvest studies have found reductions in both concentration (g kg\(^{-1}\)) and content (kg ha\(^{-1}\)) of biomass N, phosphorus (P) and potassium (K) [4,16,27] as well as a reduction in the resultant ash upon biomass pyrolysis [12,28].

A passive process that is assumed to cause additional mineral nutrient losses over winter in many delayed harvest studies is biomass nutrient leaching (BNL) [10,27,29]. Also known as throughfall in woody perennial studies [30] or field leaching in perennial grass
studies [12], biomass leaching is the passive loss of organic and inorganic nutrients from any aboveground tissue after extended leaf wetness (such as a rainy day) [31-33]. Senescence and its related loss of membrane integrity within plant cells also exacerbate BNL, therefore losses due to biomass leaching typically increase in severity with the age of the affected tissue [33,34]. Another passive nutrient loss process known to occur over winter is mass loss due to leaf drop [35]. Leaves of perennial grasses are known to contain more ash, N and calcium (Ca) (g kg\(^{-1}\)) than stems [36,37], thus loss of leaves over winter may disproportionately contribute to content reductions in those nutrients that are not susceptible to BNL.

**First question: To what extent are nutrients resorbed from senescing switchgrass leaves?**

*Objective: Develop a baseline of leaf-level mineral nutrient loss (resorption) during end-season senescence*

Aerts [38] emphasized the importance of nutrient resorption in natural ecosystems. A better understanding of the extent to which mineral nutrients are both retained and lost from senescent switchgrass biomass is necessary as a platform for more informed agronomic and harvest management decisions in the Upper Midwest [4]. A first step would be to quantify foliar nutrient changes within *in situ* switchgrass plants during end-season senescence prior to a hard frost and onset of winter, and outside any additional experimental treatments. This would establish a baseline of the way macronutrient concentrations can be expected to change temporally, and could reasonably be attributed to active nutrient resorption; this baseline can then be applied to investigate other nutrient loss processes. Additionally, because it is not agreed upon whether nutrients other than N or N-containing compounds are resorbed, leaf-level baselines should be developed for P, K, magnesium (Mg), Ca, and sulfur.
This investigation may elucidate whether other mineral nutrients are at least actively removed from the leaves, if not stored in belowground tissues.

**Second question: To what extent are nutrients leached from senescing switchgrass leaves?**

**Objective: Quantify foliar nutrient leaching in actively senescing switchgrass**

Earlier studies indicated that, in multiple plant species, BNL increased the longer the biomass was exposed to wetness and that it increased in intensity with increasing age of the tissue [34]. Collins [40] demonstrated that cut forages exposed to simulated rainfall while drying in the field had lower concentrations of nonstructural carbohydrates that they attributed to field leaching. In more recent fuel processing studies, biomass washed with water was found to have lower mineral nutrient contents as well as lower ash production [14]. These findings lend support to the assumption that BNL is a potential driver behind the loss of mineral nutrients from biomass during senescence. However, to my knowledge, no studies have been performed that specifically examine the effect of precipitation on senesced, but otherwise undamaged (i.e., unharvested), perennial grass biomass [3]. Leaves serve as a natural starting point for this investigation given their higher mineral nutrient concentrations as compared to stems. Investigating the propensity for leaf-level nutrient leaching (foliar nutrient leaching, FNL) may establish upper limits to mineral nutrient concentration changes that could reasonably be expected during strong autumn rainfalls.
Third question: To what extent does mass loss contribute to nutrient reductions in overwintered biomass?

Objective: Clarify interaction of passive processes contributing to overwinter nutrient loss

Delayed harvest recommendations often advocate for a spring harvest if the intent is to improve the quality of perennial grass biomass for combustion purposes [16]. After full senescence of aboveground biomass combined with a hard frost, active nutrient movement via the phloem will have ceased [25]. Overwinter changes in mineral nutrient concentrations can therefore ostensibly be linked to passive loss processes only. Biomass nutrient leaching is often indicated as the main process responsible for overwinter changes to mineral nutrient concentrations (g kg⁻¹) in standing perennial grass biomass [4,12]. However, yield losses in the form of leaf drop may also be affecting standing mineral nutrient content (kg ha⁻¹) overwinter in perennial grasses [35]. Often, when overwintered biomass nutrient estimates are presented, they are calculated on a whole-harvested plant basis and not separated by plant organ [41]. However, studies have found significant overwinter losses of leaves in perennial grasses left standing in the field [42,35,29]. If a nutrient is not susceptible to BNL, but estimates of its loss are presented on a content per area basis (i.e., kg ha⁻¹), it is possible that leaf drop may be driving content losses that are causally or indirectly ascribed to BNL. In order to tease apart the passive effects of nutrient leaching and mass loss in temperate climates, it is necessary to investigate both the changes in the mass (kg ha⁻¹) of switchgrass leaves, stems and panicles overwinter and the changes in mineral nutrient concentration of those morphological components during the same timeframe.
**Anticipated research impact**

Extensive agronomic protocols have been developed over several decades for annual crops like corn and soybean. While biomass for bioenergy is not a “new” phenomenon, agronomic and harvest management practices have not been as exhaustively developed for bioenergy crops such as perennial grasses. Additionally, best practices differ depending on growth environment, feedstock end use, and ecosystem service priorities. Results from my investigations will provide insight to fundamental processes governing nutrient cycling in managed perennial grass systems, as well as contribute to the rapidly expanding knowledge base for Upper Midwest producers seeking to diversify their farming operations with switchgrass.

**Thesis Organization**

My research is presented in this thesis using the following organizational structure:

Ch. 1) This introduction provides background and rationale for investigation of select active (resorption) and passive (leaching and overwinter mass loss) nutrient movement processes in switchgrass.

Ch. 2) Written as a research paper for submission to BioEnergy Research, this chapter details development of a leaf-level macronutrient concentration change (resorption) baseline during end-season senescence in switchgrass, and use of this baseline to investigate whether simulated rainfall induced foliar nutrient leaching in senescing and senesced leaves.

Ch. 3) Also written as a research paper for submission to BioEnergy Research, this chapter investigates the combined effects of two passive nutrient loss processes (biomass nutrient leaching and mass loss due to leaf drop) on mineral nutrient content changes in switchgrass over winter.
Ch. 4) The final chapter outlines general conclusions from both studies and suggestions for future research.
References


CHAPTER 2: SIMULATED RAINFALL INDUCES REAL, BUT MINIMAL, FOLIAR NUTRIENT LEACHING IN SENESCING SWITCHGRASS BIOMASS

Modified from a paper to be submitted to BioEnergy Research

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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 energy grasses until late winter reduces nutrient content, primarily due to end-season resorption, but 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 time recommendations and improve feedstock quality. Additionally, establishing a baseline of mineral loss during switchgrass senescence will improve current understanding of leaf-level nutrient resorption. We applied simulated rainfall in situ to switchgrass stands to determine if heavy precipitation can induce foliar nutrient leaching in senescing, unharvested, foliage. Five hour-long rainfall events (each ~120 mm) were simulated every two weeks from the beginning of September to a killing frost in 2014 and 2015. Foliar samples were taken from the upper and lower canopy before and after simulated rainfall and in dry (no rain) controls, then analyzed for elemental N, P, K, S, Mg and Ca. Nutrients tended to actively resorb from leaves, with resorption estimates ranging from 34\% to 81.6\%. Results from the comparison of rainfall plots to the control plot baselines indicated that lower canopy leaves, upon

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reaching ≥50% senescence, were slightly susceptible to foliar nutrient leaching, with losses ranging from 0.30 to 2.76 g kg⁻¹ (dm) for K, P, and Mg. Nitrogen, Ca and S were not susceptible to foliar leaching. Although statistically significant, these values suggest that foliar nutrient leaching is not a strong driver of nutrient loss during senescence.

**Introduction**

**Switchgrass as biomass feedstock**

Perennial grasses bring both production and conservation benefits to diversified farming systems [1,2]. When grown for bioenergy, perennial grasses can provide a high-yielding feedstock for both solid and liquid fuel applications [3,4]. A sustainable advantage of perenniality is the ability 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 1980’s, the Department of Energy identified switchgrass as a model bioenergy species and since then research has focused on increasing yield and improving harvested biomass characteristics of this plant [10,7]. A particular area of interest is reducing mineral nutrient content of the harvested biomass to not only improve crop sustainability, but also to 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 of the harvested biomass [10,13,14]. Biomass should be low in mineral nutrients not only to reduce the cost and environmental impact of
crop cultivation, but also because excess amounts can cause complications and inefficiencies in both biochemical and thermochemical conversion processes [15-17]. However, many of the essential mineral nutrients required by all plants to successfully grow and reproduce remain in the aboveground biomass of plants after senescence, even in naturally efficient perennial C4 grasses [18]. While genetic improvement approaches are underway to develop more efficient varieties, the market for biomass crops is currently small and uncertain, and slows their widespread adoption [10]. Thus, it is prudent to understand different ways to reduce mineral nutrients in biomass, either through changes in agronomic management or through pre-treatment of biomass prior to conversion [16,4,11]. 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 (also called translocation) to belowground tissues, as well as the somewhat unexplained, but empirically demonstrated, nutrient loss that occurs when biomass stands overwinter in temperate areas with a cold winter [16,8,6]. Delayed harvest can lead to yield losses from leaf drop and lodging, but it also typically provides cleaner feedstock, reduces nutrient take-off from the field, and provides ecosystem services associated with winter cover [1,8,6].

**Nutrient movement and loss processes**

Prior to complete senescence, end-season 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 the more recent ecology literature, thus advancing a more
common scientific understanding of the nuanced differences between resorption and the broader term of translocation [22,20,23,24]. Functioning phloem is required for the active process of resorption to occur [25,26]. 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 N, P, and K concentrations, resulting in a decrease in the ash concentration of the harvested biomass [8,16,27].

One process that is assumed to cause this additional mineral nutrient loss overwinter in delayed harvest studies is biomass nutrient leaching (BNL) [7,27,28], also known as throughfall in woody perennial studies and field leaching or biomass leaching in perennial grass studies. BNL is the passive loss of organic and inorganic nutrients from any aboveground tissue upon extended wetness (such as a rainy day), which increases in intensity with the age of the affected tissue [22,29,30]. This process is most notable in leaf tissue, which typically contains higher nutrient concentrations than stems in perennial energy grasses [31,32,6]. Senescence and its subsequent loss of membrane integrity within plant cells exacerbates biomass leaching [29]. While the exact mechanism is, as yet, unknown for BNL, it is likely similar to foliar nutrient uptake [33], as saturated cuticles and/or degraded membranes allow otherwise precluded molecule movement.

**Objective 1: Develop nutrient concentration baselines in senescing switchgrass leaves**

A nuanced understanding of the degree to which macronutrients are lost from aboveground biomass during senescence has not yet been developed for temperate climates, 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 BNL potential in switchgrass. Before leaching can be assessed, however, a baseline of the way leaf nutrient concentrations change during senescence (which may be largely attributed to resorption) must first be developed. Here we develop such baselines, then use them to calculate leaf-level resorption efficiency, a metric with bearing on whole-plant nutrient use efficiency. Because leaf mass and area decrease during senescence, Vergutz et al. [20] developed a mass loss correction factor (MLCF) for plant functional types that will be applied to resorption efficiency calculations in this study. Based on current understanding, we hypothesized that the phloem mobile macronutrients (N, P, S, K, and Mg) might initially decrease in actively senescing leaves and then appear to level off after complete leaf senescence has occurred. For a non-mobile mineral nutrient (such as Ca), we hypothesized concentrations may remain constant or increase (in relative terms) as other compounds are exported from the leaf during senescence.

**Objective 2: Quantify foliar nutrient leaching under simulated rainfall in senescing switchgrass leaves**

While BNL has been demonstrated to be of importance in some species [34-36] and hinted to be important in senescing switchgrass [8,6,4], no studies have yet specifically investigated precipitation effects on undamaged (not cut or conditioned) perennial grass biomass grown in temperate climates. Therefore, the second objective of this study was to assess whether *in situ* simulated rainfall would induce leaf level biomass leaching (foliar nutrient leaching - FNL) in actively senescing switchgrass during the early-fall season. We hypothesized that FNL would occur with simulated rainfall, and would increase as senescence progressed.
To address these objectives we endeavored to 1) to establish a baseline of switchgrass macronutrient concentration changes during senescence and 2) to determine whether simulated rainfall could induce foliar nutrient leaching in actively senescing switchgrass biomass.

**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 (Figure 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 two weeks apart and occurred over the course of two months, during which the switchgrass completed senescence, as indicated by disappearance of green tissue, and the occurrence of freezing temperatures.

**Study site and climate**

The experimental site was located at the 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 56 kg N ha⁻¹ every spring (46% dry urea, broadcast) and was harvested annually following senescence. Soils were a mix of fine-loamy, mixed, superactive, calcareous, mesic, Typic Endoaquolls and fine-loamy mixed superactive, mesic, Typic, and Aquic Hapludolls (Canisteo and Nicollet series) [37]. The stand was healthy and produced an average annual yield of 5.8 Mg ha⁻¹. Weather information (Figure 2) was
obtained from the ISU Agronomy and Agricultural Engineering Farm approximately 10 km from the experimental site [38].

**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 control replications that did not receive simulated rainfall. Treatments were randomly assigned to the ten plots at the beginning of each season and new plots were chosen each year, i.e., the 2014 plots were not used in 2015. Rain gauges were randomly assigned to two of the five simulated rainfall plots to measure applied simulated rainfall. The ten 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. Leaves were sampled from the interior of the plot at least 0.3 m from each edge. Simulated rainfall treatments were applied on five dates between September and November of 2014 and 2015 (Figure 2), coinciding with maximum biomass and the beginning of post-anthesis senescence of switchgrass. Date of anthesis (August 1st) 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 field rainfall simulator similar in function and design to that described by Miller (1987) (Figure 1). Briefly, the simulator was constructed of an aluminum scaffold frame with two solenoid-operated nozzles (Spraying Systems Inc. Model Number ½-HH-SS-30WSQ) centered three meters above the soil surface to consistently deliver 120 mm of simulated rain per hour 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 eight kilometers per hour. The water was obtained from the local rural water supply (Xenia Rural Water Supply, Ames, IA) and tended to have 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 the fall of 2015, water samples before and after addition of dry ice were analyzed for mineral nutrient content in order to ensure that nutrients were not inadvertently being applied to the experimental plots.

Leaf sample collection

Leaf subsamples were taken immediately before and after an hour of simulated rainfall or in the case of the control plots, before and after an hour time lapse with no simulated rainfall. In both cases, samples were labelled ‘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 canopies 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 was defined as the topmost two leaves and lower canopy was defined as anything lower than the topmost two leaves.

Leaf sample analysis

Leaf subsamples were composited by canopy layer 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 time 0 and time 60. In order to provide an estimate of visible
senescence, each combined leaf sample was visually assessed for percent green leaf area and assigned a percentage of 0, 25, 50, 75 or 100%. The samples were collected in paper envelopes and dried in a forced air dryer at 60 °C for 48 hours. After drying, the samples were ground to pass through a 1 mm screen in a Cyclone UDY sample mill (UDY Corp., Fort Collins, CO, USA) and analyzed for mineral nutrient content by Midwest Laboratories (Omaha, NE, USA). Nitrogen (N) concentration was determined via combustion analysis in a LECO 6 analyzer and phosphorus (P), potassium (K), sulfur (S), calcium (Ca) and magnesium (Mg) concentrations were determined by ICP-AES analysis following nitric acid digestion (Midwest Laboratories, Omaha, NE, USA). For all analyses, data were reported as the macronutrient concentration on a percent dry matter basis.

**Data analysis**

All data were analyzed using linear models with either the PROC GLM or PROC MIXED procedures in the SAS software (SAS 9.4, SAS institute Inc., Cary, NC, USA). Canopy level and year were considered fixed effects and analyzed separately. Main effect significance was determined at α=0.05.

**Baseline leaf nutrient concentrations and resorption efficiency estimates**

Data from the control (non-rainfall) plots were used to develop nutrient concentration baselines. This was done by first using analysis of variance in PROC GLM to determine if leaf macronutrient concentrations changed between time 0 and time 60 (without rain). No difference was found for any date or macronutrient. Therefore, the time 0 and time 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 level using analysis of variance in PROC MIXED with date as a fixed effect and the replicated plots random. An estimate of the difference between the macronutrient concentration on the first 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. Leaf level resorption efficiency estimates were calculated using the MLCF established by Vergutz et al. [20] in the following manner:

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

where \( NuR, NuS, NuG, \) and 0.713 represent nutrient resorption efficiency, nutrient concentration of senesced leaves, nutrient concentration of green leaves, and the graminoid mass loss correction factor (MLCF), respectively.

**Leaf nutrient concentrations with simulated rainfall**

Time 0 data and time 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 two-month period of study. Data were analyzed by macronutrient, year, and canopy layer 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 two-week intervals starting at the beginning of September. Because the experimental units (plots) differed in both field placement and treatment each year, they were considered
random and were nested within treatment. Equal variance assumptions among sampling dates were tested and best-fit covariance models were chosen following Littel et al. [39]. 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 was above the 30-year average (145 mm) in both years at approximately 235 mm in 2014 and 219 mm in 2015. Monthly temperatures were average and ranged from 18 to 12 °C in 2014 and 20 to 12 °C in 2015 (Figure 2).

**Baseline leaf nutrient concentrations and resorption efficiency estimates**

Three distinct trends were visible in the baseline macronutrient concentration of senescing switchgrass leaves over time (Figure 3 and 4). Two trends were similar for both canopy levels: 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 canopies, 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 level.

In the upper canopy leaves, N, P, K, and S concentrations continued to decline during the sampling period for both years (Figure 3 and 4). Averaged over the two years, upper
canopy concentrations for N decreased by 6.4 g kg\(^{-1}\), P by 0.9 g kg\(^{-1}\), K by 5.3 g kg\(^{-1}\), and S by 0.3 g kg\(^{-1}\) (Table 1). The mineral concentrations in the lower canopy leaves tended to follow similar trends as their upper canopy counterparts, but reached steady-state during the experimental period. While significant decreases were found between the first and final sampling date, N, P, K, and S concentrations leveled off rather than continuing to decline over the entire sampling period (Figures 3 and 4). Averaged over the two years, lower canopy concentrations for N decreased by 2.7 g kg\(^{-1}\), P by 1.1 g kg\(^{-1}\), K by 6.0 g kg\(^{-1}\), and S by 0.2 g kg\(^{-1}\) (Table 1). After accounting for average leaf mass loss (see discussion) upper canopy leaves experienced resorption efficiencies of 58 % N, 53 % P, 74 % K, 43% S, -1 % Ca, and 33 % Mg during the experimental period in the absence of simulated rainfall (Table 1). Lower canopy leaves experienced resorption efficiencies of 47 % N, 63 % P, 82 % K, 43% S, 16 % Ca, and 36 % Mg from the initial leaf levels (Table 1).

**Leaf nutrient concentrations with simulated rainfall**

The effect of simulated rainfall was determined by assessing deviations from the above baseline concentration values after an hour (120 mm) of simulated rainfall (short-term effects) and after all five simulations that resulted in 600 mm of rainfall in addition to natural precipitation that occurred (cumulative effects). 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 (Table 2). No consistent significant cumulative effects were observed (data not shown).

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). Fewer significant effects were found in the upper canopy than in the 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 within-sampling-date effects found for S and Ca were negative, indicating that average concentrations were higher in the rainfall plots 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 as compared to values found in the time 60 analysis.

**Discussion**

The objectives of this study were 1) to establish a baseline of switchgrass macronutrient concentration changes during senescence and 2) to determine whether simulated rainfall could induce foliar nutrient leaching in actively senescing switchgrass biomass.

**Baseline nutrient concentrations and resorption efficiency 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 phloem mobility does not guarantee that a nutrient will be resorbed in the fall [25,26]. Additionally, the very nature of end-season senescence eventually inhibits active resorption due to eventual phloem tissue breakdown [22,40]. Phloem mobile nutrients include N, P, K, Mg, and S while Ca is considered phloem immobile due to its significant structural role as a constituent of the pectin polysaccharide bonds between cell walls [25,29]. With the exception of Mg, the baselines established here for N, P, K, S, and Ca support 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 (Figures 3 and 4). Magnesium did not appear to change in concentration over the two-month sampling period in either year. While this result appears to contradict Mg’s phloem mobility, similar results have been observed in mineral resorption studies conducted with other plant species [25,41,42]. White [25] suggests 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 [43]. Additionally, a portion of the Mg pool is permanently tied up within pectin between cell walls [44]. Most likely, all three factors jointly affect the relative end-season immobility of Mg, but this is a finding that is perhaps worthy of future investigation.

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 nitrogen. For instance, some studies support the resorption of P [8,21,45] and others negate it [5,46]. The same dichotomy of opinions has been found for K [20,46-48]. 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] has made an attempt to quantify potential resorption efficiency across several plant groups for five macronutrients, establishing a mass loss correction factor for each group that can be incorporated into future calculations of resorption in other studies. In their study, nutrient resorption efficiency estimates for N (74.6%), P (82.1%), K (84.9%), Ca (32.5%), and Mg (39.8%) were similar, with the exception of Ca, to our calculated leaf-level estimates based on the nutrient resorption equation and mass loss correction factor used by Vergutz et al. [20] (Table 1). Because we observed slight increases in the baseline Ca concentration during both senescence seasons, our observed resorption efficiency estimates for that nutrient were either negative or very low. As previously explained, this was not unexpected due to its phloem immobile nature.

Another potential reason that dichotomies exist in the biomass literature regarding quantification of end-season resorption is that some studies attempt to balance aboveground nutrient loss with belowground nutrient gain [46,49,28]. 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 precludes quantification of total nutrient resorption via mass balance between above- and belowground biomass for switchgrass, but the way our baseline leaf nutrient concentrations changed during senescence
effectively supports resorption at some level. Future research in this area should focus on concomitant sampling of above and belowground 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.

**Effect of simulated rainfall on foliar nutrient leaching**

Varying significant differences were seen for short-term effects (time 60 analyses). Within these analyses, lower canopy leaves experienced the bulk of observed differences after exposure to a 1-hr, 120-mm simulated rainfall (Table 2). This may be because lower canopy leaves were further along in the senescence process earlier in the fall season than the upper canopy leaves and thus more vulnerable to leaching. Additionally, only a somewhat consistent trend was seen for K and Ca, both of which showed significant differences each year, while significant differences for P, Mg, and S were only detected in one year. Nitrogen was not observed to leach at all. Interestingly, while the differences found between the control plots and the rainfall plots were quite small, they were measureable in that they fell outside the margin of error for the analysis techniques.

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 the cytosol and phloem sap, is used for regulating osmotic potential, and remains in its ionic form throughout the plant [50]. 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 [44]. 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 the 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% overt senescence had occurred for each canopy in each year. This supports our hypothesis that leaching may increase 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 already known to be a highly 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 structurally incorporated 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 due to this reason. Furthermore, studies have demonstrated that compounds other than mineral nutrients can leach from foliage due to extended wetness, including non-structural carbohydrates (NSCs), amino acids, and other low-weight molecular compounds [33,29,51,36]. Therefore it is realistic to assume that the 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 Ca and S. 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 the rainfall plots and baseline averages. However, quantifying or identifying other leachable cell constituents was outside the scope of this study.

Because only a few significant differences were observed for cumulative effects (time 0 analyses), it appears that the simulated rainfalls did not have an overall effect on the rainfall plots during the senescence seasons (data not shown). It is important to highlight that the significant differences seen in the time 60 analyses (short-term effects) for Ca, S, P, Mg, and K occurred after at least 50% senescence of the leaves. Similar differences should have been seen in the time 0 analyses 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 also be due to the leaching effects of natural rainfall, which were not measured.

Other factors affecting end-season nutrient loss

Given that the leaf biomass in this study was fully senesced by the end of the sampling period and only weak leaching effects were found, it seems unlikely that biomass leaching is a significant driver of nutrient loss during active senescence. However, the studies that have cited biomass leaching as the cause for loss of mineral nutrients usually sampled the biomass shortly after a killing frost and then again, several months later or even in the springtime [16,48]. 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 [33,52]. Finally, delayed
harvest studies often cite nutrient changes on a total standing crop basis. It is possible that overwinter physical changes such as leaf drop are influencing the change in mineral nutrient content from the fall to the spring [53,12]. 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 does not appear to drive a significant loss in macronutrient concentrations of the leaves via foliar nutrient leaching. Instead, senescence-mediated macronutrient concentration changes in standing switchgrass biomass appear to fit the narrative of phloem mobile and non-mobile nutrients. Changes in these concentrations over the course of senescence are likely due to resorption to the stem or belowground storage tissues unless the mineral nutrient in question serves a structural role, is bound within larger weight molecular compounds, or is present in high concentrations in the soil solution already. In these cases, resorption may be delayed or inhibited altogether. Due to the potential difficulties in harvesting biomass in the 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.
Acknowledgments

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References


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. Concentrations and estimates of differences are reported in g kg$^{-1}$.

<table>
<thead>
<tr>
<th>Graph</th>
<th>Estimate $\Delta D_{\text{final}} - D_{\text{initial}}$ (g kg$^{-1}$ ± SE)</th>
<th>$P$-value (Den DF 20)</th>
<th>Average (g kg$^{-1}$)</th>
<th>NuR (% from leaf)</th>
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</thead>
<tbody>
<tr>
<td>N 2014 Upper Canopy</td>
<td>-6.6 ± 0.7</td>
<td>&lt;0.0001</td>
<td>-6.4</td>
<td>58</td>
</tr>
<tr>
<td>N 2015 Upper Canopy</td>
<td>-6.1 ± 0.5</td>
<td>&lt;0.0001</td>
<td>-6.4</td>
<td>58</td>
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<td>N 2014 Lower Canopy</td>
<td>-3.5 ± 0.6</td>
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<td>-2.7</td>
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<td>K 2014 Upper Canopy</td>
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<td>0.0634</td>
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Table 2. Analysis of variance and estimates of switchgrass leaf nutrient concentrations (g kg\(^{-1}\)) following one hour (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 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. Significance was determined at \(\alpha = 0.05\).

<table>
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<tr>
<th>Upper Canopy</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
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<td>Treatment (each date)</td>
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Figure 1. *In situ* rainfall simulator in experimental switchgrass plots in Boone County, IA. Photo credit: Martin Shipitalo.
**Figure 2.** Daily weather conditions during the fall season sampling periods in 2014 and 2015. Date of anthesis, August 1st, 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 applied rainfall (~120 mm) for both years.
Figure 3. Baseline leaf nutrient concentration (N, K, Ca) changes over time in post-anthesis switchgrass grown near Boone, IA. Graphs A and B represent upper canopy mineral nutrient concentration for 2014 and 2015 sampling periods, respectively. Graphs C and D represent lower canopy concentrations for 2014 and 2015 sampling periods, respectively. Shaded area in each graph represents percent green leaf area.
Figure 4. Baseline leaf nutrient concentration (P, S, Mg) changes over time in post-anthesis switchgrass grown near Boone, IA. Graphs A and B represent upper canopy mineral nutrient concentration for 2014 and 2015 sampling periods, respectively. Graphs C and D represent lower canopy concentrations for 2014 and 2015 sampling periods, respectively. Shaded area in each graph represents percent green leaf area.
CHAPTER 3: LEAF DROP AND LEACHING DIFFERENTIALLY DRIVE OVERWINTER CHANGES IN SWITCHGRASS MACRONUTRIENT CONTENT

Modified from a paper to be submitted to *BioEnergy Research*

Ruth Burke $^{1,2}$, Kenneth Moore $^{1,3}$, Emily Heaton $^{1,4}$

Abstract

Reducing nutrient concentrations of bioenergy crops before harvest can improve environmental sustainability and feedstock quality. The most expedient way to achieve switchgrass (*Panicum virgatum* L.) nutrient reductions in temperate climates is to delay harvest of senesced biomass until late winter or early spring. Delayed harvest takes advantage of active and passive nutrient loss processes including resorption and biomass nutrient leaching. After senescence, only passive processes can affect the biomass. No studies have yet quantified how biomass nutrient leaching and leaf drop, both passive loss processes, may affect the nutrient concentration of *in situ* switchgrass biomass overwinter in the upper Midwest. We collected monthly switchgrass biomass samples from November 2015 - March 2016. Samples were separated by morphological component (stems, leaves, panicles), weighed, and analyzed for N, P, K, Ca, Mg, and S content. Changes in mass, mineral nutrient concentration, and content were compared over sampling dates to quantify nutrient loss. Results indicate that both processes contribute to overwinter decreases in nutrient content depending on the leaching potential of the nutrient. Leaf drop was observed to be the main driver of overwinter N, Ca, and S losses whereas biomass nutrient leaching primarily caused the overwinter losses of K, P, and potentially Mg.

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Introduction

Developing agronomic protocol for perennial grasses as second generation bioenergy crops

Commensurate with the increasing importance of perennial grasses as second generation bioenergy crops [1-3], research has sought to tailor agronomic recommendations to leverage improved genetics, supply chain technology and technoeconomic efficiency [4-7]. Technoeconomic efficiency, i.e., the efficiency with which biomass is converted to energy and the cost of doing so, can be increased by reducing residual mineral nutrient content in the feedstock [8-11]. Delayed harvest, the practice of leaving standing biomass in the field until late winter or early spring, has been proposed as a method of reducing mineral nutrient content in harvested biomass [12,3,13]. However, this practice comes with potential drawbacks, most notably loss of biomass in temperate climates as crops deteriorate overwinter [14-17].

Delaying harvest until early winter or early spring

Delayed harvest recommendations for the perennial grass switchgrass (Panicum virgatum L.) take advantage of active and passive end-season nutrient loss processes [18,19,13]. End-season nutrient resorption, the process through which nutrients are actively resorbed from the aboveground biomass and transported to storage tissues over a dormant period, is the primary driver of biomass nutrient loss during annual senescence [20,21,7]. Passive nutrient loss processes such as biomass nutrient leaching [18,22] and volatilization [23-25] may also be affecting aboveground biomass nutrient loss during end-season senescence. Some have advocated for delaying harvest beyond senescence, finding
overwintered perennial grass biomass has improved (lowered) ash content and mineral nutrient concentrations in the spring [8,26,13].

Given that phloem is no longer functioning after complete senescence, only passive processes could be affecting mineral nutrient content of overwintered biomass [21,27]. It may be that multiple freeze/thaw cycles are needed for biomass nutrient leaching to become a stronger driver of nutrient loss from biomass. Thus, nutrient leaching may become increasingly important over winter. Structural elements (e.g., cell wall membranes) that may have retained nutrients even in dead tissue degrade over winter, possibly making nutrients more vulnerable to leaching [28]. In addition to leaching, organ loss from standing biomass (also called litter fall) may also be contributing to changes in harvested biomass nutrient content. Lewandowski and Kicherer [29] found that *Miscanthus × giganteus* leaves contain more ash, nitrogen, and calcium (g kg$^{-1}$) than stems, which corroborates similar findings for switchgrass [4]. Studies of other perennial grasses have also found significant overwinter losses of leaves [30,31]. Therefore, it is possible that leaf drop may be driving overwinter biomass nutrient changes. A common problem with previous work, however, is that overwintered biomass nutrient estimates are presented from harvested whole plants [12,18,8] and not individual organs, making it difficult to discern mechanisms of overwinter nutrient reduction.

In chapter 2, we investigated whether foliar nutrient leaching (passive biomass nutrient leaching from the leaves) would exacerbate nutrient loss from *in situ* senescing switchgrass biomass exposed to simulated heavy rainfall, finding it was not a significant driver of leaf level nutrient loss during senescence. What about over winter? Are the changes in biomass nutrient content seen by others a function of nutrient loss from plant organs
(leaching), or a loss of organs from standing biomass (mass loss)? In this chapter we use switchgrass to address these questions, but expect elucidating the relative importance of nutrient changes due to leaching vs. leaf drop may be translatable to other temperate perennial grasses.

**Objective of paper**

To our knowledge, no studies have been published that establish baselines for overwinter changes in the mass, concentration, and mineral nutrient content of the individual plant organs of *in situ* switchgrass in the upper Midwest and more specifically, Iowa. The objectives of this study were two-fold: 1) Investigate how the mass (kg ha\(^{-1}\)) of switchgrass stems, leaves, and panicles changes during an Iowa winter, and 2) Investigate how the macronutrient concentration (g kg\(^{-1}\)) and content (kg ha\(^{-1}\)) of the individual morphological components changes in the same time period and location.

**Materials and Methods**

**Study overview**

In this study, standing switchgrass biomass samples were collected at approximately one-month intervals during the winter of 2015-2016 to assess the effects of two passive nutrient loss processes (biomass leaching and mass loss due to leaf drop) on the individual morphological components of switchgrass.

**Study site and climate**

The experimental site consisted of a 0.5 hectare field of “Cave-In-Rock” switchgrass that had been established in 2009, located at the Woodruff Research Farm in Boone County,
Iowa (41°59'10.0"N 93°41'26.8"W). It received 56 kg N ha⁻¹ (46% dry urea, broadcast) each spring and was harvested annually following senescence. The stand was healthy and produced an annual yield of 6 Mg ha⁻¹. The soils were a mix of fine-loamy, mixed, superactive, calcareous, mesic Aquic and Typic Hapludolls and Typic Endoaquolls (Canisteo and Nicollet series) [32]. Total precipitation (rain and melted snow) during the sampling period was 305.4 mm (Figure 1). Weather information was obtained approximately 10 km from the experimental site at the ISU Agronomy and Agricultural Engineering Farm (ISU-AAEF) [33].

**Biomass collection and mineral nutrient analysis**

Overwinter biomass samples were collected beginning in November of 2015 and continued monthly until March of 2016. On each sampling date, four 0.25 m² replicate quadrat samples of biomass were randomly selected and stems cut by hand approximately 8-10 cm above ground. As a result of heavy snow cover in February 2016, cutting height was increased to 45 cm. Harvested biomass samples were separated into three morphological subsamples (stems, leaves and panicles) for a total of 12 subsamples. Subsamples were dried in cloth sacks in forced-air ovens for 48 hours at 60 °C. After drying, weights were recorded using an analytical balance (Denver Instrument XL-3100D, Denver Instrument Company) and leaves and panicles were ground in a Cyclone UDY sample mill (UDY Corp., Fort Collins, CO, USA) to pass through a 1-mm mesh screen. The stems were ground to one mm in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) due to their size and coarseness. Mixed aliquots were selected from each subsample for a total of 60 subsamples (5 dates*4 quadrats*3 morphological components) and sent to Midwest Laboratories (Omaha, NE, USA) for mineral nutrient content analysis. Nitrogen (N) concentration was determined via
combustion analysis in a LECO 6 analyzer (LECO Corp., St. Joseph, MI, USA) and phosphorus (P), potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg) concentrations were determined by ICP-AES analysis following nitric acid digestion (Midwest Laboratories, Omaha, NE, USA). All mineral nutrient concentrations were reported as fraction of dry matter.

**Data analysis**

The data were analyzed by morphological component as a completely randomized design (n=4) with sampling date as the fixed variable. A standard analysis of variance (PROCS MIXED and GLM) was used to make three pre-planned comparisons. The first compared changes in leaf, stem, and panicle dry mass (kg ha\(^{-1}\)) over sampling dates. The second compared the changes in leaf, stem, and panicle mineral macronutrient concentration (g kg\(^{-1}\)) over sampling dates. Finally, the third compared the changes in leaf, stem, and panicle mineral macronutrient content (kg ha\(^{-1}\)) over sampling dates. Least square means were calculated over sampling dates for each morphological part, as well as the weighted total in all three a priori comparisons. Comparison of least square means was done for each sampling date and significance was determined at \(\alpha=0.05\). Fractional changes in nutrient concentrations from the initial to the final sampling dates were calculated as:

\[
\text{Eq 1: } \frac{\text{Concentration (initial)} - \text{Concentration (final)}}{\text{Concentration (initial)}} \times 100
\]

Data were analyzed and reported on a physiological time scale (days after anthesis) to aid interpretation in light of plant development. Date of anthesis, August 1\(^{st}\) 2015, was the date by which 50% or more of the switchgrass panicles in the experimental site showed anthers. Sampling dates of 105, 133, 166, 203, and 238 days after anthesis correspond with
November 13th and December 11th of 2015, and January 13th, February 19th, and March 25th of 2016. Heavy snowfall preceding the February sampling date (Figure 1) caused significant lodging. Consequently, data obtained from February samples were not used in the mass or mineral content analysis. Nevertheless, since mineral concentrations in the morphological components would not be affected by smaller sample size, the February data were included in the nutrient concentration analysis.

Results

Change in biomass dry weight over sampling dates

Total, stem, and panicle biomass dry weights did not change over sampling dates (Figure 2). Leaf biomass dry weight decreased significantly (P= 0.0003) by a total of 1,469.0 kg ha⁻¹ between the November and March harvests.

Change in nutrient concentration over sampling dates

The largest nutrient concentration changes observed over the duration of the experiment occurred in stems (Figure 3 and Table 1). Phosphorus, K, and S concentrations in the stems decreased by 1.1, 4.8, and 0.1 g kg⁻¹, representing changes of 71, 89, and 29 % from initial concentrations, respectively. Calcium concentrations in the stems increased by 0.4 g kg⁻¹ (24 %), while N and Mg did not change. In the leaves, N and S concentrations increased by 1.4 and 0.1 g kg⁻¹, representing changes of 18 and 14 %, respectively. Phosphorus and Ca concentrations did not change in the leaves. In contrast, K and Mg concentrations in the leaves decreased by 1.2 g kg⁻¹ (57 %) and 0.7 g kg⁻¹ (39 %). Panicle concentrations changed the least, with P, K, and Mg decreasing by 0.4, 0.6, and 0.1 g kg⁻¹, respectively. Those changes represented decreases of 40, 63, and 7 % over initial
concentrations for those nutrients. No other nutrient concentrations in the panicles had significant changes between the November and March sampling dates.

**Change in nutrient content over sampling dates on an area basis**

Nitrogen content from the stems and panicles did not change over sampling dates but the content from leaves decreased significantly by 10.7 kg ha\(^{-1}\) between the November and March harvests (Table 2 and Figure 4). Despite this difference, the total standing biomass N content did not change over time. Phosphorus content decreased significantly by 6.1, 1.2, and 7.4 kg ha\(^{-1}\) in the stems, leaves, and weighted total (standing total crop), respectively, but did not differ significantly in the panicles between November and March (Table 2 and Figure 5). Potassium content decreased significantly in all three morphological components as well as the weighted total (Table 2 and Figure 6): stems, leaves and panicles decreased by 29.3, 4.2, and 0.3 kg ha\(^{-1}\) respectively. The total weighted K content changed by 33.8 kg ha\(^{-1}\). In contrast to the reduction trend, Ca content over winter both increased and decreased significantly, depending on the morphological part (Table 2 and Figure 7). In the leaves, Ca content decreased by 10.4 kg ha\(^{-1}\) whereas in the stems, Ca content increased by 5.9 kg ha\(^{-1}\). Calcium content did not change significantly in the panicles nor in the weighted total between November and March. Magnesium content decreased by 2.8 and 0.3 kg ha\(^{-1}\) in the leaves and panicles but did not change appreciably in the stems (Table 2 and Figure 8). Standing total Mg content decreased by 3.7 kg ha\(^{-1}\). Sulfur content decreased minimally between the initial and final sampling dates, by 1.0 kg ha\(^{-1}\) in the leaves and by 1.1 kg ha\(^{-1}\) in the weighted total (Table 2 and Figure 9). Sulfur content in the stems and panicles did not change significantly.
Discussion

In order to better understand nutrient cycling in perennial grasses grown for bioenergy, this study addressed how leaf drop and biomass nutrient leaching may together contribute to overwinter mineral nutrient content changes in switchgrass.

Teasing apart the effects of passive nutrient loss processes

Studies have cited overwinter biomass leaching as the cause of reduced mineral nutrient concentration in spring-harvested perennial grass biomass [18,1]. However, overwinter mass loss in the form of leaf drop has also been documented from delayed harvest of perennial grasses [34,26,30]. Estimates of reduced nutrient concentration in overwintered biomass are usually either obtained from or presented as whole-plant samples. This makes it difficult or impossible to separately quantify both leaching and leaf drop [6]. In this experiment, we have focused on biomass nutrient leaching and leaf drop as the main causes of change to biomass nutrient concentration and content, respectively, because other passive processes such as volatilization and microbial degradation [35,23,24] either affect only one nutrient (volatilization affects N) or are decreased significantly in the cold temperatures of the upper Midwest (e.g., microbial activity is negligible at low temperatures).

How can overwinter mass loss affect nutrient estimates in standing biomass?

Leaf biomass significantly decreased over the duration of this experiment, declining by 1,469.0 kg ha⁻¹, while stem, panicle and total biomass did not change appreciably in that same period (Figure 2). A similar trend was seen by Adler et al. [26]. The incongruous reduction of leaf biomass while total biomass stayed constant can be explained by snowfall, which, by late winter, had buried or flattened many switchgrass plants. Our March samples
were randomly selected from the few areas of the field where plants had not lodged. This likely led to more robust, denser samples taken in March, as such plants were the only ones still standing. This theory is supported by the fact that stem and panicle biomass dry weight increased slightly overwinter, although the differences were not significant.

The decrease in leaf biomass overwinter found here likely influences the content (i.e., kg ha\(^{-1}\)) estimates of those mineral nutrients that are not subject to overwinter leaching from the aboveground biomass. In these cases, a reported content loss of a non-leaching nutrient may be attributed to passive overwinter leaching when in reality, mass loss in the form of leaf drop may be to blame. In a similar vein, standing biomass (whole plant) nutrient concentration estimates (i.e., g kg\(^{-1}\)) may be affected by leaf drop as well. In situations where standing biomass nutrient concentrations are reported to change over winter, these changes may result either from leaching, a drop of high-concentration morphological components, or both and are thus easily conflated with nutrient content estimates (kg ha\(^{-1}\)). Therefore, it is also important to determine how the concentrations of mineral nutrients change over winter within the different morphological components so as to better differentiate between leaching effects and mass loss effects.

**Which nutrients are subject to overwinter biomass leaching in this study?**

All six macronutrients studied here leach to varying degrees in several plant species [36-38]. Other organic cell constituents such as amino acids and simple sugars are also susceptible to leaching [35] depending on several factors including tissue age, stress, rain pH, and plant species [39-41]. In this study, we found apparent overwinter leaching of P, K, and Mg, rates of which differed by morphological component (Table 1). Nitrogen and Ca did not
appear to leach and it remains unclear whether S is susceptible to leaching. In this study, it is possible that a few characteristics may affect the leachability of a mineral nutrient: the form of transport within a plant, water solubility of that form, the presence of a structural role, and incorporation into large weight molecular compounds. The role of these characteristics is discussed below.

Of the three nutrients observed to leach here, K’s susceptibility is fairly straightforward: it is the only mineral nutrient within plants that is not incorporated into any structural component or biochemical compound [42]. During the growing season, K travels through the plant strictly in its water soluble, ionic form (K⁺) and is primarily used as an osmotic balance regulator [43]. It decreased over winter in all three morphological components (Table 1 and Figure 3) and the magnitude of its reductions (the highest being 89% in the stems) were higher than the leaching losses observed for either P or Mg (Table 1). Other studies have found similar K concentration decreases [18,26].

Phosphorus and Mg seemed to be slightly less susceptible to biomass leaching, or perhaps have organ-specific responses; reductions were observed in two of the three morphological parts for both nutrients (Table 1 and Figure 3). Phosphorus is often transported throughout the plant in its inorganic form, as a simple phosphate ester, or in a high-energy pyrophosphate bond such as in adenosine triphosphate (ATP) [43]. Organic P has a structural role in lipid membranes, and also is incorporated into DNA and RNA molecules [44,39]. While inorganic phosphate and structurally incorporated P may not be susceptible to leaching due to insolubility, the relative polarity of DNA, RNA, ATP and simple phosphate sugars lend support to the partial leaching of those vulnerable molecules. Like K, Mg serves as a regulator of cation-anion balance, turgor pressure, and cellular pH.
and travels throughout the plant in its soluble, ionic form (Mg\(^{2+}\)) [45]. However, Mg also has a slight structural role within plants, serving as the central, stabilizing atom in chlorophyll molecules as well as being incorporated within pectin between cell walls [43,39]. These traits may reduce its susceptibility to biomass leaching, as can be seen from the comparably minimal reductions for Mg (Table 1). Again, Mg and P reductions observed here are similar to those found in other, delayed harvest studies of perennial grasses [26,13].

In the case of Ca and N, it is likely that Ca’s significant structural role in pectin and membrane stabilization [46] and N’s incorporation into large-weight molecular compounds as well as cell wall proteins [39] prevent either nutrient from leaching to any noticeable degree. Instead, the concentrations of both nutrients appeared to increase slightly – N in the leaves and Ca in the stem (Table 1 and Figure 3). This can be explained by leaching of other plant compounds that change organ mass and thus the concentration of non-leached constituents. Apparent overwinter increases in the concentrations of some non-leaching mineral nutrients may be due, in part, to the losses of other leachable mineral nutrients as well as amino acids and non-structural carbohydrates. However, the physiological role of N and Ca within plants may also contribute to their general lack of leaching.

Calcium is not mobile throughout the growing season and is present at very low quantities in its ionic form within the cell [43]. The bulk of Ca can be found embedded between cell walls, bound to pectin polysaccharides as structural support [39], and also plays a significant role in stabilizing the plasma membrane [47]. As for N, it is the element required in largest quantity by plants after carbon [43]. Used for the construction of proteins, co-enzymes, nucleic acids, secondary metabolites, and phytohormones, N is most often thought about in reference to chlorophyll and the rubisco enzyme [43,39]. Nitrogen is
primarily transported throughout the plant as reduced amino-N, and in studies that have considered end-season nutrient resorption in perennial grasses, it is generally agreed that N is resorbed to overwintering tissues [48-50]. It is possible that the bulk of N movement occurs prior to senescence in the form of end-season resorption. After death of aboveground tissues, any N left over may likely be that which is incorporated into larger weight molecules or structural proteins that are not susceptible to leaching. Our results are similar with studies that have considered biomass leaching of N and Ca [8,18,36,30].

Sulfur appeared to leach minimally in the stems, but increased in concentration to a similarly small degree in the leaves (Table 1 and Figure 3). Necessary for the construction of S-containing co-enzymes and secondary metabolites, S is also a primary component of the amino acids cysteine and methionine and moves throughout the plant during the growing season in both its reduced, ammino-S form and its inorganic form, sulfate [51]. Because S is also present in a structural role as sulpholipids in cellular membranes and is a primary constituent of larger weight secondary metabolites [43], it is possible that it is not susceptible to leaching to any measureable degree. However, in the few studies that have considered biomass leaching of S, significant, if minimal, losses were seen [36,26]. It is important to note that most foliar and biomass leaching studies are conducted on tissue that has been damaged in some way – usually cut and either left in the field, leached in a laboratory setting, or washed prior to combustion or conversion [36,11,52,53]. In situ studies are rare with the exception of some throughfall work done with deciduous trees [38]. This study is unique in that it was conducted on standing switchgrass (not exposed to mechanical damage), which likely reduced the general susceptibility of the biomass to leaching.
How did mineral nutrient content change overwinter in this study and which processes affected these changes?

Nutrient content is a product of the nutrient concentration and biomass production of each morphological component. We have attempted to clarify how decreases in nutrient content overwinter may have been affected by two passive processes: mass loss due to leaf drop and biomass nutrient leaching, but it was not always possible to separate effects of the two processes. The only morphological component that significantly decreased in mass overwinter was leaves. Therefore, in the instances where leaf nutrient concentrations decreased as well (signifying leaf level nutrient leaching for that mineral nutrient), it would not be possible to differentiate how leaf drop and foliar nutrient leaching affected the overall reduction in content for leaves. However, in many instances, leaf level nutrient leaching was not significant. Therefore, in that circumstance, any significant leaf content changes could ostensibly be attributed to mass loss of the leaves. Likewise, decreases to content over winter in the stems and leaves can reasonably be linked to biomass nutrient leaching because overall mass changes between November and March were not significant for those morphological components.

The only significant overwinter change for N content was observed in the leaves (Table 2 and Figure 4). Because leaching was not observed in the leaves for N (Table 1 and Figure 3), mass loss in the form of leaf drop may be the primary driver of nutrient loss in this case. Phosphorus content decreased in the stems and leaves for a total content decrease of 7.4 kg ha\(^{-1}\) (Table 2 and Figure 5). This standing crop decrease in P content was affected by both passive processes, as stem level P content changes were primarily driven by biomass nutrient leaching whereas leaf level content changes may have been due solely to leaf drop.
The total content loss of 33.8 kg ha\(^{-1}\) in K was driven primarily by biomass nutrient leaching, as can be seen by the leaching driven decreases in the stems and panicles (Table 2 and Figure 6). Both passive processes contributed to the additional leaf level content decrease for K. Calcium content increased in the stems and decreased in the leaves but did not change appreciably in the panicles or in the standing crop total (Table 2 and Figure 7). The content increase in the stems is likely due to the previously mentioned leaching loss of other stem constituents (such as other mineral nutrients, non-structural carbohydrates, etc.). Because no leaf level leaching was found for Ca (Table 1 and Figure 3), the content decrease of Ca in the leaves can be attributed to leaf drop. For Mg, the total overwinter content change of 3.7 kg ha\(^{-1}\) can be attributed to both passive processes (Table 2 and Figure 8). The leaf level decrease was likely due to both nutrient loss processes whereas the panicle level decrease may be attributed to biomass leaching. In contrast to the other nutrients, the total standing content decrease of 1.1 kg ha\(^{-1}\) for S can be primarily linked to leaf mass loss overwinter given that S content decrease was only found in the leaves and this change was attributed to leaf drop due to a lack of significant concentration change overwinter (Table 2 and Figure 9).

While it is not possible to estimate total nutrient content loss overwinter due to the interaction of leaf drop and leaf level nutrient leaching, it is possible to estimate the additional content of nutrients that could potentially have been harvested had the significant leaf loss not occurred. For example, we have both an estimate for leaf mass loss overwinter (1,469.0 kg ha\(^{-1}\)) and a final (March) N concentration estimate for leaves (Figure 3). By multiplying these two values and dividing the resultant figure by a factor of 1000 (to conserve kg as the unit of mass), we can estimate that an additional 13.7 kg ha\(^{-1}\) of N would have been harvested had leaf mass loss not impacted harvestable N levels. Additional
estimates for leaf level nutrients that could have been harvested are presented in Table 3. Potential harvests were relatively low for most of the nutrients, averaging an additional 1.5 kg ha\(^{-1}\) for P, K, Mg, and S. However, potential harvests of N and Ca (11.5 kg ha\(^{-1}\)) were much larger, which signifies that overwinter leaf drop can have an important impact on nutrient loss for certain, non-leaching nutrients. These results support recent conclusions that stemmy morphotypes of switchgrass may be advantageous over leafy morphotypes in the selection process for plants better suited to bioenergy purposes as leaves may contribute to higher nutrient removal in earlier harvests [4].

**Conclusions**

We found that both nutrient leaching and mass loss from leaf drop contribute to overwinter decreases in biomass feedstock nutrient content. Nutrient leaching appeared to vary by morphological component, and leaf drop appeared to be the primary driver of non-leaching nutrient overwinter reductions in a standing switchgrass crop. Based on this study, leaf drop may be driving estimates of overwinter content changes previously attributed solely to leaching. Nutrient loss that was primarily driven by biomass leaching included K, P, and potentially Mg. Nutrient loss that was primarily driven by mass loss due to leaf drop included N, Ca, and S. Leaves consistently had higher concentrations of N, Ca, Mg, and S overwinter and by spring, also had marginally higher concentrations of K and P as well. Future research should include similar overwinter studies in other Midwest states with multiple switchgrass genotypes to broaden the scope of these results.
Acknowledgments

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References


Table 1. Differences of the mean nutrient concentrations for each morphological component between the final and initial sampling date with corresponding fractional change reported as percent change from the initial concentration. Negative signs indicate a reduction in concentration over time and positive signs indicate an increase. ‘NS’ indicates there was no significant change in nutrient concentration for that value (significance determined at $\alpha = 0.05$).

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<th>Nutrient</th>
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<th>(% Change From Initial)</th>
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<td>Nitrogen</td>
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<td>Magnesium</td>
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<tr>
<td>Calcium</td>
<td>0.4 (+ 24%)</td>
<td>NS</td>
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</table>
Table 2. Differences of the mean nutrient contents for each morphological component between the final and initial sampling date with the corresponding nutrient loss process indicated within parentheses. Negative values indicate a reduction in content over time and positive values indicate an increase. Acronyms within parentheses implicate the proposed nutrient loss process and represent leaching (L), mass loss due to leaf drop (ML), neither process (N), and both processes (B). ‘NS’ indicates there was no significant change in nutrient content for that value (significance determined at $\alpha = 0.05$).

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<th>Nutrient</th>
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<th>Panicle</th>
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</tr>
<tr>
<td>Magnesium</td>
<td>NS</td>
<td>-2.8 (B)</td>
<td>-0.3 (L)</td>
<td>-3.7</td>
<td></td>
</tr>
<tr>
<td>Sulfur</td>
<td>NS</td>
<td>-1.0 (ML)</td>
<td>NS</td>
<td>-1.1</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Additional leaf level nutrient content that could have been harvested had leaf drop not occurred overwinter. Value is calculated using the final sampling date nutrient concentration multiplied by the estimate of leaf mass loss between the initial and final sampling date (1,469.0 kg ha\(^{-1}\)) and divided by a factor of 1000.

<table>
<thead>
<tr>
<th>Mineral Nutrient</th>
<th>Potential Harvest (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>13.7</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.5</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>11.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.5</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Figure 1. Daily precipitation (rain + melted snow), maximum temperature, and minimum temperature experienced during the winter months of 2015 and 2016. Anthesis occurred August 1st 2015.
Figure 2. Stem, leaf, panicle, and total biomass dry weight over sampling dates. Anthesis occurred August 1st 2015. Due to the presence of deep snow in the field, Day 203 (February 19) was not included. Comparisons of least square means were done for total dry mass and the dry mass of each morphological component over sampling dates. Significance was determined at $\alpha=0.05$. Entries in a row followed by the same lowercase letter are not significantly different. Columns signify total biomass weighted by morphological part contribution. Columns marked by the same capital letter in the figure are not significantly different.
Figure 3. Nutrient concentration in each morphological component over sampling dates. Anthesis occurred August 1\textsuperscript{st} 2015. Panels A, B, C, D, E and F correspond with N, Ca, K, P, Mg, and S (respectively).
Figure 4. Nitrogen content (kg ha⁻¹) for each morphological component over sampling dates. Anthesis occurred August 1ˢᵗ 2015. Due to the presence of deep snow in the field, Day 203 (February 19) was not included. Comparisons of least square means were done for each morphological component as well as the weighted total content over the sampling dates. Significance was determined at α=0.05. Entries in a row followed by the same lowercase letter are not significantly different. Columns signify total content weighted by morphological part contribution. Columns marked by the same capital letter in the figure are not significantly different.
Figure 5. Phosphorus content (kg ha⁻¹) for each morphological component over sampling dates. Anthesis occurred August 1ˢᵗ 2015. Due to the presence of deep snow in the field, Day 203 (February 19) was not included. Comparisons of least square means were done for each morphological component as well as the weighted total content over the sampling dates. Significance was determined at α=0.05. Entries in a row followed by the same lowercase letter are not significantly different. Columns signify total content weighted by morphological part contribution. Columns marked by the same capital letter in the figure are not significantly different.
Figure 6. Potassium content (kg ha$^{-1}$) for each morphological component over sampling dates. Anthesis occurred August 1$^{st}$ 2015. Due to the presence of deep snow in the field, Day 203 (February 19) was not included. Comparisons of least square means were done for each morphological component as well as the weighted total content over the sampling dates. Significance was determined at $\alpha=0.05$. Entries in a row followed by the same lowercase letter are not significantly different. Columns signify total content weighted by morphological part contribution. Columns marked by the same capital letter in the figure are not significantly different.

<table>
<thead>
<tr>
<th>Days Post Anthesis</th>
<th>105</th>
<th>133</th>
<th>166</th>
<th>238</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>33.8a</td>
<td>16.1b</td>
<td>5.0c</td>
<td>4.5c</td>
</tr>
<tr>
<td>Leaf</td>
<td>4.8a</td>
<td>1.4b</td>
<td>0.8b</td>
<td>0.6b</td>
</tr>
<tr>
<td>Panicle</td>
<td>0.5a</td>
<td>0.2b</td>
<td>0.2b</td>
<td>0.3b</td>
</tr>
</tbody>
</table>
Figure 7. Calcium content (kg ha⁻¹) for each morphological component over sampling dates. Anthesis occurred August 1st 2015. Due to the presence of deep snow in the field, Day 203 (February 19) was not included. Comparisons of least square means were done for each morphological component as well as the weighted total content over the sampling dates. Significance was determined at α=0.05. Entries in a row followed by the same lowercase letter are not significantly different. Columns signify total content weighted by morphological part contribution. Columns marked by the same capital letter in the figure are not significantly different.
Figure 8. Magnesium content (kg ha$^{-1}$) for each morphological component over sampling dates. Anthesis occurred August 1$^{st}$ 2015. Due to the presence of deep snow in the field, Day 203 (February 19) was not included. Comparisons of least square means were done for each morphological component as well as the weighted total content over the sampling dates. Significance was determined at $\alpha=0.05$. Entries in a row followed by the same lowercase letter are not significantly different. Columns signify total content weighted by morphological part contribution. Columns marked by the same capital letter in the figure are not significantly different.
Figure 9. Sulfur content (kg ha\(^{-1}\)) for each morphological component over sampling dates. Anthesis occurred August 1\(^{st}\) 2015. Due to the presence of deep snow in the field, Day 203 (February 19) was not included. Comparisons of least square means were done for each morphological component as well as the weighted total content over the sampling dates. Significance was determined at \(\alpha=0.05\). Entries in a row followed by the same lowercase letter are not significantly different. Columns signify total content weighted by morphological part contribution. Columns marked by the same capital letter in the figure are not significantly different.
CHAPTER FOUR: GENERAL CONCLUSIONS

Switchgrass Harvest Management

As our energy future becomes more uncertain, developing a knowledge base for the production of alternative feedstocks for bioenergy will become more critical. Unlike major annual crops, agronomic and harvest management protocol is not well defined for perennial grasses. My research has directly contributed to the growing body of work on switchgrass harvest management by exploring nutrient movement and loss processes to improve switchgrass feedstock quality. In these experiments, I addressed three questions:

1. To what extent are nutrients resorbed from senescing switchgrass leaves?

2. To what extent are nutrients leached from senescing switchgrass leaves?

3. To what extent does mass loss contribute to nutrient reductions in overwintered biomass?

First question: To what extent are nutrients resorbed from senescing switchgrass leaves?

To address this question, I endeavored to develop a baseline for leaf-level nutrient loss during end-season senescence in switchgrass grown in temperate climates. These results could then ostensibly be linked to the active process of end-season nutrient resorption. Over two autumn seasons in 2014 and 2015, I observed similar trends in the resorption of the six macronutrients (N, P, K, Ca, Mg, and S) that appeared to depend on their phloem mobility or a lack thereof. Leaf-level resorption efficiency estimates from this study ranged from 30% (Mg) to 80% (K) and were similar to values found in the relatively scarce literature that exists on the topic. Structurally bound nutrients like Ca may not resorb at all, while Mg may
have minimal resorption due to its already large presence in the soil as well as a slight structural role alongside Ca between cell walls. Future studies should use tracers (e.g., isotope labels) and concomitantly sample above- and belowground biomass in an attempt to balance nutrient loss from aboveground plant organs (such as leaves and stems) with nutrient gains in belowground organs such as roots and rhizomes.

**Second question: To what extent are nutrients leached from senescing switchgrass leaves?**

To address this question, I endeavored to quantify foliar nutrient leaching in actively senescing switchgrass in temperate climates. During two senescence seasons in 2014 and 2015, heavy simulated rainfall events were applied bi-monthly to undamaged *in situ* switchgrass plots. Leaf measurements were taken before and after simulated rainfall to determine whether macronutrient concentrations changed significantly due to the additional rainfall. While some significant differences were found, most notably for K, the small magnitude and infrequency of these differences do not support the hypothesis that end-season foliar nutrient leaching drives a biologically significant loss of nutrients during senescence. It is possible multiple freeze/thaw cycles and other passive loss processes (such as volatilization and microbial degradation) are required in concert with full senescence in order for passive biomass leaching to become more prominent. Additionally, in the few studies that have assessed biomass nutrient leaching, damage (intentional or otherwise) from harvest and conditioning likely impacted leaching potential of the biomass. Because this study was applied to standing, undamaged switchgrass, it is possible that biomass leaching effects were not as pronounced.
Third question: To what extent does mass loss contribute to nutrient reductions in overwintered biomass?

To answer this question, I endeavored to clarify the interaction of two passive nutrient loss processes that contribute to overwinter nutrient loss: biomass nutrient leaching and mass loss due to leaf drop. Because estimates of overwinter leaching in perennial grasses are often reported on a whole-standing-crop basis, my work focused on parsing biomass leaching effects in the different morphological components of the aboveground biomass, as well as to quantify mass losses experienced overwinter in the same plant organs. Aboveground switchgrass biomass samples were collected monthly from November 2015 to March 2016 and individual morphological parts were weighed and tested for their macronutrient concentrations. Overwinter changes in yield, concentration, and content were assessed for each macronutrient. Biomass nutrient leaching varied by morphological component and was the primary driver of overwinter nutrient loss for K, P, and potentially Mg. Potassium was most affected by overwinter leaching, with a standing crop content loss of 33.8 kg ha\(^{-1}\) that was primarily driven by biomass leaching and only slightly affected by leaf drop. In contrast, I determined that mass loss in the form of leaf drop was the primary cause of N, Ca, and S content decreases overwinter. Significant overwinter leaching was not observed for these nutrients in the different morphological parts, but leaf-level content losses occurred, which are likely due to the concurrent significant leaf mass loss. Reverse calculations indicate that had significant overwinter leaf drop not occurred in this study, an additional 13.7 kg ha\(^{-1}\) of N and 11.5 kg ha\(^{-1}\) of Ca would have potentially been harvested in March. Future research should attempt to broaden the scope of this research by including multiple years, perennial grass species, and locations.
Future Research Endeavors

While valuable, my research conclusions are limited in geographic scope to Central Iowa. Were these experiments to be repeated, I would advocate for additional locations in the upper Midwest as well as multiple grass species. Furthermore, nutrient resorption efficiency estimates are limited by uncertainties regarding where nutrients are moved to once resorbed from the leaf. Therefore, both aboveground and belowground biomass samples should be taken from spring through full senescence in order to more accurately account for nutrient deposition. These samples should be separated by morphological component: stems, leaves and panicles for aboveground biomass and roots and rhizomes for belowground biomass. Finally, because delaying harvest overwinter can lead to yield losses and harvest difficulties, a study assessing maximum appropriate levels of feedstock macronutrient contents should be conducted for varying types of biorefineries and combustion facilities. It is possible that in the Midwest, earlier harvests in the late fall will yield biomass feedstock of sufficient quality as to negate the need for a spring harvest while maximizing potential biomass yields.
ACKNOWLEDGMENTS

Three years of graduate studies and research do not happen within a vacuum. A great deal of people helped me in my efforts over the last few years and I shall endeavor to thank them all here without writing another novel. First I would like to thank my major professor, Emily Heaton, for all her support and assistance. Emily guided me whenever I had lost sight of a goal and provided candid and honest advice when I needed it most. Whether our discussions were in an office, on a trail walk, or in a booth at the local pub, Emily never failed to keep me interested and on track with my studies and research. Furthermore, she has given me confidence in my abilities as a researcher and scientist; she always tells me that I ask the best questions! She was more than an advisor; she was (and is) a friend.

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