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Nutrient Removal in Willow Biomass Crops is Impacted Over Multiple Rotations, Timing of Harvest, and Harvesting System

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NUTRIENT REMOVAL IN WILLOW BIOMASS CROPS IS IMPACTED OVER MULTIPLE ROTATIONS, TIMING OF HARVEST, AND HARVESTING SYSTEM

by

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Dedication

When I became a father, I realized that all my dreams were fulfilled. Since then, nothing that I do, accomplish, or achieve is for me. All I want now is to see my children happy and help them to fulfill their dreams. I understand now that a father's dream is to see his children grow and wonder what they'll be in the future. Today, I live my dreams through my kids, and hope to always be present to see them fulfilling their goals.

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The interest in bioenergy as an alternative to fossil fuels is projected to increase in the future given environmental and climatic concerns related to greenhouse gases (GHG) emissions. Short rotation woody crops (SRWC), including shrub willow, are dedicated energy crops established to produce woody biomass for the generation of bioenergy and bioproducts. Despite initial commercial-scale deployment of shrub willow crops in the Northeast U.S. region, especially in New York State (NY), uncertainties exist about the intensity of the crop's nutrient management and inputs. This dissertation studies nutrient removal in shrub willow crops under different scenarios and scales. Nutrient removal via harvested biomass in 18 cultivars planted at two sites was different across three rotations. Later rotations removed significantly more K, Ca, and Mg compared to earlier rotations, while N and P removals did not change over rotations among five top yielding cultivars. Soil total N (-18%) and P (-51%) decreased significantly over three rotations at one site (Belleville), while soil K (+30%) levels increased in the other site (Tully), after three rotations (~10 years). Biomass production and nutrient removals were impacted by timing of harvest. Harvesting during leaf-off season had higher biomass production (+36%) and reduced nutrient removal (-19% N, -16% P, -33% K, -21% Ca, -22% Mg, and -30% S) compared to leaf-on harvests. However, cultivar varieties responded differently to harvest dates and will influence nutrient management guidelines. The amount of dropped biomass after a mechanized harvest (7-15% of total standing biomass) could contribute as a significant source of nutrients and other elements being returned to the willow crop (5-17 kg N ha^{-1}, 1.0-3.3 kg P ha^{-1}, 3.3-9.6 kg K ha^{-1}, 8-57 kg Ca ha^{-1}, 0.5-2.4 kg Mg ha^{-1}, 0.5-1.6 kg S ha^{-1}) for subsequent rotations. Although research and experiments provide insight for nutrient management guidelines, commercial harvest operations, soil conditions, and weather are not considered. Timing of harvest, dropped biomass after mechanical harvest, and soil nutrient levels need to be accounted for when developing nutrient management plans.

Keywords: *Salix*, nutrient concentration, short rotation woody crops, woody biomass, nutrient content.

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CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1. Introduction

Fossil fuels remain the dominant energy source despite current environmental, social, and economic concerns; however, renewable energy sources, including biomass, have been receiving more attention in recent years. Biomass is the largest component of worldwide renewable energy production, representing approximately 50% of the total [1]. Interest in bioenergy as an alternative to fossil fuels has been developed in recent years as a response to issues and concerns regarding energy security, energy independence, and environmental and climate impacts [2], [3].

Dedicated energy crops are established to produce large amounts of biomass per hectare [4] that can then be converted into bioenergy, biofuels and bioproducts. According to the U.S. Department of Energy (USDOE) [5], perennial grasses, trees, shrubs, and some annual crops can be grown specifically to supply large volumes of uniform, consistent-quality feedstock. In the US woody species managed as short rotation woody crops (SRWC), such as willow (*Salix* spp.), poplar (*Populus* spp.), pine (*Pinus* spp.), and eucalypt (*Eucalyptus* spp.), are the focus of attention. There are 330 – 500 willow species worldwide with a wide range of genetic variability [6], [7], which gives willow great capacity to be grown in a wide variety of conditions, including marginal agricultural land, and opportunities for genetic improvement. In the northern temperate regions of the US, shrub willow has been a primary focus because of several characteristics (high yields, short rotations, ease of propagation, ability to resprout following multiple harvests) that make some of these species ideal bioenergy feedstocks [8].

The cultivation of willow as a locally produced, renewable feedstock for bioenergy and bioproducts has been stimulated in NY and the northeastern US [9] due to continuing development of hybrid cultivars [10], incentive programs [11], bioremediation and alternative applications [12]–[14], and opportunities to promote biodiversity and produce bioenergy [15], [16], and concerns focused on environmental and climatic impacts. In NY, shrub willow crops have been commonly planted on abandoned or marginal agricultural land [16], which are typically considered not profitable to agriculture, mainly due to poor drainage.

1.2. Nutrient management in shrub willow crops

Shrub willow crops are commonly managed with more intensive cultural practices compared to traditional forestry, but less intense than agricultural systems. The crop is commonly planted at a density of about 13,500 plants ha^{-1}, coppiced after the first growing season to promote the regeneration of multiple and more robust stems, and harvested every 3-4 years for up to seven rotations [17]. Despite several decades of research in shrub willow crops, these intense techniques, coupled with frequent whole plant harvests, have raised concerns about nutrient removals, long-term site conditions, and willow productivity over multiple rotations [9], [17].

Nutrient removal in shrub willow crops has been an area of research for many years and in different regions [18]–[22]; however clear guidelines for nutrient management in willow crops in North America have yet to be developed. Currently, the application of 100 kg N ha^{-1} in the spring following a harvest is the only recommendation for nutrient management in shrub willow crops in the US [16]. Nonetheless, the majority of the research on nutrient removal focused on the first or first two rotations of willow crops, leaving the remaining rotations and potential impacts of long-term nutrient removal unknown. Furthermore, this research used a wide array of willow cultivars, most of which were established as part of hybrid development programs, that included high and low yielding cultivars, [23], [24] many of which are no longer used. The currently deployed and high yielding cultivars in commercial sites have only received minor assessment. Hence, a better understanding of long-term nutrient removal dynamics, coupled with the management practices and plant genetics that impact them, is required in order to develop clear and accurate nutrient management guidelines for shrub willow crops.

1.3. Harvesting of shrub willow crops

Despite relatively recent interest in shrub willow in the United States and the absence of a stable market for biomass and bioenergy, harvesting equipment and techniques for SRWC have been developed and studied [25]–[27]. However, due to limited scale of SRWC deployment, evolving technology, and management objectives, no dominant system exists [25], [28], though the most common system in the United States is the single-pass, cut-and-chip coppice header (130FB) attached to a Case New Holland (CNH) FR Series forage harvester.

Similar to nutrient management in shrub willow, guidelines and methodologies for harvesting are still being developed. The existing harvesting recommendations state that (1) harvesting should occur after leaf-fall, during the plant's dormancy stage, and (2) operate during frozen ground conditions to protect the soil from compaction [29], [30]. However, following these recommendations is not always possible. In the northeast US, marginal sites where shrub willow crops are commonly planted frequently have drainage limitations, which results in seasonal saturation or near saturation [16]. Additionally, consistent freezing temperatures in NY have been unreliable, sometimes occurring after the first significant snowfall, further limiting access of harvesting machinery to fields. As a result, some willow growers have been conducting harvests during the late growing season (from August until October/November) when leaves are still fully or partially on the plants as well as during the dormant season. The foliage of plants, including shrub willow, has higher nutrient concentrations than other above-ground biomass components of the plants [31]–[34], which raises concerns about the amount of nutrients being removed in leaf-on harvests.

The effect of timing of harvest on shrub willow crop growth and survival has been studied in other crops and regions [35], [36]; however, the effects of timing of harvest on nutrient removal in shrub willow crops in the US has not been studied, other than observations on the seasonal variation of the nutrients in the plant [31], [32]. Given the current harvesting practices being used by willow growers in NY, it is important to understand how timing of harvest impacts shrub willow growth and yield as well as nutrient removal via harvested biomass. The removal of nutrient rich plant parts, such as the foliage, could have serious consequences in the crop's long-term productivity and nutrient management. Although shrub willows are harvested as whole plants, leaving in theory no residues to decompose and supply nutrients to the soil, it has been observed that between 8 – 28% [25], [37] of the total standing biomass is dropped by single pass cut and chip harvesting systems and will remain on site, returning nutrients to the soil.

Commercial harvest operations and research field harvests follow completely different guidelines. Research fields are commonly harvested by hand using brush saws, while commercial sites are harvested with large harvesting machines. Additionally, research fields provide the majority of study results and information used to develop guidelines for commercial sites. In this sense, nutrient removal via harvested biomass in research fields is calculated using strict guidelines and procedures, in which the plants to be harvested are selected according to the diameter range observed, are carefully removed from the field, all its parts are collected, sampled, and analyzed for nutrient content (see Chapter 2 for more information). On the other hand, commercial harvest operations use larger equipment, with vibrating and rotating parts and saws, resulting in broken limbs, stems, and twigs that will remain on site, as previous observations have shown [25], [37]. Thus, nutrient removal results obtained from hand harvested research trial might differ from nutrient removal rates in commercial sites. In fact, although 8 – 28% of the total standing biomass might be dropped and can represent economic losses, the nutrient content of the dropped biomass could be an important input to maintain soil nutrient levels and the crop's long-term productivity.

1.4. Research Objectives

The goal of this dissertation is to study nutrient removal patterns in shrub willow crops depending of the rotation, timing of the harvest, and harvesting method and its impact on soil nutrient levels. The five specific objectives are to:

1. Determine the concentrations in the biomass and total removals of N, P, K, Ca, Mg, and S via harvested aboveground biomass of 18 willow cultivars planted at two locations over three consecutive three-year rotations;
2. Examine if the soil concentrations of N, P, K, Ca, and Mg at two shrub willow trials changed after three three-year rotations;
3. Determine the impact that the timing of harvest has on nutrient removal and aboveground biomass production in four shrub willow cultivars in NY;
4. Investigate the differences between nutrient removal by hand harvesting compared with mechanized harvesting of a commercial willow crop;
5. Estimate the amount of biomass dropped after a mechanized harvest and how this affects estimates of nutrient removal in shrub willow crops

This dissertation is divided into five chapters designed to meet the objectives listed above. Chapter 1 consists of the Introduction with background and objectives. Chapters 2 – 4 are each a separate manuscript, formatted as standard journal manuscripts. Chapter 2 relates to the determination of concentration and removal of N, P, K, Ca, Mg, and S via harvested biomass of 18 willow cultivars at two sites in NY over three three-year rotations and the impacts of the nutrient removal on soil N, P, K, Ca, and Mg levels after three three-year rotations. Chapter 3 reports on the effects of six different harvest dates on nutrient removal and aboveground biomass production of four shrub willow cultivars in a site located in NY. Chapter 4 addresses the specific objectives 4 and 5 by studying the differences between nutrient concentration and removal in shrub willow hand-harvested biomass following research methodology and mechanically harvested biomass in a commercial site, as well as estimating the amount of shrub willow biomass dropped after a mechanized harvest and the nutrient content in this dropped biomass. Chapter 5 consists of overall conclusions of the dissertation as well as some recommendations and considerations for future research.

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CHAPTER 2: NUTRIENT REMOVAL IN SHRUB WILLOW BIOMASS AND CHANGES IN SOIL NUTRIENT CONCENTRATION OVER THREE ROTATIONS

Abstract

The pressing need to mitigate climate change and find alternative uses for marginal agricultural land have stimulated the establishment of short rotation woody crops (SRWC), like shrub willow, in both North America and Europe. There is limited research on the dynamics of nutrient removal over several rotations in these systems and little is known about the long-term impacts of repeated whole-plant harvesting on soil nutrient concentrations. This study compared nutrient removals among 18 cultivars of willow harvested across three three-year rotations at two sites and changes in the soil nutrient concentrations. Nutrient removal was statistically different among rotations for all studied elements in the following order 2011 ≤ 2017 < 2014. For example, K removal was 7 kg ha^{-1} year^{-1} in 2011, 14 kg ha^{-1} year^{-1} in 2017, and 20 kg ha^{-1} year^{-1} in 2014 at the Belleville site. Additionally, significant effects of site (for N and Ca) and cultivar (all elements) were observed. A significant decrease in soil concentrations among years was observed for total N (1,986 g kg^{-1} in 2008 and 1,633 g kg^{-1} in 2017) and P (6.9 g kg^{-1} in 2008 and 3.4 g kg^{-1} in 2017) at one site (Belleville) while a significant increase was observed for K (44 g kg^{-1} in 2008 and 57 g kg^{-1} in 2017) at the other site (Tully). These results show that shrub willow crops are not negatively impacting extractable nutrient reserves and are capable of recycling nutrients effectively over a 10-year period. Adequate nutrient management guidelines for commercial willow sites should be site specific, consider the selection of cultivars deployed given the high variation in nutrient removal among cultivars, and the soil nutritional status.

Keywords: *Salix*, short rotation woody crops, nutrient management, soil fertility, long term productivity

2.1. Introduction

The interest in and establishment of shrub willow as a short rotation woody crop (SRWC) for bioenergy in the Northeast and Midwest was stimulated by the need to reduce greenhouse gases (GHG) emissions, mitigate climate change, replace fossil fuels with renewable energy sources, and find alternative uses for marginal land that will support rural economic development. Since the mid-1980s the development of willow biomass crops in the northeastern US has spurred research and improvements in the system, such as the development of hybrid cultivars [1]–[3], harvesting technologies [4], [5], conversion techniques [6], utilization for bioremediation, environmental benefits, and alternative uses [7]–[11], and programs to incentivize commercialization [12].

Fast-growing woody species, such as shrub willow (*Salix* spp.), are grown in short rotations with more intensive management techniques compared to traditional forestry (e.g. shorter rotations [3-5 years], the use of coppicing, and frequent fertilizer applications), to promote higher yields over multiple-rotations. However, the high growth rates obtained with such techniques may increase nutrient removals (kg ha^{-1} $year^{-1}$) at harvest [13] compared to traditional forestry systems. Studies have suggested that nutrient removal in harvested biomass in natural and planted forests could have potential negative effects on future nutrient availability and productivity depending on harvest techniques and timing, crop age and species, and site conditions [14]–[16]. In a literature review, Eisenbies et al. [14] concluded that, although unclear and site dependent, the removal of harvesting residues from US southern pine forest sites could present a potential negative long-term effect on forest productivity and that an increase of 45-60% in mid-rotation fertilization rates might be needed to replace nutrients removed via harvested biomass that would otherwise serve as a nutrient source for future stands. Similarly, Johnson et al. [17] found that, 33 years after harvesting, soil nutrient concentrations were consistently lower at the whole-tree harvest site compared to a stem only.

In contrast to traditional forest management, SRWC such as shrub willow, are commonly managed using coppicing techniques, which implies the removal of the aboveground biomass at frequent intervals (typically three to four years) with no need to replant due to willow's ability to resprout after being cutback. The biomass harvested from coppice stands may have higher nutrient concentrations compared to the equivalent biomass from a mature forest whole tree harvest [18] due in part to the higher bark:wood ratio of the smaller diameter stems [19] in coppice systems and the higher nutrient concentration of the bark [20], [21]. Additionally, Lodhiyal and Lodhiyal [22] noted that concentrations of nitrogen (N), phosphorus (P), and potassium (K) in poplar trees decreased with age in trees from one to four years, probably as a result of the higher proportion of wood, compared to bark, as the poplar's diameters increased. Hence, on a yearly basis (kg ha^{-1} $year^{-1}$), the harvest of coppice stands at early development stages can potentially remove higher amounts of nutrients than a whole-tree harvest of a mature forest [23], even though the total biomass harvested (Mg ha^{-1}) might be greater in the whole tree harvest.

Nutrient management in willow biomass production systems has been an area of research for many years and focusing on different issues, including species and cultivars [13], [24]–[29]. According to those studies, willow responds differently to fertilization depending on soil type and quality [13], [24]–[27], [29], harvests remove considerable amounts of nutrients in the harvestable biomass [13], [28], and produces high yields with no or minimal fertilization [26], [27]. Furthermore, willow has the capacity to produce positive soil-ecological effects, such as increasing carbon storage and reducing nutrient losses into ground water [30], [31]. However, these results are based on data from one or two three-year rotations, leaving questions about the long-term nutrient demand to support high yield, nutrient removal from soil, and effect on soil quality.

Nutrient removal via harvested biomass in forests and shrub willow crops is positively related. Higher yields results in higher nutrient removal, and vice-versa [13], [24], [28], [32], [33]. Additionally, in recent research, Sleight et al. [34] reported that shrub willow yield at two sites in NY state remained constant over three rotations when first rotation yield was between 8-12 Mg ha^{-1} $year^{-1}$. These results contrast with earlier studies where increasing shrub willow yield trends over two or three rotations are commonly reported [35]–[39], especially when first rotation yield results <8 Mg ha^{-1} $year^{-1}$ [40]. However, considering the results of Sleight et al. [34] (given the geographic location of the study, the cultivars used, and the reported first rotation yields [10-11 Mg ha^{-1} $year^{-1}$]; see [34] for more information) it could be inferred that nutrient removal rates will be consistent over several rotations, or even over the entire life cycle in a shrub willow crop (seven three-year rotations or ~21 years). Changes in nutrient concentrations of willow at a single site over multiple rotations have not been reported. Given the probable consistency in nutrient removal rates, a decline in soil nutrients in later rotations might be expected, which would need to be replaced through fertilization.

The coppicing techniques used in shrub willow crops, where whole plants are harvested and removed from the site raises questions about soil nutrient depletion and the effects it could have on the crop's long-term productivity. Additionally, the constant research and development of higher yielding cultivars [1]–[3] raises concerns about a possible increase in the amounts of nutrient removed during harvest. With the limited knowledge on the long-term nutrient removal dynamics in shrub willow crops and on the impacts caused on soil nutrient levels, site degradation and nutrient depletion could occur if nutrients are not managed correctly. Furthermore, depending on the possible impact of nutrient removal on soil nutrient levels, management costs could increase if additional fertilization is required. The establishment of SRWC in the United States [41] will demand more precise nutrient management and fertilizer guidelines based on recent and local data obtained from studies conducted using the most recently developed and commercially available cultivars, to ensure that maximum yields are obtained and soil nutrient levels are preserved, which could contribute to minimized or optimized costs. Additionally, understanding nutrient management is an important part of developing these recommendations and guidelines. In this context, the objectives of this project are (1) to determine the concentrations in the biomass and total removal rates of N, P, K, Ca, and Mg via harvested aboveground biomass of 18 willow cultivars planted at two locations (Tully and Belleville, NY) over three three-years rotations and (2) to examine if the soil concentrations of N, P, K, Ca, and Mg at the two shrub willow trials changed after three three-year rotations.

2.2. Materials and methods

2.2.1. Site description

The two study sites in this project were established in May 2005 in Tully, NY (42º47’30”N, 076º07’30”W) and Belleville, NY (43º47’19”N, 076º07’49”W). The soil at Tully is a Palmyra gravelly loam, well-drained to excessively well-drained, fine-loamy over sandy or sandy-skeletal, mixed, active mesic Glossic Hapludalf, and a depth to water table and to bedrock greater than 203 cm [42]. Root pit excavations at this site at the end of the third rotation indicated that soil depth at this site was limited by a shale layer at 40-60cm depth. The soil at Belleville is defined as a well-drained to moderately well-drained Galway silt loam, coarse-loamy, mixed, superactive, mesic Typic Eutrudept, with a depth to seasonal high water table ranging from 46 to 102 cm and a depth to bedrock of 51-102 cm [43] (See [44] for more information in soil characteristics at the two sites). Mean annual precipitation (1039 – 1104 mm) and annual growing degree days (967 – 1193 GDD) fall within similar ranges for both locations (see [40] for more information).

Both sites were planted with the same suite of 18 shrub willow cultivars (see [34] for more information in cultivars origins, species, and diversity groups). The site was planted in four blocks with one plot of each cultivar planted at each block. In May 2005, twenty-five-centimeters-long dormant willow cuttings were planted in double rows with spacing of 1.5 m between double rows, 0.76 m within double rows, and 0.61 m between plants, creating 6.86 x 7.92 m cultivar plots consisting of 78 willow plants each distributed in three double rows and a planting density of 14,400 plants ha^{-1}. Each plot had an effective measurement area of 12.54 m^2, which was the center of the plot consisting of 18 plants in the center double row. A border area of a single double row on each side and the remaining plants at the extremes of the rows surrounded the measurement area. Management techniques of the trials consisted of herbicide applications with oxyfluorfen (Goal 2XL, 1.1 kg ai ha^{-1}) and simazine (Princep, 2.2 kg ai ha^{-1}) immediately after planting, coppicing after the first growing season in January 2006, and on the application of 100 kg N ha^{-1} application as urea after coppicing and after each harvest.

2.2.2. Field activities

Harvests occurred every three years in the same year/season at both locations. Each harvest occurred during the dormant season after leaves had dropped. The first harvest occurred in December 2008, after the fourth growing season (third post-coppice growing season), when the plants were three years old above ground and four years old below ground. The data for the project were collected during the second, third, and fourth harvests, which occurred in the winters of 2011-2012, 2014-2015, and 2017-2018, when the plants were seven, ten, and thirteen years old below ground, respectively, and three years old above ground. All above ground material was removed during harvests, which were performed by cutting the stems in the measurement plots approximately 5-15 cm above the soil surface with brush saws. The aerial parts of the plants were completely removed from the field (stems, bark, branches, and twigs) leaving few residues to decompose and provide nutrient to the soil other than the leaf material lost by senescence. The harvested biomass was collected in bundles and the fresh weight was measured using a hanging field scale. A 1-2 kg sample from each plot, obtained from chipping three stems with different diameters (small, medium, and large, relative to the diameter range at each plot), was collected in paper bags and weighed in the field, and later dried at 60ºC to a constant weight to determine its moisture content. Using the moisture content value, the dry yield was calculated based on the amount of biomass weighed from the measurement area divided by the size of the measurement area; annual yield was then calculated dividing dry yield by the rotation period (3 years) and scaled up to megagrams (Mg) per hectare per year. Stems in the border rows were harvested using a single pass cut and chip harvester.

Soil samples were collected in the Fish Creek and SX64 plots at both sites in the spring after the harvests performed in 2008 and 2017. Soil augers were used to collect two samples in the plots (one between double rows and one within a double row) and at two different depths (0-20 cm and 20-40 cm). Samples from the same depths were combined into one sample in a paper bag, generating two samples per plot, for a total of 16 samples per site and per harvest.

2.2.3. Laboratory procedures

Nutritional analyses of the plant biomass were performed on a subsample of the chips collected during the harvest. Representative samples were obtained from the chipped and dried samples and ground in a Willey Mill using a 40-mesh screen to produce 300 – 400 g samples. Samples of 3-5 grams were used for the nutritional analyses of the biomass, which were performed at the Agricultural Analytical Services Lab at the Pennsylvania State University. Determination of total N was done through the micro-Kjeldahl method while the determination of P, K, Ca, and Mg was performed through the microwave acid digestion method and the inductively coupled plasma atomic emission spectrometry (ICP-AES). The soil samples were air-dried for 10-15 days, after which soil aggregates were ground and crushed with a mortar and pestle, and sifted to separate rocks and roots from the soil. A subsample of each soil sample was analyzed at the Dairy One Soil Testing Laboratory in Ithaca, NY. The Morgan method was used to determine P, K, Ca, and Mg, while Total N was calculated through the combustion method using a LECO analyzer.

2.2.4. Statistical analyses

Nutrient concentration can be defined as the amount of nutrient in the biomass, expressed as grams of nutrient per kilogram of biomass (g kg^{-1}). Using the nutrient concentration and yield values nutrient removal was determined for each plot for each of the three harvests. Nutrient removal is the amount of nutrient removed from the site by the crop at the time of harvest, expressed as kilograms of nutrient removed per hectare (kg ha^{-1}).

The experimental design, crop management, and harvesting techniques have been the same for both sites since the crops' establishment; hence, differences in nutrient removal and soil nutrient concentration can be attributed to year, site, cultivar, and genotype x environment effects, eliminating possible effects caused by extraneous variables. Both sites consisted of a randomized complete block design (RCBD) with four blocks and 18 cultivars per block, resulting in a random effect of blocks nested within sites. Also, for the soil nutrient concentration analysis, the two depths (0-20 and 20-40 cm) were analyzed separately, in order to better observe the interaction between the variables and have a cleaner result. The annual yield (Mg ha^{-1} $year^{-1}$), defined as the average of the sum of the yields of the four blocks divided by the rotation period (three years), was calculated for each cultivar at each site. Additionally, annual nutrient removal (kg ha^{-1} $year^{-1}$) of each element, defined as the average of the total removal on the four blocks divided by the rotation period, was calculated for each cultivar by multiplying nutrient concentration by annual yield. The statistical analyses were performed with SAS® version 9.4 at a critical level α of 0.05. Interactions terms were tested at an α level of 0.15, in order to reduce chances of committing type I error [45]. Mixed models were built using Generalized Linear Mixed Models (GLMM) analysis to estimate the effects of year, site, cultivar, and the interactions year:site (YxS), year:cultivar (YxC), site:cultivar (SxC), and year:site:cultivar (YxSxC) on nutrient concentration and removal. The GLIMMIX procedure for GLMM from SAS 9.4® was used, since it allows for random effects in the model. Year, site, cultivar, depth, and the interactions between them were considered as fixed effects, while blocks nested within sites (for nutrient removal) and depth nested within blocks nested within sites (for soil nutrient concentration) were considered as random effects.

2.3. Results

Results of the annual biomass production and nutrient concentration in the biomass will be briefly mentioned in this section, since they are crucial for the determination of nutrient removal via harvested biomass; however, considering that the objective of this research is to determine the pattern of nutrient removal and its impact on soil nutrient concentration over three rotations, no further discussion will be elaborated on the annual biomass production or biomass nutrient concentration unless necessary whenever they provide relevant insight into the removal pattern.

2.3.1. Annual biomass production

Overall, mean annual biomass production across years, sites, and cultivars was 8.9 Mg ha^{-1} $year^{-1}$. Significant effects of year, cultivar, YxS, YxC, and SxC were observed (Table 2.1). A significant effect of the interaction YxS could be observed by the greater biomass production observed at Belleville in 2011 (10.6 Mg ha^{-1} $year^{-1}$) compared to Tully (9.1 Mg ha^{-1} $year^{-1}$), which was not observed in 2014 and 2017, when yield was similar at both sites. The YxC interaction was statistically significant (Figure 2.1A) with eleven of the cultivars across a range of the diversity groups showing a decrease in yield over the three rotations while six cultivars showed no significant change in yield. One cultivar (S25) showed a slight increase then decrease in yield, but the yields of this cultivar were low (< 6 Mg ha^{-1} $year^{-1}$) in all rotations. The SxC interaction was significant with cultivars 9837-77, Fish Creek, Millbrook, and SV1 having significantly higher yield at Belleville compared to Tully, while no differences were observed on the other cultivars (Figure 2.1B). Across sites and cultivars, mean annual biomass production was significantly greater in 2011 (9.85 Mg ha^{-1} $year^{-1}$), followed by 2014 (8.97 Mg ha^{-1} $year^{-1}$), and lower in 2017 (7.79 Mg ha^{-1} $year^{-1}$) (Figure 2.1A). Across years and sites, the highest yielding cultivar was Oneonta (11.3 Mg ha^{-1} $year^{-1}$), followed by SX61 (10.9 Mg ha^{-1} $year^{-1}$), and SV1 (10.7 Mg ha^{-1} $year^{-1}$); while the lowest yielding were 9837-77 (5.8 Mg ha^{-1} $year^{-1}$), 9832-49 (4.8 Mg ha^{-1} $year^{-1}$), and S25 (4.1 Mg ha^{-1} $year^{-1}$) (Figure 2.1A).

Table 2.1. ANOVA results for the effect of year, site, and cultivar on yield and nutrient concentration of different elements. Main effect significance determined using α=0.05 and interactions with α=0.15. Significant effects are presented in bold format.

	p-values						
Parameters	Year (Y)	Site (S)	Cultivar (C)	YxS	YxC	SxC	YxSxC
df	2	1	17	2	34	17	34
Yield	**<0.0001**	0.7599	**<0.0001**	**0.0004**	**0.0832**	**<0.0001**	0.6992
N	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0175**	**0.0099**	**0.1271**
P	**<0.0001**	**0.0084**	**<0.0001**	**<0.0001**	**0.0062**	**0.0002**	**0.04**
K	**<0.0001**	0.0987	**<0.0001**	**<0.0001**	**0.0026**	**0.0726**	0.211
Ca	**<0.0001**	**0.0014**	**<0.0001**	**<0.0001**	**<0.0001**	**0.1069**	0.3395
Mg	**<0.0001**	0.2975	**<0.0001**	**<0.0001**	**<0.0001**	0.1907	**0.0031**

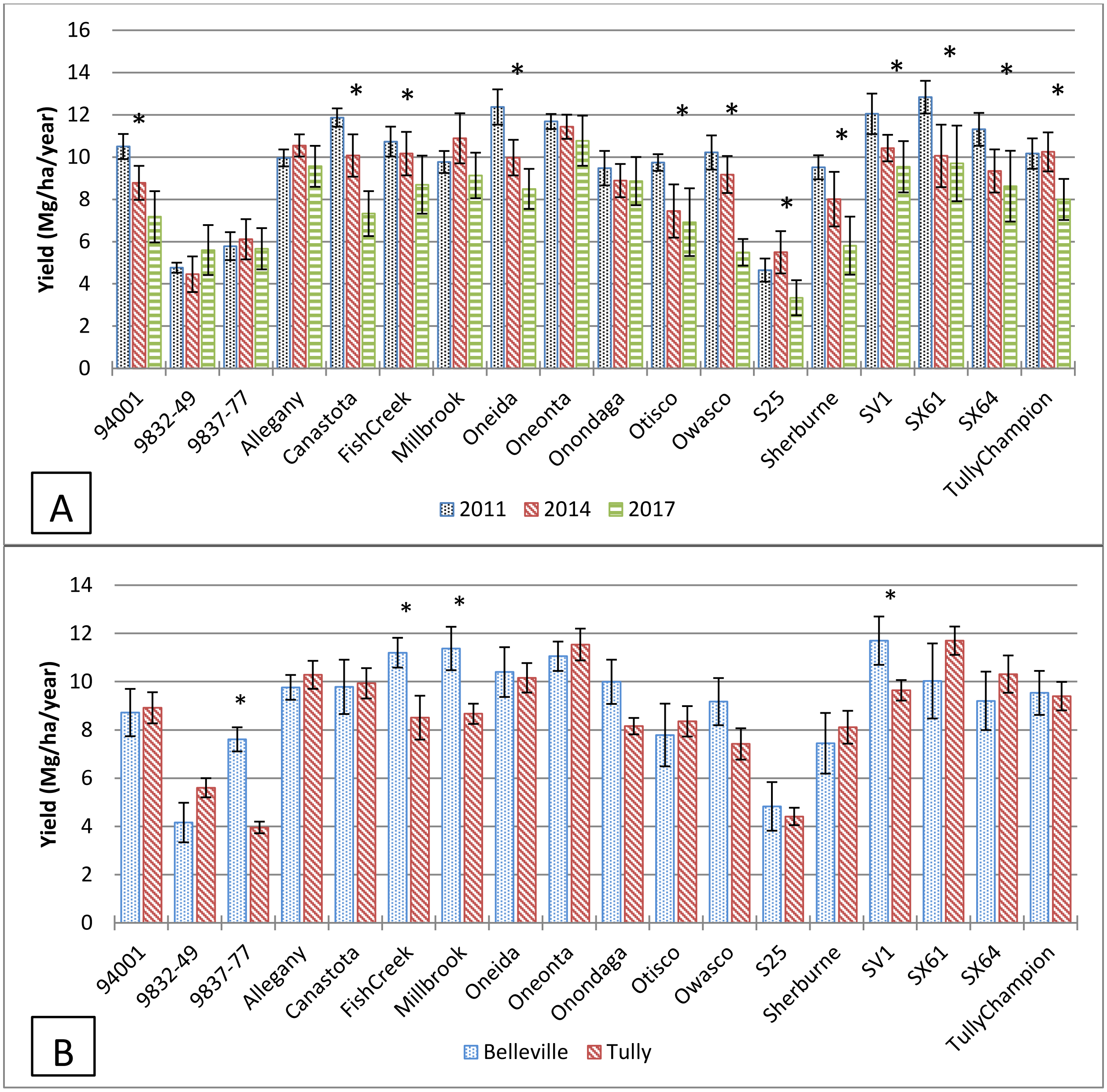

Figure 2.1. Average annual biomass production (mean ± Standard Error [SE]) of 18 willow cultivars: (a) over three rotations (rotations 2, 3, 4) across two sites and (b) across years at two sites averaged across three rotations. Statistically significant differences among years (A) and between sites (B) are indicated by asterisks (*).

2.3.2. Nutrient concentration

Nutrient concentration varied widely depending on year, site, and cultivar. Nutrient concentration ranges were 3-3.9 g N kg^{-1}, 0.5-0.7 g P kg^{-1}, 1.2-1.9 g K kg^{-1}, 3.4-9.2 g Ca kg^{-1}, 0.2-0.4 g Mg kg^{-1}, and 0.2-0.3 g S kg^{-1} across years and sites. The overall ranking of average element concentration followed the order Ca > N > K > P > Mg, which was also observed across sites and cultivars for the years 2014 and 2017, while for 2011 the order was N > Ca > K > P > Mg. Statistically significant effects of year, site, cultivar, YxS, YxC, SxC, and YxSxC were observed in nutrient concentrations in the harvested biomass (Table 2.1). The year effect was statistically significant for all elements, as well as the interaction YxS. At Belleville, the concentration of P, K, Ca, and Mg in the biomass followed the pattern 2014 > 2017 > 2011 (Figure 2.2), whereas the concentration of N was 2017 = 2014 > 2011. On the other hand, the observed biomass nutrient concentration at Tully either increased over the years (K, Ca, Mg) or was constant in 2011 and 2014 but higher in 2017 (N, P) (Figure 2.2). Overall nutrient concentrations of all elements were significantly lower in 2011 than in 2014 and 2017 (Figure 2.2). Cultivar and YxC effects were statistically significant for all elements (Table 2.1). Pair-wise comparisons indicated that, overall, cultivars S25 (5 g N kg^{-1}; 0.8 g P kg^{-1}; 0.4 g Mg kg^{-1}) and Canastota (2.0 g K kg^{-1}; 9.3 g Ca kg^{-1}) had significantly higher nutrient concentrations in their biomass. However, the YxC interaction resulted in different cultivars having highest and lowest concentration in their biomass, depending on the year and nutrient considered. Site had significant effects on the concentration of N, P, and Ca (Table 2.1), resulting in consistently higher levels in the biomass harvested at Belleville than at Tully.

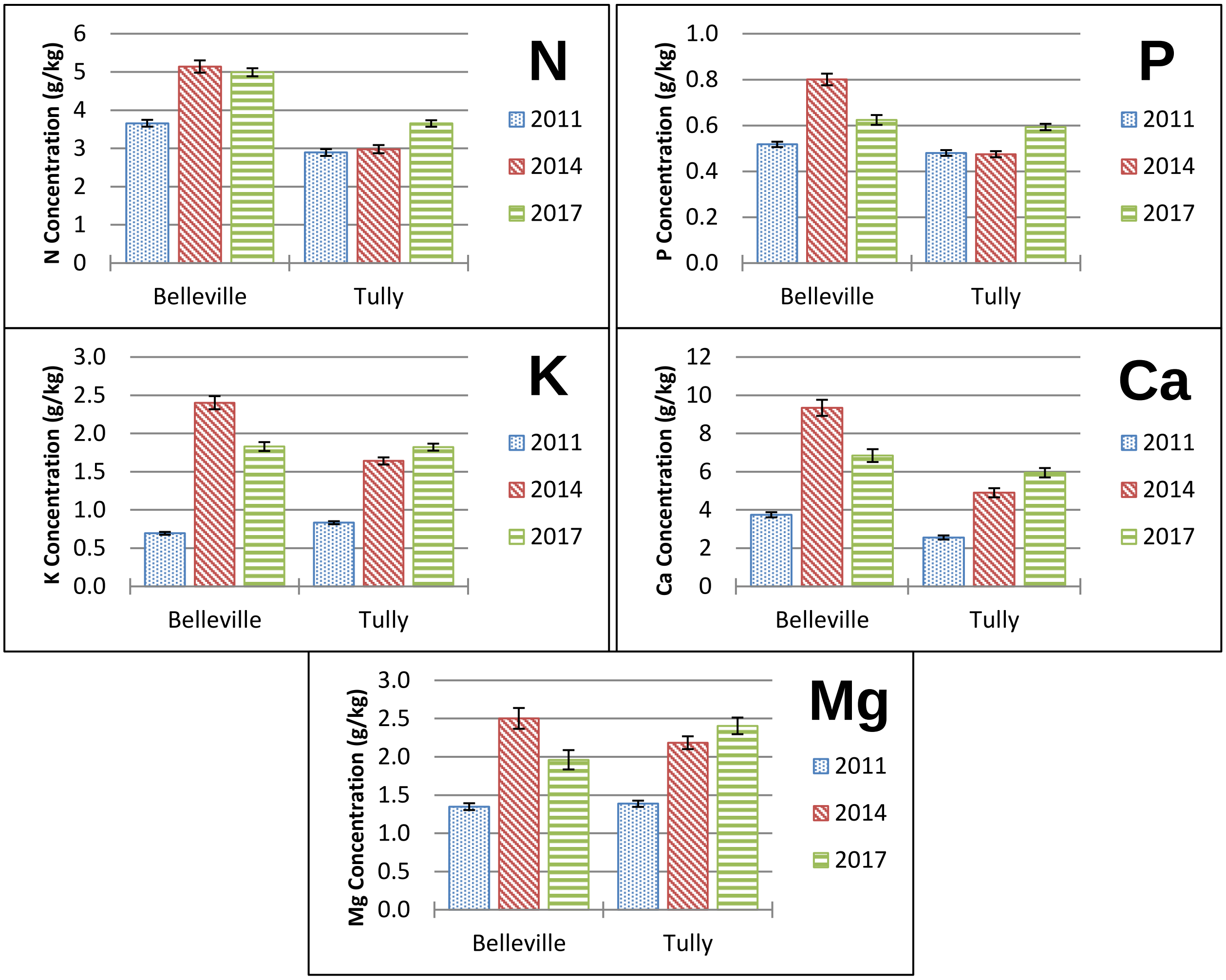

Figure 2.2. Nutrient concentration (mean ± SE) in shrub willow biomass by year and site. Effects of year, site, and the interaction between year and site (YxS) are visible.

Finally, the interaction SxC was significant for N, P, K, and Ca (Table 2.1), while the interaction YxSxC was significant for N, P, and Mg indicating that a two-way interaction (YxS, YxC, or SxC) varies across the levels of the third variable. Using N as an example, simple effects of year showed N in 2017 = 2014 > 2011, while the main effect of site showed higher N at Belleville compared to Tully. However, there was a year by site interaction than influenced these patterns (Figure 2.3). Additionally, the interaction YxS was inconsistent, depending on the cultivar considered (Figure 2.3), explaining the three-way interaction.

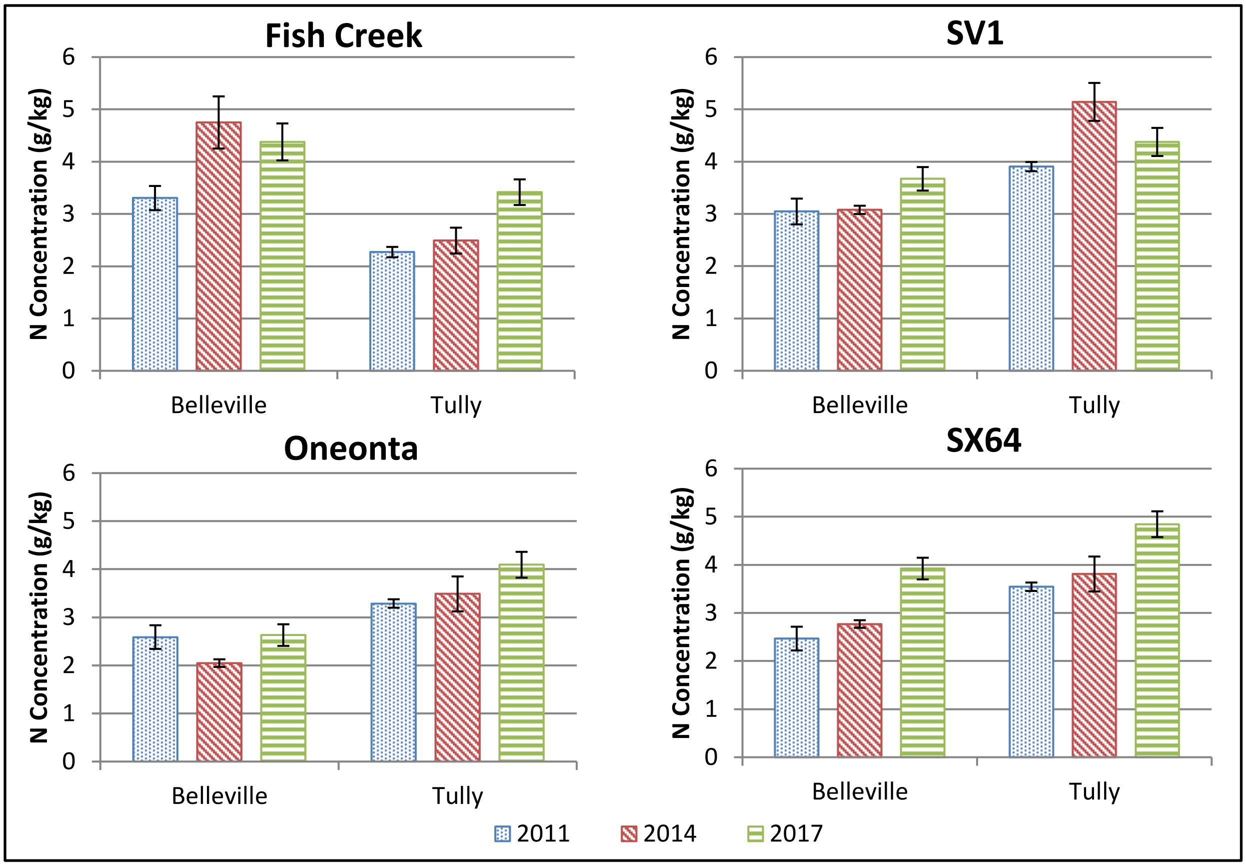

Figure 2.3. Nitrogen concentration (mean ± SE) on four different cultivars, displaying the effects of the YxS and YxSxC interactions. Threeway interaction (YxSxC) can be observed on the different patterns depending on year, site, and cultivar.

2.3.3. Nutrient removal

Average nutrient removal showed a varying behavior depending on the element observed and the year, site, and cultivar. Overall, a slight positive relationship was observed between yield and nutrient removal for all studied elements, indicating higher nutrient removal by higher yielding cultivars (Figure 2.4). Across years and sites annual nutrient removal ranges were 21.5-43.8 kg N ha^{-1} $year^{-1}$, 3.4-6.3 kg P ha^{-1} $year^{-1}$, 7.2-19.3 kg K ha^{-1} $year^{-1}$, 23.8-85.1 kg Ca ha^{-1} $year^{-1}$, and 1.3-2.6 kg Mg ha^{-1} $year^{-1}$ (Figure 2.5). Overall, the ranking of average nutrient removal across years, sites, and cultivars followed the order Ca > N > K > P > Mg, which was also observed across sites and cultivars at each individual year (2011, 2014, and 2017) (Figure 2.5).

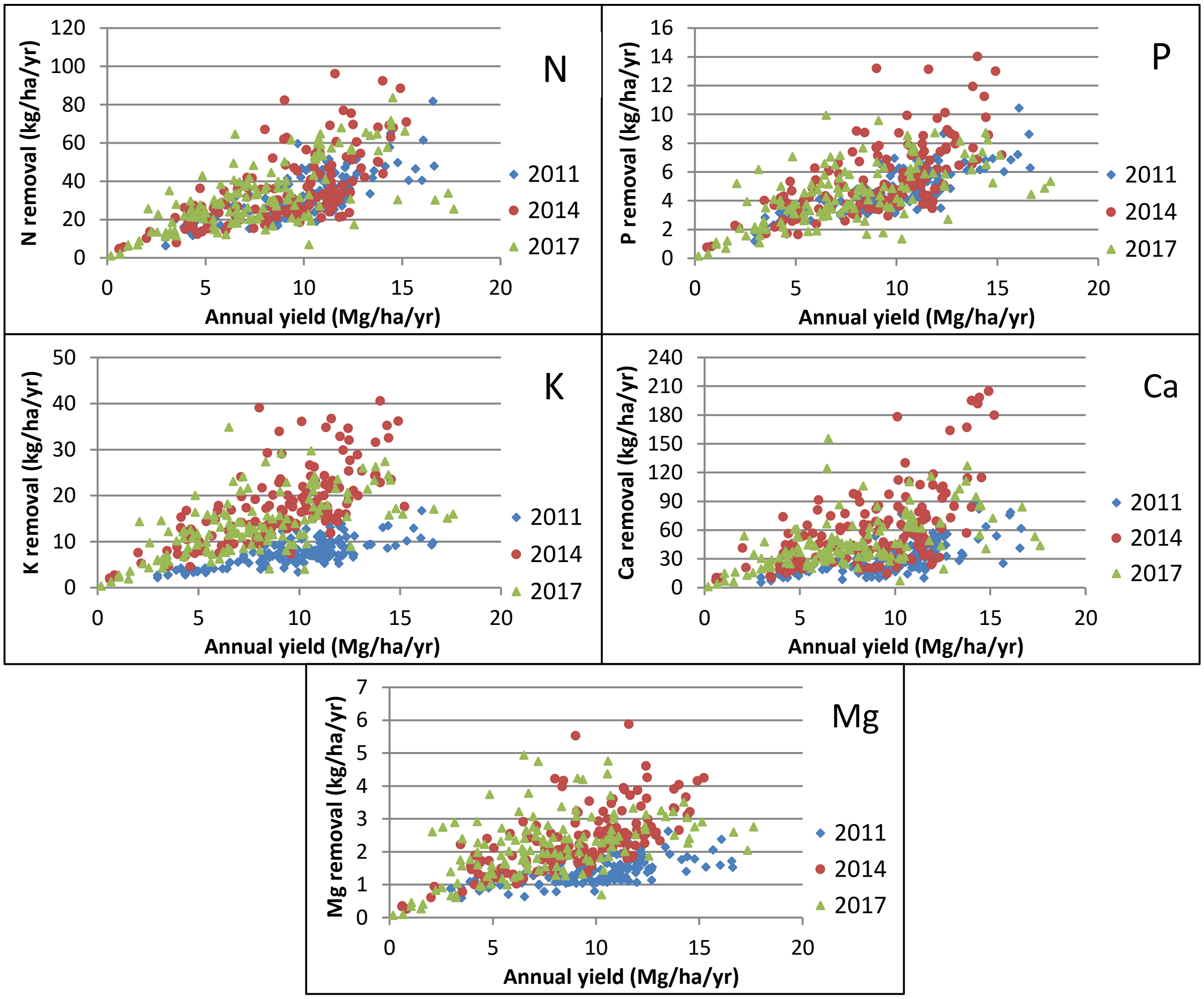

Figure 2.4. Scatterplot illustrating positive linear relationship between removal of different nutrients and annual yield for willow biomass crops over three rotations.

The YxS interaction was significant for removal of all the nutrients (Table 2.2, Figure 2.6). At Belleville, a pattern of peak nutrient removal was observed in 2014 for all elements, except for N which was not significantly different over the years, but followed a similar pattern with the largest removal occurring in 2014. At Tully, more variability was observed among the elements studied, where N and P removals were constant over the years, K and Ca increased from 2011 to 2014, remaining constant in 2017, and Mg increased over the years. Similar to the observations on nutrient concentration, nutrient removals during the year of 2011 were significantly lower than removals in 2014 and/or 2017.

Table 2.2. Analysis of variance for nutrient removal from 18 willow cultivars at two sites and harvested over three rotations. Main effect significance determined using α=0.05 and interactions with α=0.15. Significant effects are presented in bold format.

Parameters	*p*-values						
	Year (Y)	Site (S)	Cultivar (C)	YxS	YxC	SxC	YxSxC
df	2	1	17	2	34	17	34
N	**0.0444**	**0.0448**	**<0.0001**	**0.0612**	0.3026	**0.003**	0.3863
P	**<0.0001**	0.0697	**<0.0001**	**<0.0001**	0.8114	**0.003**	0.842
K	**<0.0001**	0.3543	**<0.0001**	**<0.0001**	0.2689	**0.0516**	0.6355
Ca	**<0.0001**	**0.0194**	**<0.0001**	**<0.0001**	0.2019	**0.1221**	0.5445
Mg	**<0.0001**	0.7136	**<0.0001**	**<0.0001**	0.282	**<0.0001**	0.3861

The interaction SxC was significant for all elements. Cultivars removed more N, P, K, and Ca at Belleville than at Tully, while two cultivars had higher Mg removals at Tully and two at Belleville (Figure 2.5). The site effect on the removal of N and Ca showed removals significantly higher at Belleville (N: 40.2 kg N ha^{-1} $year^{-1}$; Ca: 56.8 kg Ca ha^{-1} $year^{-1}$) than at Tully (N: 26.6 kg N ha^{-1} $year^{-1}$; 37.5 kg Ca ha^{-1} $year^{-1}$) (Figure 2.6).

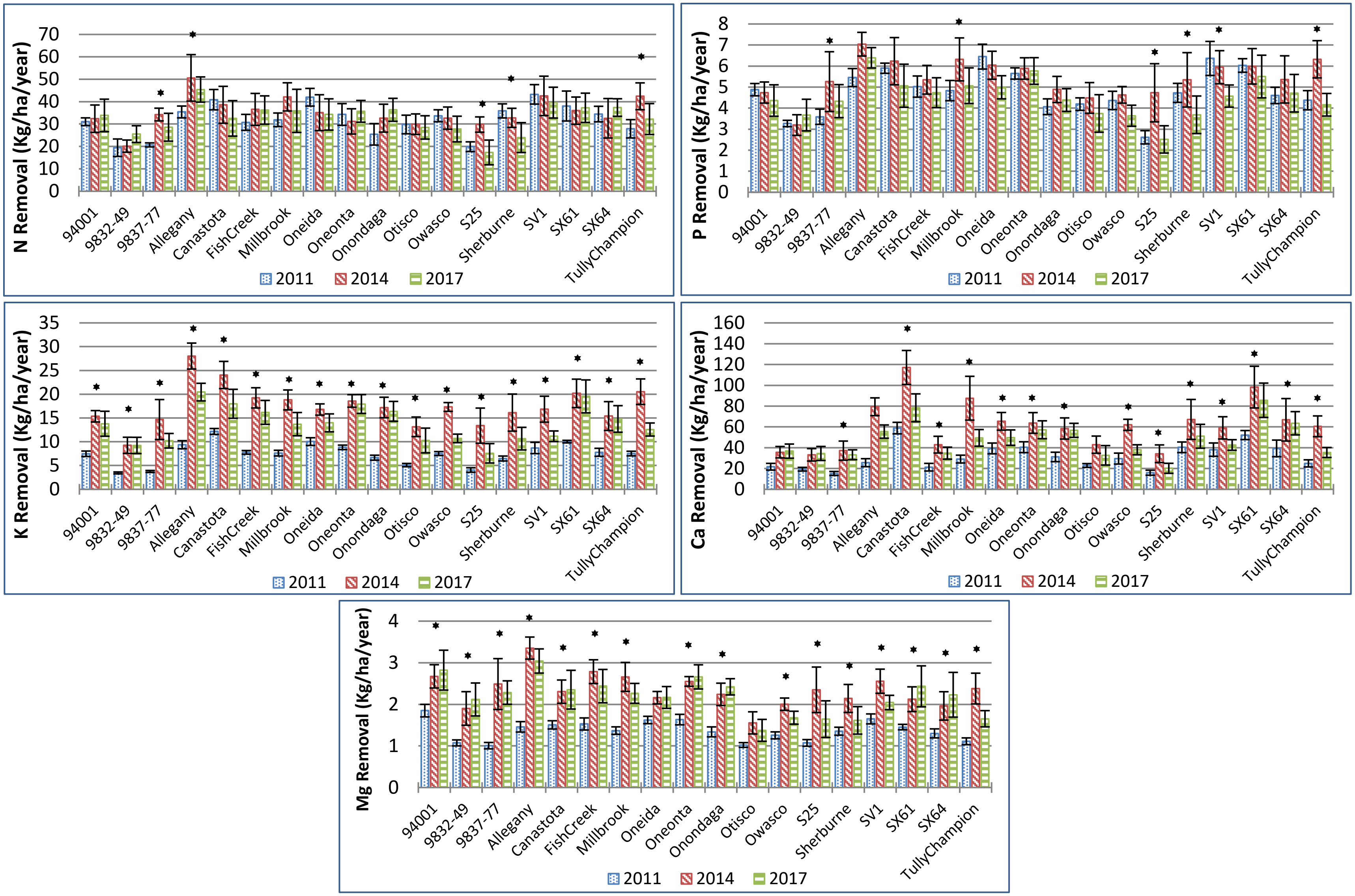

Figure 2.5. Removal of N, P, K, Ca, and Mg via harvested biomass (mean ± SE) of 18 shrub willow cultivars during the years of 2011, 2014, and 2017 and across two sites. Statistically significant differences among years are indicated by asterisks (*) and determined using α=0.05.

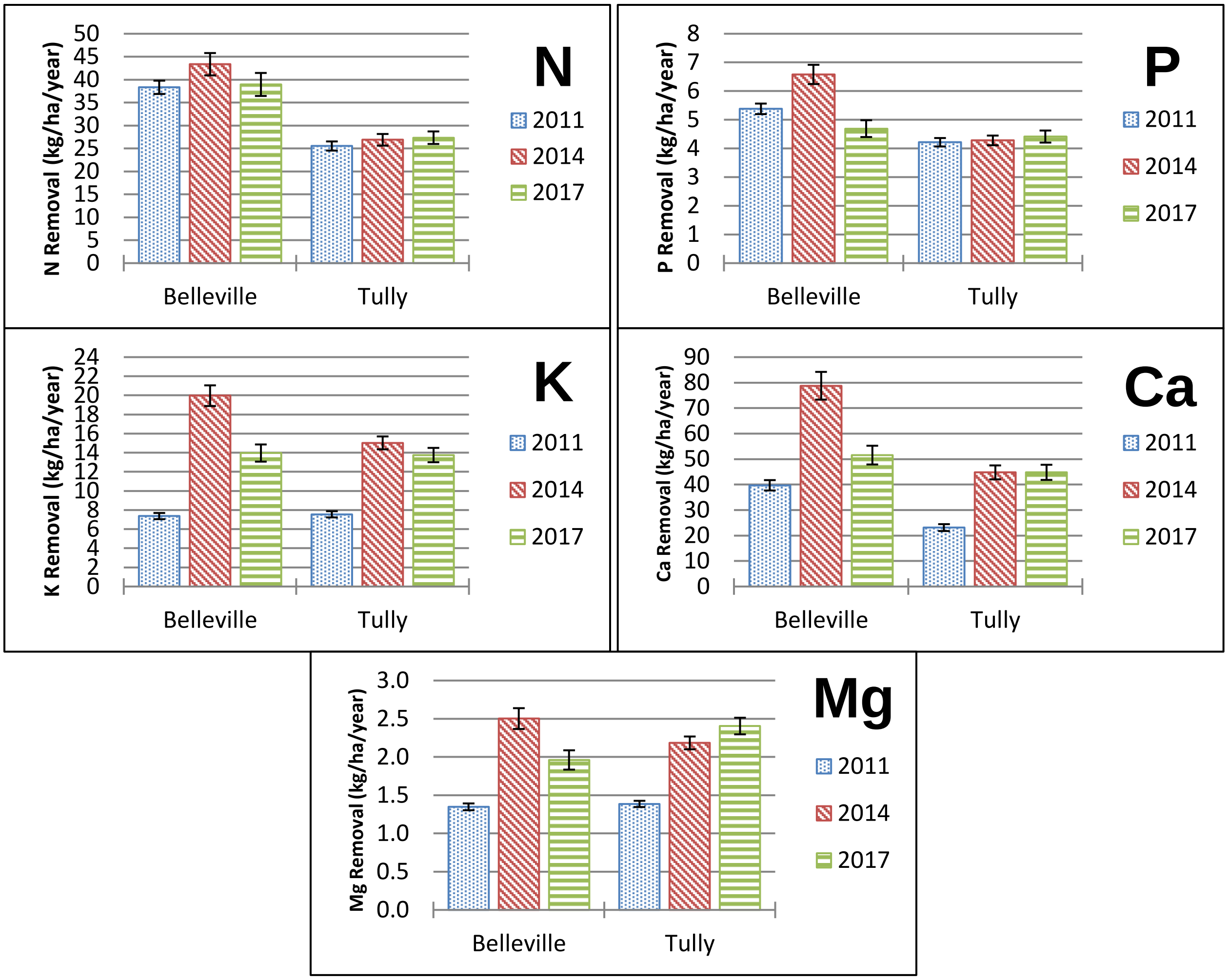

Figure 2.6. Nutrients removed **(mean ± SE)** in harvested willow biomass crops over three rotations at two sites in NY (YxS interaction). Significant differences observed between sites for N and Ca.

2.3.4. Soil nutrient concentrations

Soil nutrient concentrations at the two depths showed different results depending on the element observed and the year, site, and cultivar (Table 2.3). The ranking of average overall soil nutrient concentration across years, sites, cultivars, and depth was Ca > N > Mg > K > P. In 2017, the same ranking was observed, while in 2008 it was N > Ca > Mg > K > P, which highlights the decrease in total N levels from 2008 to 2017. Overall, average soil nutrient concentration ranges across years, sites, and depth was 1,622.5-1,736.8 mg N kg^{-1}, 3.1-3.5 mg P kg^{-1}, 52.1-52.5 mg K kg^{-1}, 1,568.3-1,801.7 mg Ca kg^{-1}, and 61.6-69.7 mg Mg kg^{-1} (Table 2.4). The YxS interaction was statistically significant for N and P on the 20-40 cm layer and for K on the 0-20 cm layer (Figure 2.7). The concentrations of N and P at the 20-40 cm layer at Belleville were higher in 2008 (1,869 mg N kg^{-1} and 5.0 mg P kg^{-1}) compared to 2017 (1,196 mg N kg^{-1} and 1.8 mg P kg^{-1}) (Table 2.4), but the concentration of these elements did not change over time at Tully and at the 0-20 cm layer at Belleville. Concentrations of K at Tully increased from 2008 to 2017 at the 0-20 cm layer but not at Belleville, while a reduction occurred at both sites at the 20-40 cm layer. There were no significant changes in Ca and Mg over time at the two sites. Concentrations of Ca were more than 2x higher at Belleville than Tully but there was no difference in Mg at the two sites.

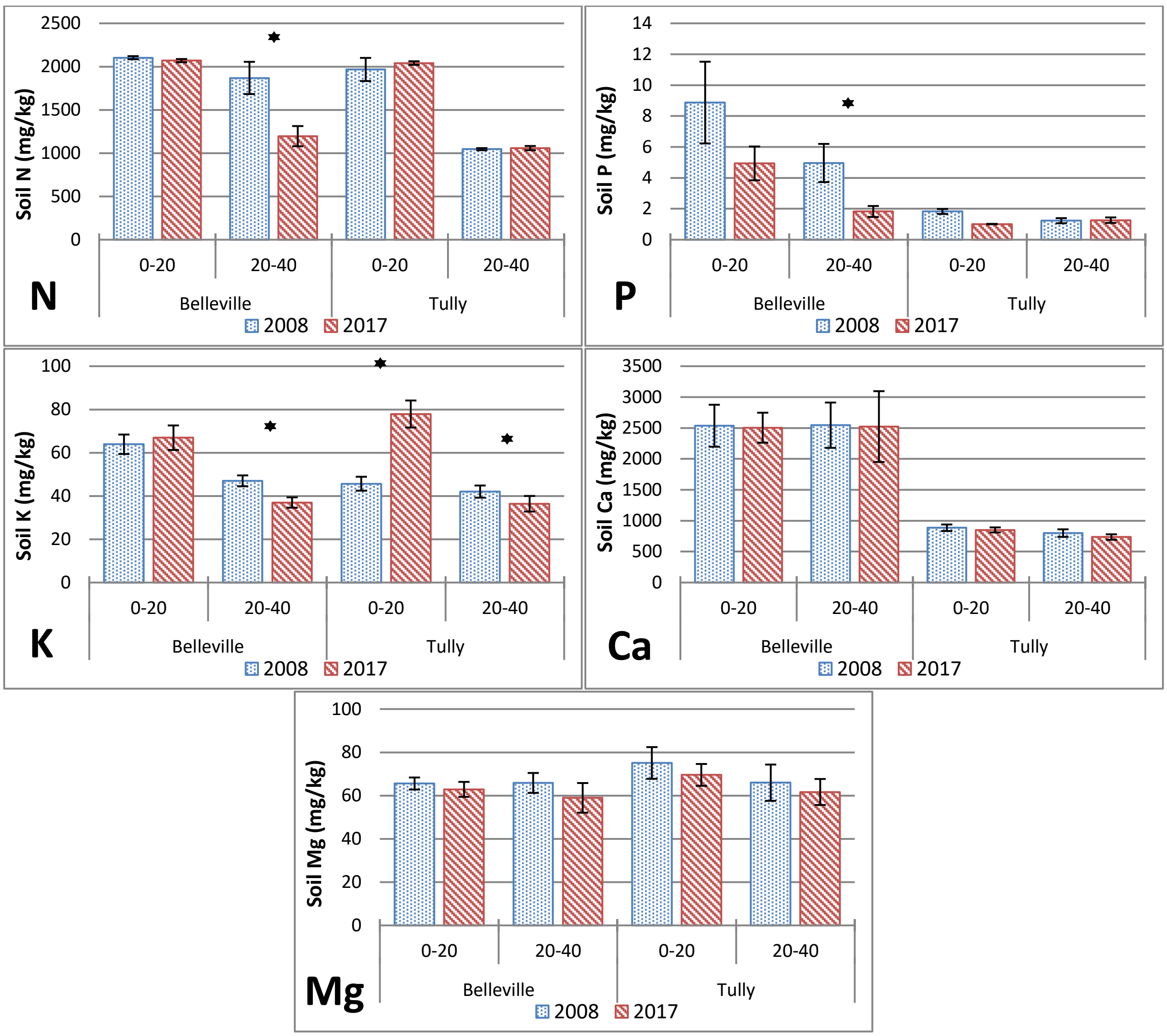

Figure 2.7. Soil nutrient concentration (mean ± SE) under shrub willow crops by year, site, and depth. Visible significant effect of year, site, and YxS. Significant differences between years are indicated by asterisks (*)

Table 2.3. Summary of analyses of variance for the effect of year, site, cultivar, and the interactions between the main effects on soil nutrient concentration at two depths. Significant values tested at α=0.05 for main effects and α=0.15 for interactions and presented in bold.

	Year	Site	Cultivar	YxS	YxD	Year	Site	Cultivar	YxS	YxD
	0-20 cm					20-40 cm				
Df	1	1	1	1	1	1	1	1	1	1
N	0.7808	0.2603	0.8565	0.4571	0.4633	**0.0029**	**<0.0001**	0.0564	**0.0026**	0.2003
P	0.1286	**0.0013**	0.8124	0.311	0.7621	**0.0392**	**0.0047**	0.4283	**0.0299**	0.4593
K	**0.0015**	0.4675	0.6521	**0.0066**	0.773	**0.0042**	0.3686	0.2802	0.4963	0.7501
Ca	0.8815	**<0.0001**	0.6485	0.9941	0.8725	0.908	**<0.0001**	0.2635	0.9552	0.8113
Mg	0.4347	0.1304	0.4729	0.7873	0.8168	0.4381	0.8964	0.058	0.7704	0.9303

Table 2.4. Soil nutrient concentration at two depths and under two shrub willow cultivars plots at Tully and Belleville after the first (2008) and fourth (2017) rotations. Numbers in parenthesis represent standard errors

	pH		N		P		K		Ca		Mg	
			mg kg^{-1}									
Belleville	2008	2017	2008	2017	2008	2017	2008	2017	2008	2017	2008	2017
Fish Creek												
0-20 cm	6.4(0.4)	6.6(0.4)	2110(26)	2094(28)	8.2(4.7)	5.0(2.2)	56.6(5.8)	62.1(7.1)	2428(456)	2427(399)	65.6(4.5)	61.4(3.7)
20-40 cm	6.5(0.4)	6.9(0.3)	2199(84)	1294(238)	4.1(1.5)	1.9(0.5)	45.4(5)	34.1(2.8)	2286(269)	2059(228)	63.1(7.1)	53.2(10.6)
SX64												
0-20 cm	6.6(0.3)	6.9(0.3)	2095(27)	2044(19)	9.5(3.2)	4.9(1.0)	71.3(5)	71.8(9.2)	2645(568)	2584(335)	65.6(4)	64.3(6.4)
20-40 cm	6.8(0.3)	7.1(0.2)	1538(287)	1098(21)	5.9(2.1)	1.8(0.6)	48.7(1.7)	39.9(3.7)	2803(714)	2988(1156)	68.6(6.7)	64.7(9.2)
Tully												
Fish Creek												
0-20 cm	4.9(0.1)	5.3(0.1)	1893(282)	2056(43)	1.7(0.1)	1.0(0.0)	52.2(4.2)	79.1(11.2)	856(97)	863(71)	70.1(11.6)	68.5(8.3)
20-40 cm	5.0(0.1)	5.5(0.1)	1042(23)	1029(10)	1.1(0.1)	1.2(0.2)	48.1(4.2)	41.4(6.5)	711(107)	700(71)	55.1(9.6)	54.2(8.2)
SX64												
0-20 cm	4.9(0.0)	5.2(0.0)	2040(14)	2025(7)	2.0(0.3)	1.0(0.0)	39.2(1.5)	76.7(7.5)	915(54)	839(51)	80.1(10)	70.7(7.1)
20-40 cm	5.1(0.1)	5.4(0.2)	1048(32)	1086(46)	1.4(0.3)	1.3(0.3)	37.5(1.6)	31.5(1.3)	866(62)	772(58)	74.1(12.2)	69.2(8)
Overall	5.8(0.2)	6.1(0.2)	1768(92)	1591(89)	4.3(0.9)	2.3(0.4)	49.9(2.2)	54.6(4)	1720(198)	1654(215)	68.2(3)	63.3(2.7)

2.4. Discussion

As previously mentioned, given the objective of the project, the discussion will focus on the nutrient removal pattern across rotations and its relationship to soil nutrient concentration changes. Additionally, while the results of nutrient removals for 18 cultivars was provided and analyzed, the discussion section will focus on the nutrient removals of the three top yielding cultivars (Oneonta, SV1, and SX61) and the two cultivars where soil samples were taken (SX64 and Fish Creek), which will be referred to as “top cultivars”. These cultivars, in addition to being connected to the soil nutrient concentration results, are commercially available and could be the most likely to be recommended for commercial scale plantings. Hence, by focusing on these five cultivars the discussion is focused on potential nutrient removal scenarios in larger plantings.

2.4.1. Nutrient removal patterns

When focusing on the top cultivars no significant differences in N and P removals were observed among rotations, while significantly higher removals of K, Ca, and Mg during 2014 and 2017 compared to 2011 occurred (Figure 2.8). The reason for the significant difference could be explained by higher yield, given the observed positive linear relationship between yield and nutrient removal [13], [24], [28], [46]. However, this relationship, although observed, does not explain the removals of all nutrients. Overall, higher yields were observed in 2011, compared to 2014 and 2017 (Figure 2.8), while higher removal rates were observed in 2014 and 2017 compared to 2011. According to the positive relationship between yield and nutrient removal, it would be expected to see higher removals in 2011. Regression analyses between nutrient removal, nutrient concentration, and yield (data not shown) indicated that nutrient removal is highly related to both nutrient concentration and yield and is well determined by the combination of both factors; however, depending on the element observed, either nutrient concentration or yield will have a stronger effect and higher impact on nutrient removal patterns. Research has suggested that nutrient concentration and yield share an inverse relationship, in which higher nutrient concentrations are observed in lower yielding cultivars, as a result of lower wood:bark ratio as the stems grow larger in diameter and higher nutrient levels found in the bark [20], [22], [47], [48]. There is evidence of a trend towards higher number stems with smaller diameterfor each successive harvest at Tully and Belleville (data not shown), however, the reduction in the wood:bark ratio is not significant enough to explain the large differences between 2011 and 2014 and 2017 removals of K, Ca, Mg, and S. On the other hand, perhaps the more developed root system from the third and fourth rotations are capturing more nutrients (in comparison to the second rotation), especially K, Ca, and Mg, given the high supply of these elements by the soil parent material and the observed stability or increasing availability of these elements in the soil, which could lead to increased concentration in the harvested biomass

No studies reporting nutrient removal patterns in willow biomass crops over, at least, two rotations were found. However, one study [13] reported yield and nutrient removal by cultivar SV1 in a previous existing experiment at Tully at three different rotation lengths (1-,2-, and 3-year). Comparing our results to Adegbidi's [13] three-year rotation, we can observe that both the yield and nutrient removal values of our results are considerably lower (Table 2.5). One explanation for the large differences is that Adegbidi's experiment received two applications of 224 kg N ha^{-1} as ammonium nitrate, 112 kg P ha^{-1} as treble superphosphate, and 224 kg K ha^{-1} as muriate of potash during the springs of 1991 and 1992 (before and after the first harvest [1-year cycle]) and 224 kg N ha^{-1} during the spring of 1993 (after the second harvest [2-year cycle]), while our experiment received 100 kg N ha^{-1}, as urea, once every three years in the springs after harvests (2009, 2012, and 2015). It has been shown that fertilization tends to increase nutrient removal via harvested biomass, either by means of increased yield or increased nutrient concentration in the biomass [13], [24]; which explains the larger removals observed by Adegbidi's compared to this study.

Table 2.5. Comparison of yield and removals of N, P, K, Ca, and Mg by cultivar SV1 at Tully between Adegbidi et al., 2013, and this study. Number in parenthesis are SE

Source	Harvest cycle/rotation	Yield (Mg ha^{-1} yr^{-1})	N	P	K	Ca	Mg
			kg ha^{-1} year^{-1}				
[13]	3-year rotation	21.7 (1.4)	83 (4)	10.6 (1.1)	32 (2)	79 (5)	5.3 (0.4)
This research	Second (2011)	12.4 (0.9)	43 (7)	6.4 (0.8)	9 (1)	38 (6)	1.7 (0.1)
	Third (2014)	10.4 (0.6)	43 (6)	5.9 (0.8)	17 (3)	59 (11)	2.6 (0.3)
	Fourth (2017)	9.5 (1.2)	40 (7)	4.6 (0.5)	11 (1)	43 (5)	2.0 (0.2)

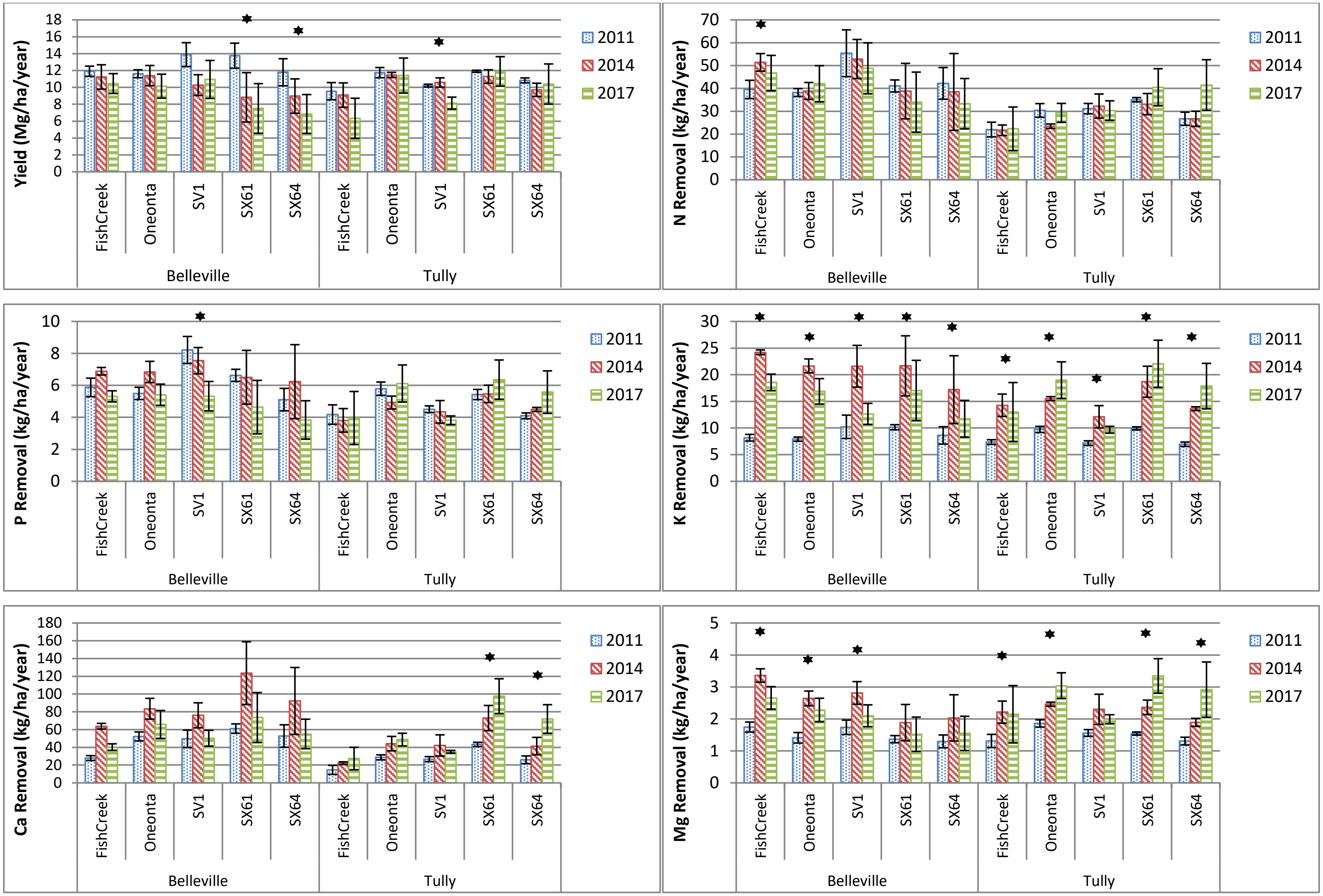

Figure 2.8. Yield and removal of N, P, K, Ca, and Mg (mean ± SE) via harvested biomass of top five shrub willow cultivars at Tully and Belleville and over three harvests. Significant differences among rotations are indicated by asterisks (*).

Overall, the observed nutrient removal rates were comparable to other studies focusing on nutrient removal by shrub willow crops in different regions of the world and with different cultivars (Table 2.6). However, removals of K and Mg in the second rotation, in addition to being significantly lower compared to the third and fourth rotations, corresponded to the lower range of or were below results reported in the literature. The significant differences observed on removals of K, Ca, and Mg between 2011 and 2014 and 2017 could indicate that later rotations could remove higher levels of nutrients; however, the ranges of K, Ca, and Mg removals in the third and fourth rotations were similar to the values reported in the literature (Table 2.6). In fact, depending on the cultivar considered, the amount of nutrients removed decreased in 2017 at Belleville in comparison to 2014 and, either stayed similar or increased at Tully (Figure 2.8), which could be an indication of reduced or constant amount of nutrients removed in future rotations.

Table 2.6. Results of nutrient removal from shrub willow crops from studies across North America.

Aboveground age (rotation)	Yield ($Mg\ ha^{-1}\ year^{-1}$)	N	P	K	Ca	Mg	Source
		kg ha^{-1} $year^{-1}$					
1, 2, 3 (first rotation)	4 - 22	26 - 83	4 - 11	15 - 32	19 - 79	3 – 5	[13]
3 (second rotation)	7 – 23	48 - 176	6 - 21	20 - 71	44 - 112	4 - 14	[24]
3 (first rotation)	5 – 9	14 - 26	3 - 4	11 - 19	29 - 54	3 – 6	[28]
5 (second rotation)	Not reported	18 - 54	3 - 9	7 - 26	10 - 117	1 - 5	[46]
Belleville							
3 (second rotation)	12 – 14	38 – 55	5 – 8	8 – 10	28 – 61	1 - 2	
3 (third rotation)	9 - 11	39 – 53	6 – 8	17 – 24	64 – 124	2 – 3	This study*
3 (fourth rotation)	7 - 11	33 - 49	4 - 5	12 - 19	40 - 74	2 – 3	
Tully							
3 (second rotation)	10 – 12	22 – 35	4 – 6	7 – 10	15 – 43	1 – 2	
3 (third rotation)	9 – 11	22 – 33	4 – 5	12 – 19	22 – 73	2 – 3	This study*
3 (fourth rotation)	6 – 12	22 – 42	4 – 6	10 – 22	27 – 98	2 – 3	

*Values across top cultivars

Our results indicate that for an average biomass production of 32 Mg ha^{-1} across sites, cultivars, and rotations, a total of 109 kg N ha^{-1}, 16 kg P ha^{-1}, 43 kg K ha^{-1}, 162 kg Ca ha^{-1}, 6 kg Mg ha^{-1}, and 8 kg S ha^{-1} were removed from the soil by the top cultivars at each harvest (Figure 2.8). Considering the results observed at Tully, where yield [40] and removals of N and P remained constant across rotations, we would estimate a total of 218 Mg ha^{-1} over seven rotations (21 years) and removals of 628 kg N ha^{-1}, 102 kg P ha^{-1}, 276 kg K ha^{-1}, 906 kg Ca ha^{-1}, 45 kg Mg ha^{-1}, and 44 kg S ha^{-1} via harvested biomass only. These values alone, however, do not provide much insight about the relevance or long-term impact of the removals on the soil's nutrient availability and crop productivity.

The results observed are crucial to understand the long-term nutrient removal in a shrub willow crops. Another concerning subject is how timing of harvest could impact nutrient removal. Harvesting of shrub willow is recommended after leaf fall, and before leaf set [49] to avoid removing from the site the plants' nutrients rich foliage and limiting nutrient removal at harvest. All harvests for this study occurred after leaf drop but harvesting schedules for commercial scale operations are highly unpredictable and subjected to ground conditions, weather, and machine availability, which can delay, hinder, or preclude the harvest to occur during winter or leaf-off seasons. If harvests are to occur during leaf-on stages, the long-term nutrient removal patterns and impacts on soil nutrient concentration could be different from our results. Hence, the relationship between timing of harvest and nutrient removal should be a focus for future research.

2.4.2. Changes in soil nutrient concentration over three rotations

Reports of previous land use for both sites indicate that Tully was a field that was mowed periodically but not used for any active crop or tree production, while Belleville was actively managed for corn production, which probably included regular fertilizer applications. The differences between soil N (20-40 cm layer), P (both layers), and K (0-20 cm layer) concentrations at both sites in 2008 are probably a result of the intensive management and fertilizer applications at Belleville. In 2017 however, soil N and P levels at the 20-40 cm layer at Belleville were similar to the levels observed at Tully (Figure 2.7). This decrease in soil N and P at Belleville could be explained by higher removals by the shrub willow crop at Belleville compared to Tully and by high losses via leaching [13], [31], [50], [51]. The observed change in soil K at the 0-20 cm layer at Tully from 2008 to 2017 indicated a significant increase in K levels; although not expected, this result is not surprising. Soil K is mainly sourced through mineral weathering [51], [52], especially in K rich soil such as the ones in the study sites. Additionally, the balancing nature of soil K, in which nonexchangeable, exchangeable, and soluble K occurs in equilibrium, is perhaps sourcing more K than the plants need to grow. Finally, another important K source is through foliage leaching (by rainwater) and decomposition (as leaf litter) [51]–[53], and perhaps after several years of no use/management at Tully, the addition of the shrub willow crop in Tully is adding K into the soil surface (0-20 cm layer). Additionally, leaf litter is known to have high concentrations of nutrients in its biomass, especially N, K, and Ca [22], [54], [55], and it has been shown that on average 48% (N) and 50% (K) of annual nutrient uptake is returned to the soil through litter fall [22], [28], which is also contributing to the observed stable N and increasing K levels at Tully over the years.

Management of SRWC falls between intensive agricultural management and the lower intensity forest management, and biomass and nutrient removal rates are probably somewhere between these two systems. Still, the use of other crop or trees' nutrient management guidelines in shrub willow crops might provide some insight towards the crop's nutritional needs. Soil P levels decreased from 2008 to 2017, especially at Belleville (both depths); however, considering the P recommendation for Christmas trees from the Phosphorus Guidelines for Field Crops in New York [56], there was still be enough P available and no fertilizer application would be required. Nonetheless, the levels at Tully would be close to requiring fertilizer applications to maintain higher yields and the profitability of the system.

Among all the macronutrients, N and P are probably the ones that most often limit plant's growth rate, generally receiving more attention and being of higher concern compared to the others [18], [52]. According to our results, N, P, and K presented a significant decrease after three rotations (Figure 2.7); still, this result was forced by decreases of both elements at Belleville, while no significant change was observed at Tully for any studied element. Furthermore, significant differences between sites were observed for soil N (20-40 cm layer) and P (both soil depths) in 2008, but not in 2017. The effect of fertilization on shrub willow yield has been an area of constant research [24], [27], [28]; however, inconsistencies in yield responses to fertilization have been observed, possibly as a result of site specific soil and climate conditions. Quaye and Volk, (2013) [27] observed no significant effect of fertilization on first rotation shrub willow yield at three different sites. Conversely, Labrecque and Theodorescu, 2003 [24], reported increased yield by shrub willow crops at two contrasting (sandy and clayey) sites, as a response to wastewater sludge application (equivalent to 100 kg of available N ha^{-1}).

Decreases in soil total N and P at Belleville corresponded with a decrease in yield over three rotations, while no change in yield was observed at Tully (Figure 2.8). Overall yield at Belleville in 2008 (12.6 Mg ha^{-1} $year^{-1}$) was significantly higher than at Tully (10.9 Mg ha^{-1} $year^{-1}$), but not in 2017 (9.2 Mg ha^{-1} $year^{-1}$ at Belleville and 9.8 Mg ha^{-1} $year^{-1}$ at Tully). The yield decrease observed at Belleville followed a similar trend to soil N and P levels, indicating a potential loss in productivity as a result of lower nutrient availability. If higher yields are desired, the addition of fertilizers, additionally to the 100 kg N ha^{-1} after each harvest, could be considered. However, the cost of fertilizing can substantially impact profitability, accounting for up to 10% or more of the total system cost [57], and the desired response of increased yield might not be sufficient to offset these costs or increase the system's profitability. Hence, careful considerations should be made when deciding whether to fertilize or not, since higher yields can potentially be achieved without fertilization and an increased yield cannot be guaranteed when fertilizer is applied [26], [27].

No specific nutrient management recommendations related to shrub willow crops in New York State have been developed; still, the application of 100 kg N ha^{-1} following a 3-year rotation harvest appears to be a common practice [40], [58], and has been applied to Tully since its establishment in 2005. The results observed at Tully (constant yield, N and P concentration and removal, and soil nutrient levels) indicate the high efficiency of shrub willow crops to utilize and recycle nutrients, and potentially proving the addition of fertilizer N after each 3-year harvest unnecessary. Fertilizer application is estimated at \$160 ha^{-1} [59] occurring every three years, during the spring following a 3-year rotation harvest. Using EcoWillow2.0 [59] to perform a quick economic evaluation, with the software's suggested and default values and considering fertilizer applications every three years, an Internal Rate of Return (IRR) <0% and a Net Present Value (NPV) of -\$12,749 were calculated over a 22-year period in the base scenario (see [59], [60] for detailed information on different scenarios) and IRR of 6.4% and NPV of \$3,797 at an optimistic scenario. However, when fertilization is removed from the calculations, an IRR of 2.6% and NPV of -\$5,647 are observed in the base scenario, while 8.8% of IRR and \$10,188 of NPV are observed in the optimistic scenario. Hence, if shrub willow crops and soil parent material are recycling and supplying sufficient nutrients to support high yield production over several rotations, the application of fertilizer could be discontinued, improving the profitability and economics of the system.

Nutrient removal rates (NRR) have served as loose guidelines for long-term fertility management, indicating the quantities of nutrients removed off the field via harvested biomass and the amounts needed to replace them through fertilization [61]. The results observed in our study indicate that NRR in shrub willow crops in NY directly impact soil nutrient levels; however, these impacts are both negative and positive, depending on the element and site considered. A quick analysis relating soil nutrient levels at 2008 and 2017 and total nutrient removals via harvested biomass over the three harvests shows that the observed decrease in soil N and P, as well as the increase of K and unchanged levels of Ca and Mg, do not correspond directly to the observed total removals (Table 2.7). In addition to removals in harvested biomass other processes including mineral weathering, recycling of the nutrients (via leaf litter decomposition, root turnover, and microbial activity), and atmospheric deposition play important roles in these systems [62], [63]. Recent observations have found that nutrient content in decomposing foliage and fine and coarse woody biomass in a shrub willow plot at the end of a 3-years rotation are 38.4-99.5 kg N ha^{-1}, 4.2-11.6 kg P ha^{-1}, 6.9-11.0 kg K ha^{-1}, 59.2-412.3 kg Ca ha^{-1}, 4.2-8.8 kg Mg ha^{-1}, 3.7-9.4 kg S ha^{-1}, and 4.0-16.7 kg Al ha^{-1} (Personal data, not published). This will likely contribute as nutrient supply for the upcoming rotation. Considering NRR as nutrient management guidelines at Tully, an application of P could be beneficial immediately to adjust soil nutrient levels (Table 2.8). However, the observed interaction between nutrient removal via harvested biomass, nutrient recycling and sources, and the soil nutrient levels, indicate a stable source of nutrient to support the crop's nutritional need in upcoming rotations, with no fertilizer applications need.

Table 2.7. Partial soil nutrient budget to 40 cm depth after three 3-year rotations at Tully and Belleville obtained from soil samples taken in 2008 and 2017.

	Observed initial soil nutrients (2008)$	Removed over three harvests*	Net soil balance (after harvest)^	Observed net soil Balance (2017)&	% Change in soil nutrients#
N (kg ha^{-1})					
Belleville	8640.0	385.0	8255.0	7103.0	-17.8
Tully	5683.7	268.9	5414.8	5730.1	0.8
P (kg ha^{-1})					
Belleville	30.1	53.9	-23.8	14.7	-51.1
Tully	5.7	43.8	-38.1	4.2	-26.5
K (kg ha^{-1})					
Belleville	241.3	137.0	104.3	226.1	-6.3
Tully	162.6	118.2	44.4	211.3	30.0
Ca (kg ha^{-1})					
Belleville	11053.4	580.6	10472.8	10937.3	-1.1
Tully	3127.7	388.1	2739.6	2934.2	-6.2
Mg (kg ha^{-1})					
Belleville	285.8	18.3	267.5	265.0	-7.3
Tully	261.9	19.4	242.5	242.6	-7.4

$Assumed a soil bulk density of 1.45 Mg m^{-3} at both sites
*Nutrients removed via harvested biomass during the three harvests.
^Theoretical balance only considering removals at harvest and not considering inputs
&Soil nutrients calculated from soil samples analyses done in 2017
#Change in soil nutrients = ((2008 soil nutrients – 2017 soil nutrients) / 2008 soil nutrients) * 100

2.5. Conclusions

Research on nutrient removal over multiple rotations in shrub willow biomass has been very limited. Our results indicate that a combination of five high yielding commercially available shrub willow cultivars, the removals of N and P will remain constant over three rotations, while removals of K, Ca, and Mg will likely increase from the second to the third and fourth rotations, depending on the site. The higher removals of K, Ca, and Mg could be explained by a combination of higher nutrient availability in the soil given the rich K, Ca, and Mg soil parent material of the sites, a better developed root system in later rotations (especially after the second rotation) capable of capturing higher amounts of the cations, not only present in the soil solution in available form, but also on soil colloid surfaces in exchangeable form, and finally by the crop's trend towards higher number of stems with smaller diameter, implicating lower wood:bark ratio and higher nutrient levels observed in the bark. Still, the values observed during the third and fourth rotations resulted in similar ranges compared to other studies on nutrient removals by shrub willow crops.

Soil nutrient concentration results were different between sites, which may be associated with previous land use (corn production at Belleville and no active crop or tree production at Tully). A significant decrease in soil N and P was observed at one site (Belleville), but may be associated with the higher initial soil levels of both elements and higher removal via harvested biomass. On the other hand, an increase in soil K at the other site (Tully) was probably a result of increased K supply through K rich soil parent material weathering and leaf K leaching and recycling. Similar explanation can be given to the stable Ca and Mg observed at both sites. Still, higher yields were observed at Belleville in the second rotation, compared to the third and fourth and Tully. The higher initial soil N and P observed at Belleville could have resulted in higher yield production by the shrub willow, and the decreasing soil N and P levels observed in 2017 resulted in decreased yield, in a range similar to the observed at Tully. It is possible that applications of N and P fertilizers at these sites would support higher yields produced by the shrub willow crop. However, the benefits of applying fertilizers at ~\$160 rotation^{-1} should be weighed against the potential increase in the profitability of the system by reaching higher yields.

Nutrient removal rates have constantly been used as a guideline to determine shrub willow crop's nutrient needs. However, our results were obtained from leaf-off, hand harvested biomass, and given the possible need to perform shrub willow harvests during leaf-on or growing stages it would be important to focus future research on the implications of timing of harvest on nutrient removal patterns and levels and on soil nutrient concentrations.

Our results show the great capacity that shrub willow crops have to recycle nutrients in the system, as well as the importance of considering soil mineral weathering depending on the soil parent material. Our nutrient removal results would have implicated the depletion of P after the fourth rotation and K in a near future; however, the observed soil nutrient net balance in 2017 indicated that more nutrients were added to the system then removed via harvested biomass. Hence, we can assume that nutrient removal rates via harvested biomass should not be considered as a guideline to determine shrub willow nutrient needs.

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CHAPTER III: GROWING SEASON HARVESTS OF SHRUB WILLOW HAVE HIGHER NUTRIENT REMOVALS AND LOWER YIELDS COMPARED TO DORMANCY SEASON HARVESTS

Abstract

The recent establishment of shrub willow crops at commercial scale in New York State has raised concerns about nutrient removal via harvested biomass. Furthermore, the marginal condition (related to hydrologic limitations) of the sites where the crops are established has resulted in both leaf-on and leaf-off harvests, with limited knowledge on the implication this could have in nutrient export from the site and in the crop's long-term productivity. This study examined the effects of six harvest dates (June, August, September, October, January, and April) on the nutrient removal and second rotation biomass production of four shrub willow cultivars in NY. Biomass production was significantly different across harvest dates (p-value= 0.0027) with higher production when harvests occurred in April (104 Mg ha^{-1}), January (93 Mg ha^{-1}), and October (94 Mg ha^{-1}) compared to June (77 Mg ha^{-1}), August (78 Mg ha^{-1}), and September (85 Mg ha^{-1}). A significant interaction between harvest date and cultivars was observed, indicating variable responses to harvest date. There was a significant effect of harvest date on the removal of N, K, Ca, Mg, and S. Willow harvested in October removed higher amounts of N (77.1 kg ha^{-1} $year^{-1}$, P (11.2 kg ha^{-1} $year^{-1}$), Ca (163.7 kg ha^{-1} $year^{-1}$), Mg (9.9 kg ha^{-1} $year^{-1}$), and S (8.9 kg ha^{-1} $year^{-1}$) than plants harvested in other months. Willow K removal was greater for plants harvested in June and August (51.2 and 52.5 kg ha^{-1} $year^{-1}$ respectively), and Al removal was greater for April harvests (0.15 kg ha^{-1} $year^{-1}$). The significant interactions observed between harvest date and cultivar for both biomass production and nutrient removal indicate that a careful selection of cultivars to be deployed in commercial field

could ensure high biomass production and limited nutrient removal across a variety of harvest dates.

Keywords: *Salix*, short rotation woody crops, biomass production, harvesting equipment, nutrient management

3.1. Introduction

Short rotation woody crops (SRWC), including shrub willow, are considered potential biomass feedstocks to replace the fossil fuels that dominate energy supply in the U.S. [1], [2]. Initial establishment of willow crops at commercial scales has occurred in the U.S. [3], [4]. Establishment has been facilitated by research and improvements in the system through the development of high yielding cultivars, implementation of incentive programs, demonstration of environmental services and alternative applications, and opportunities to promote biodiversity and produce bioenergy [5]–[10]. Despite the interest, the establishment and development of a robust market for solid biomass has not yet occurred. A consistent supply of biomass must be guaranteed in order to support such a market. However, several issues related to shrub willow management and harvesting need to be addressed to ensure that shrub willow producers can deliver biomass to end users year-round.

Harvesting of woody biomass in forests or SRWC removes nutrients from the site and can impact soil fertility and forests or SRWC long-term productivity [11], [12]. Common strategies to reduce nutrient losses during forest biomass harvest operations include [13]: (a) retain adequate quantities of slash (coarse and fine woody debris) on-site; (b) retain or leave tree foliage on-site to retain nutrients; and (c) replace removed nutrients by fertilizing biomass sites with wood-ash or other sources. Shrub willow crops are generally used for bioenergy (electricity and heat) production, which relies on the utilization of the whole plant. Despite concerns about the effects of whole-plant harvesting and utilization on soil nutrient levels and the long-term productivity of the crop, shrub willow harvest operations have shown to leave 1.6 – 4.5 Mg ha^{-1} (see Chapter 4) of residual biomass on the site, which could support the nutrient balances of the soil. Nonetheless, harvesting of shrub willow is recommended after leaf fall, and before bud set [14] to avoid removing nutrient rich foliage, thus limiting nutrient removal at harvest. By harvesting after leaf fall most nutrients have been translocated from the leaves to the root system and stem, while the nutrients not translocated are returned to the soil in the foliar litter [15].

The timing of willow harvests can impact coppice regeneration and regrowth, impacting the willow crop profitability. The effect of timing of harvest on coppice has arisen as a key research question and which may have different implications in different regions, and among different SRWC species [21]–[25], including shrub willow cultivars. However, the reasons for differences in coppicing due to timing of the harvest is yet not fully understood [16]. Dormant-season harvest is recommended to ensure maximum sprout vigor, compared to growing-season harvest, given the higher availability of carbohydrate reserves in roots after leaf fall, which will support the initial growth of new sprouts after harvest [17]. The initial growth rate of shrub willow coppiced stems is very dependent on solar radiation, temperature, and water availability, however, studies have shown that the vigor of sprouts and the sprouting ability severely decreased when plants were harvested during an actively growing stage [16], [17], [21].

What few existing recommendations and guidelines for shrub willow suggest is that harvest during winter, after leaf fall has occurred, is the most effective way to maintain the long-term productivity of the crop [13], [14]. However, harvesting schedules are highly unpredictable and subjected to ground conditions, weather, and machine availability, which may delay, hinder, or even preclude the harvest to occur during winter or leaf-off season. Shrub willow crops in NY have commonly been planted on abandoned or marginal agricultural land, typically considered not profitable for agriculture mostly because of poor drainage. Additionally, operating on frozen ground is key for protecting the soil from displacement [13], [14]; however, freezing temperatures in northern NY have been unreliable, sometimes occurring after the first significant snowfall, precluding access of harvesting machinery to fields. Willow growers have responded by conducting harvests during the late growing season when leaves are still on the willow plants. In theory this removes all the above ground biomass, including leaves, which can potentially impact the crop's long-term productivity. Given the reality of commercial harvesting operations and the importance of nutrient management strategies that ensure the system's production over multiple coppice cycles, the objective of this project is to determine what effects the timing of harvest has on nutrient removal and above ground biomass production in several cultivars of shrub willow crops in New York State.

3.2. Materials and methods

3.2.1. Site description

A willow stand located in Canastota, NY (43º03'05"N, 075º44'19"W) was selected to perform this study. The soils at the site are classified as Cazenovia silt loam (Fine-loamy, mixed, active, mesic Oxyaquic Hapludalfs) that are moderately well drained with a depth to water table ranging from 61 to 121 cm, and a depth to bedrock of more than 200 cm [22], [23]. The climate is temperate humid and cold, with an average annual precipitation ranging from 973-1017 mm [24].

The site was established in the spring of 2002 with a suite of six cultivars (Table 3.1) planted in monoclonal blocks in a north-south orientation in double-rows with a spacing of 1.5 m between double-rows, 0.8 m within double-rows, and 0.6 m between plants, for a density of 14,400 plants ha^{-1}. After the first growing season the crop was coppiced to induce the growth and development of new sprouts. At the beginning of the second growing season 100 kg N ha^{-1} were applied as urea. Treatment plots were installed in each cultivar block, with exception of cultivars 95311 and SX61 due to low survival rate after the first growing season. The plots consisted of five double rows in width and length of 14 plants per double-row for a total area of 49 m^2, except the plots in cultivar 9882-25, which consisted of four double rows and 39 m^2, due to lower number of rows in the block. A subplot with an effective measurement area of 12.6 m^2 consisting of 18 plants was installed centered in each plot (center three double-rows and two double-rows in the 9882-25 plots). Hence, the measurement plot was buffered by one double-row in each side, three plants at the southern end and one plant at the northern end. The plots were arranged in a south-north orientation, in a fashion so the earliest cut plots would be in the southern end of each replication, avoiding shading impacts from the remaining standing plants, which could have an impact on the second rotation growth (Figure 3.1). Additionally, the remaining area of the site was harvested during the Jun harvest, to simulate clear-cut conditions and avoiding shading effects on the harvest date plots. The study consisted of six treatments (Table 3.2) replicated three times for each cultivar. The experimental design consisted of three blocks (replications) in a 4x6 factorial experiment with a strip-strip-plot design, comprised by four strips, formed by the cultivars, crossed by six strips, formed by the harvest dates (Figure 3.1).

Table 3.1. Shrub willow cultivars included in the time of harvest study in Canastota, NY.

Cultivar ID	Diversity group	Species/pedigree
9882-25	PUR	*S. purpurea*
9870-40	MIYA	*S. miyabeana*
9871-41	MIYA	*S. miyabeana*
95311	ERIO	*S. eriocephala*
SX61	MIYA	*S. miyabeana*
SX67	MIYA	*S. miyabeana*

Figure 3.1. Aerial view of the site with the four cultivars used in the study and layout of plots and replications. The area shaded in yellow was harvested at the same time as the first harvest date (Jun). Plots with white border were harvested in the date specified. Plots with black border are the subplots were measurements and biomass samples were taken.

Table 3.2. Timing of harvest, age of plants in months, growing degree days (GDD) for each harvest date, and stage of plant growth used to study the impacts of timing of harvest on nutrient removal and coppice regrowth of shrub willow crops

Treatment	Harvest date	Plant stage	First rotation age (months)	Rotation GDD*	Second rotation age (months)	Rotation GDD*
Jun	June 2007	After full leaf out	54	7,187	54	6,745
Aug	August 2007	After bud set	56	7,739	52	6,146
Sep	September 2007	Starting senescence	57	8,164	51	5,747
Oct	October 2007	Mid-fall (leaves dropping)	58	8,366	50	5,552
Jan	January 2008	Dormant	61	8,417	47	5,508
Apr	April 2008	Before leaf out starts	64	8,420	44	5,460

*Calculated using 10°C as base temperature

3.2.2. Harvest activities

Harvesting was conducted by hand using a brush saw on the dates specified by the treatments (Table 3.2). The plants in the measurement area of the plot were cut at approximately 5-15 cm above the soil surface and the aerial parts of the plants were removed from the site (stem, branches, twigs, and bark, and leaves whenever present), bundled, and weighed to estimate wet yield. A 1-2 kg sample from each plot, obtained from chipping three stems with different diameters (small, medium, and large, relative to the diameter range at each plot), was collected in paper bags, weighed in the field, and later dried at 60ºC to a constant weight to determine its moisture content. For the growing season harvest treatments (Jun, Aug, and Sep), leaves from the three stems collected for moisture content were stripped from the stems by hand and weighed separately from the rest of the biomass. Moisture content and mass of the leaves were determined so shoot:leaf ratios could be established for each cultivar at each harvest date, which were then used to determine the mass of foliage and woody material at each harvest. The leaves from the three stems with different diameters were stripped off, weighed, collected in paper bags, and dried following the guidelines used with the woody samples. Using the chips' moisture content value, the dry yield was calculated based on the amount of biomass weighed from the measurement area divided by the size of the measurement area; yield was then calculated by dividing production by the length of the rotation in GDD as a proportion of the rotation length in years (Table 3.2) and scaled up to Mg per hectare per year. The site was harvested again in December of 2011, in order to calculate the yield at the end of the rotation after the harvesting date treatments were applied, using the same methodology used in the previous harvests.

3.2.3. Laboratory procedures

Nutritional analyses of the biomass were performed on a subsample of the chips collected during the harvest in order to determine concentration in and removal via harvested biomass. A representative sample of chips from each plot was dried and ground in a Willey Mill using a 40-mesh screen to produce a 300 – 400 g sample. Samples of 3-5 grams were used for the nutritional analyses of the biomass, which were performed at the Agricultural Analytical

Services Lab at the Pennsylvania State University. Determination of total N was done through the micro-Kjeldahl method, while the determination of P, K, Ca, Mg, Al, and S was performed through the microwave acid digestion method and the inductively coupled plasma atomic emission spectrometry (ICP-AES).

3.2.4. Statistical analyses

The total biomass production and annual yield (Mg ha^{-1} $year^{-1}$) of each treatment, defined as the average of the yields of the three blocks divided by the rotation period, was calculated for each cultivar. Nutrient removal (kg ha^{-1} $year^{-1}$) of each element for each treatment was calculated for each cultivar by multiplying the nutrient concentration values by yield. Analysis of variance was performed with SAS® version 9.4 at a critical level α of 0.05. Interactions terms were tested at an α level of 0.15 [25]. Mixed models were built using Generalized Linear Mixed Models (GLMM) analyses to estimate the effects of the six treatments (Table 3.2) and its interaction with cultivars (TxC) on total biomass production, annual yield, nutrient removal, and crop survival. First rotation biomass production and yield were used as covariates to determine second rotation biomass production and yield. The GLIMMIX procedure for GLMM was used, since it allows for random effects (blocks) in the model. Significant differences among treatments were determined based on Least Square Means (LS Means) rather than actual means.

3.3. Results

3.3.1. Effects of timing of harvest on biomass production

Total biomass production (first rotation + second rotation) ranged from 61.0 Mg ha^{-1} for cultivar 9882-25 in Jun to 122.2 Mg ha^{-1} for 9870-40 in Apr (Figure 3.2). A significant harvest date effect is observed (Table 3.3), where total biomass was significantly higher in Apr, followed by Oct and Jan, and significantly lower in Jun and Aug (Figure 3.2). Additionally, despite a substantial increase in biomass production in the second rotation compared to the first rotation for Jun and Aug harvest dates, the total biomass production in Jun and Aug was significantly lower than any other harvest date (Figure 3.2). The harvest date effect on total biomass was also observed individually in cultivars 9870-40 and 9871-41 (Figure 3.2); however, cultivars 9882-25 and SX67 did not show significant differences among harvest dates. In fact, SX67 produced more total biomass in Jun, followed by Oct and Aug.

Table 3.3. Summary of analyses of variance for the effects of harvest date (T) , cultivar (C), TxC, and previous yield (used as a covariate) on shrub willow total biomass production and annual yield. Significant effects tested at α=0.05 for main effects and α=0.15 for the interaction effect and are presented in bold format.

Source	DF	Total biomass (first + second rotation)		Second rotation yield	
		F value	*p-values*	*F value*	*p-values*
Harvest Date (T)	5	4.41	**0.0027**	7.52	**<0.0001**
Cultivar (C)	3	1.32	0.3324	2.51	0.1321
TxC	15	1.26	0.2707	1.77	**0.0774**
Pre-harvest yield (covariate)	1	-	-	61.81	**<0.0001**

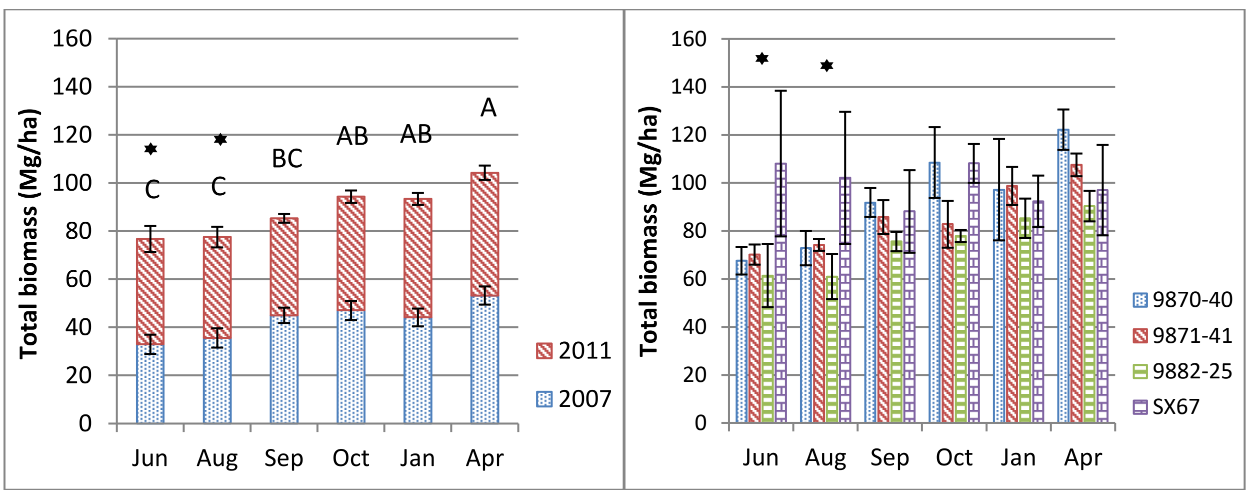
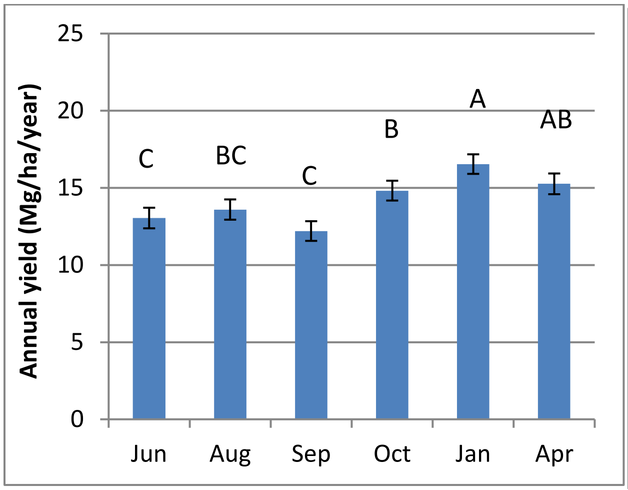


Figure 3.2. Total shrub willow biomass production (mean ± SE) over two rotations across all cultivars for different harvest dates (left graph). Total biomass for harvest dates with similar letters (determined by Tukey HSD) were not significantly different and significant difference between first and second rotation are indicated by asterisks. Total biomass production of two rotations for each cultivar at each treatment (right graph). Significant differences among cultivars within a given date are indicated by asterisks.

3.3.2. Effects of timing of harvest on annual yield

The TxC interaction and harvest date were both significant for second rotation yield; first rotation yield was a significant covariate (Table 3.3). Annual yields ranged from 10.8 Mg ha^{-1} yr^{-1} for a Sep harvest of 9871-41 to 18.3 Mg ha^{-1} yr^{-1} for 9882-25 harvested in Oct (Figure 3.3). The significant TxC effect was driven by the significant difference between the second rotation yield of cultivars 9870-40 and 9882-25 across harvest dates, while no significant differences were observed for cultivars 9871-41 and SX67 (Figure 3.3). When averaged across all cultivars, harvesting in Jan resulted in higher yield (16.5 Mg ha^{-1} $year^{-1}$), followed by Apr and Oct, indicating better yield results when harvesting during the crop's dormancy stage (Figure 3.3).



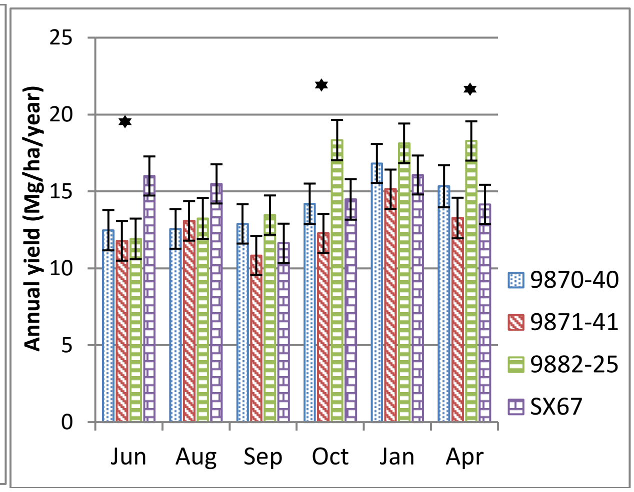


Figure 3.3. Average annual yield (mean ± SE) after 3 – 3.9 growing seasons (end of second rotation) across all cultivars for different harvest dates (left graph). Annual yields for harvest dates with similar letters (determined by Tukey HSD) were not significantly different. Annual yield at the end of the second rotation for each cultivar at each treatment (right graph). Significant differences among cultivars within a given date are indicated by asterisks.

3.3.3. Effects of timing of harvest on nutrient removal

Significant TxC interaction effects occurred for total removal (wood+foliage) of all nutrients, harvest date was significant for all nutrients except P and cultivar was only significant for N, Ca, Mg, and S (Table 3.4). As expected, nutrient removals were higher for woody biomass (44.3-73.0 kg N ha^{-1}, 7.4-10.1 kg P ha^{-1}, 31.8-42.6 kg K ha^{-1}, 112.3-153.5 kg Ca ha^{-1}, 5.1-8.5 kg Mg ha^{-1}, 4.8-7.1 kg S ha^{-1}, 0.05-0.15 kg Al ha^{-1}) compared to foliage biomass (11.8-21.1 kg N ha^{-1}, 1.2-2.7 kg P ha^{-1}, 6.0-11.3 kg K ha^{-1}, 13.9-22.8 kg Ca ha^{-1}, 2.5-3.2 kg Mg ha^{-1}, 2.6-3.0 kg S ha^{-1}, 0.01-0.02 kg Al ha^{-1}), despite concentrations 3 – 10 times higher in the foliage (Appendix 3.2 – 3.4).

Table 3.4. Summary of analyses of variance for the effects of treatment, cultivar, and TxC on total nutrient and Al removal (wood+foliage) on the first rotation harvest. Main effects significance tested at α=0.05, while interaction significance was tested at α=0.15.

Element	Harvest Date (T)	Cultivar (C)	TxC
		p-values	
N (kg ha^{-1} year^{-1})	**0.0246**	**0.0803**	**0.0369**
P (kg ha^{-1} year^{-1})	0.0599	0.1134	**0.1451**
K (kg ha^{-1} year^{-1})	**0.0002**	0.0546	**0.1367**
Ca (kg ha^{-1} year^{-1})	**0.0053**	**0.0015**	**0.0119**
Mg (kg ha^{-1} year^{-1})	**0.0035**	**0.0119**	**0.0157**
S (kg ha^{-1} year^{-1})	**0.0021**	**0.0385**	**0.0366**
Al (kg ha^{-1} year^{-1})	**<0.0001**	0.4920	**0.0350**
DF	5	3	15

3.3.3.1. Nitrogen

Significant effects of harvest date, cultivar, and TxC were observed on total N removal (Table 3.4), with the highest removal observed by cultivar SX67 in Jan (110 kg N ha^{-1} year^{-1}) and the lowest by 9870-40 in Jan (46 kg N ha^{-1} year^{-1}; Figure 3.4). Removals by cultivar SX61 were significantly higher than the removals of all the other cultivars in Jan and statistically similar to cultivar 9870-40 in Jun and Aug. On the other hand, removals in Sep, Oct, and Apr were statistically similar for all cultivars. Overall, total N removal was lower in Apr (57 kg N ha^{-1} year^{-1}) compared to Oct (78 kg N ha^{-1} year^{-1}) and Jan (73 kg N ha^{-1} year^{-1}). Total N removal for the other months was not significantly different. When just considering woody biomass, the lowest removal was in Jun (44 kg N ha^{-1} year^{-1}), Aug (45 kg N ha^{-1} year^{-1}), and Sep (46 kg N ha^{-1} year^{-1}). An increase in N removal was observed as the crop approached and reached the dormancy stage (from Jun to Oct-Jan) until a decrease was observed before the growing season started (Apr) (Figure 3.4). On the other hand, foliar N removal was lower in Oct (12 kg N ha^{-1} year^{-1}) and Sep (14 kg N ha^{-1} year^{-1}), and a linear decrease was observed as the crop approached the dormancy stage and began losing foliage, showing a contrasting behavior to woody N removals.

3.3.3.2. Phosphorus

The TxC interaction was significant for total P removal (Table 3.4), with higher removals by SX67 compared to the other cultivars in Jun (13.9 kg P ha^{-1} year^{-1}), Aug (13.0 kg P ha^{-1} year^{-1}), and Jan (12.7.0 kg P ha^{-1} year^{-1}), while removals in Apr were higher by cultivar 9882-25 (11.6 kg P ha^{-1} year^{-1}) (Figure 3.5). No significant effect of harvest date or cultivar was observed (Table 4). While harvest date effect had a p-value of 0.0599 in the ANOVA, analysis indicated that total P removal was higher in Oct (11.7 kg P ha^{-1} year^{-1}) than either Sep (8.7 kg P ha^{-1} year^{-1}) or Apr (8.2 kg P ha^{-1} year^{-1}) (Figure 3.5). The removal of P in just the woody biomass had a significant increase as the crop reached the dormancy stage (from Jun to Oct-Jan), and dropped again in Apr to similar levels observed in Jun, Aug, and Sep; while removals via foliage biomass reduced significantly as the plants started to shed their leaves (Figure 3.5).

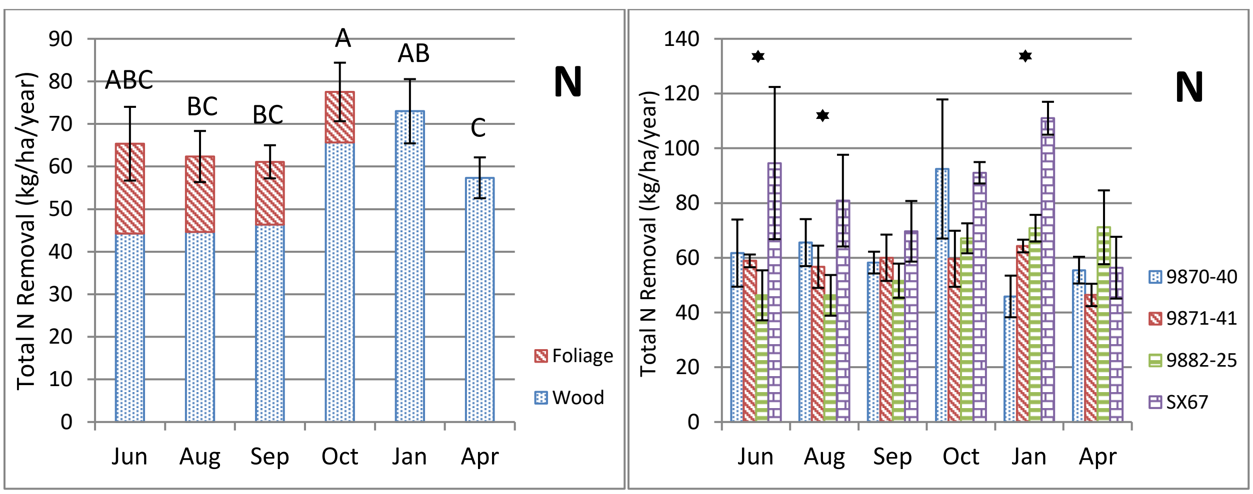


Figure 3.4. Removal of N (mean ± SE) in shrub willow wood and foliage in the first rotation for different harvest dates (left). Total N removal for treatments with similar letters (determined by Tukey HSD) are not significantly different (α=0.05). Right figure shows interaction of six harvest dates and four willow cultivars on total N removal. Significant differences among cultivars within a treatment are indicated by asterisks (*).

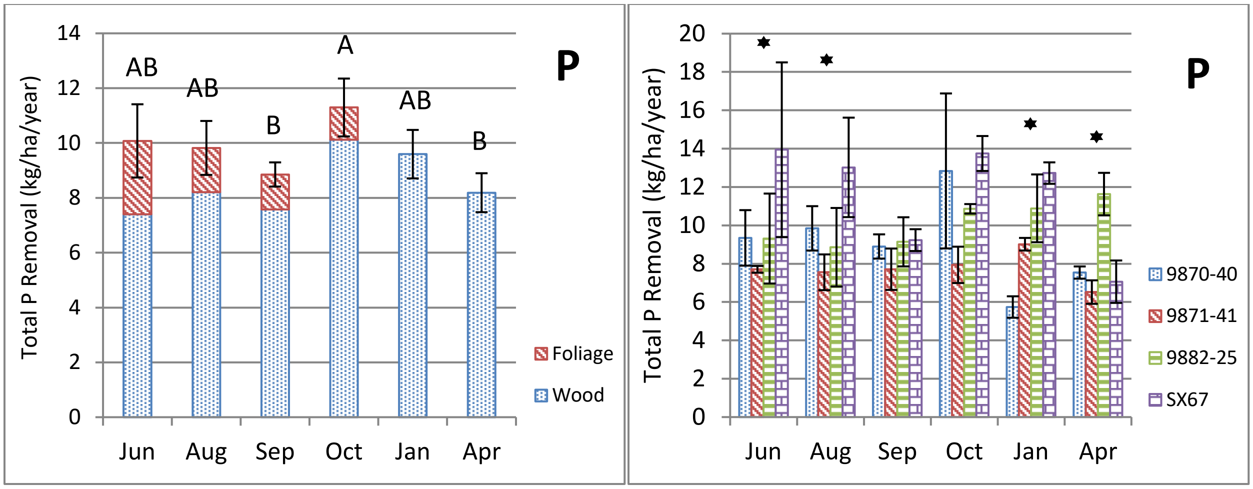


Figure 3.5. Removal of P (mean ± SE) in shrub willow wood and foliage in the first rotation for different harvest dates (left). Total P removal for treatments with similar letters (determined by Tukey HSD) are not significantly different (α=0.05). Right figure shows interaction of six harvest dates and four willow cultivars on total P removal. Significant differences among cultivars within a treatment are indicated by asterisks (*).

3.3.3.3. Potassium

The total removal of K was significantly affected by harvest date and TxC, while no cultivar effect was observed (Table 3.4). The TxC interaction showed significantly higher removals by cultivars 9870-40 and SX67 for Jun (54.1 kg K ha^{-1} $year^{-1}$ for 9870-40 and 67.8 kg K ha^{-1} $year^{-1}$ for SX67) and Aug (64.0 kg K ha^{-1} $year^{-1}$ for 9870-40 and 63.7 kg K ha^{-1} $year^{-1}$ for SX67) , significantly higher removal by 9870-40 in Oct (63.2 kg K ha^{-1} $year^{-1}$) , and significantly higher removal by SX67 in Jan (52.9 kg K ha^{-1} $year^{-1}$) (Figure 3.6). The harvest date effect observed showed that total removal of K was significantly lower in Apr (31.8 kg K ha^{-1} $year^{-1}$) compared to the other harvests, except Jan (32.3 kg K ha^{-1} $year^{-1}$). In contrast to the results of N and P, the removal of K via woody biomass was higher during the full-leaf out stages of the crop (40.0 kg K ha^{-1} $year^{-1}$ in Jun and 42.6 kg K ha^{-1} $year^{-1}$ in Aug), compared to the late season harvests (Sep, Oct, Jan, and Apr). Similarly, K removals via foliage were higher in early season (11.3 kg K ha^{-1} $year^{-1}$ in Jun) and decreased as the crop approached the dormancy stage (Figure 3.6).

3.3.3.4. Calcium

Significant effects of harvest date, cultivar, and TxC were observed in total removals of Ca (Table 3.4). The TxC interaction showed that cultivars 9870-40, SX67, and 9871-41 removed significantly higher amount of Ca in all harvests compared to cultivar 9882-25 (Figure 3.7), but that the cultivar with the highest removal varied. Cultivar SX67 had the highest removals in Jun (173.9 kg Ca ha^{-1} $year^{-1}$), Aug (193.2 kg Ca ha^{-1} $year^{-1}$) and Jan (230.3 kg Ca ha^{-1} $year^{-1}$) while 9870-40 had the highest removal in Oct (231.4 kg Ca ha^{-1} $year^{-1}$). Across all cultivars total Ca removals were higher in Aug (152.2 kg Ca ha^{-1} $year^{-1}$), Oct (164.6 kg Ca ha^{-1} $year^{-1}$) and Jan (153.5 kg Ca ha^{-1} $year^{-1}$) than in Jun (126.2 kg ca ha^{-1} $year^{-1}$) and April (121.0 kg Ca ha^{-1} $year^{-1}$). Among cultivars, SX67 removed higher total Ca (173.1 kg Ca ha^{-1} $year^{-1}$) and 9882-25 removed significantly less (77.3 kg Ca ha^{-1} $year^{-1}$). Wood only Ca removals were lower in Jun (112.3 kg Ca ha^{-1} $year^{-1}$) and Sep (115.1 kg Ca ha^{-1} $year^{-1}$) and higher in Jan (153.5 kg Ca ha^{-1} $year^{-1}$), showing an increasing pattern from early season harvests (Jun, Aug, Sep) to late season harvests (Oct and Jan) until a decrease in Apr. Foliage only Ca removals were significantly lower in Jun (13.9 kg Ca ha^{-1} $year^{-1}$) compared to the other harvests, and increased slightly as the crop approached the dormancy stage (Figure 3.7), which is different than observations for foliar removals of N, P, and K.

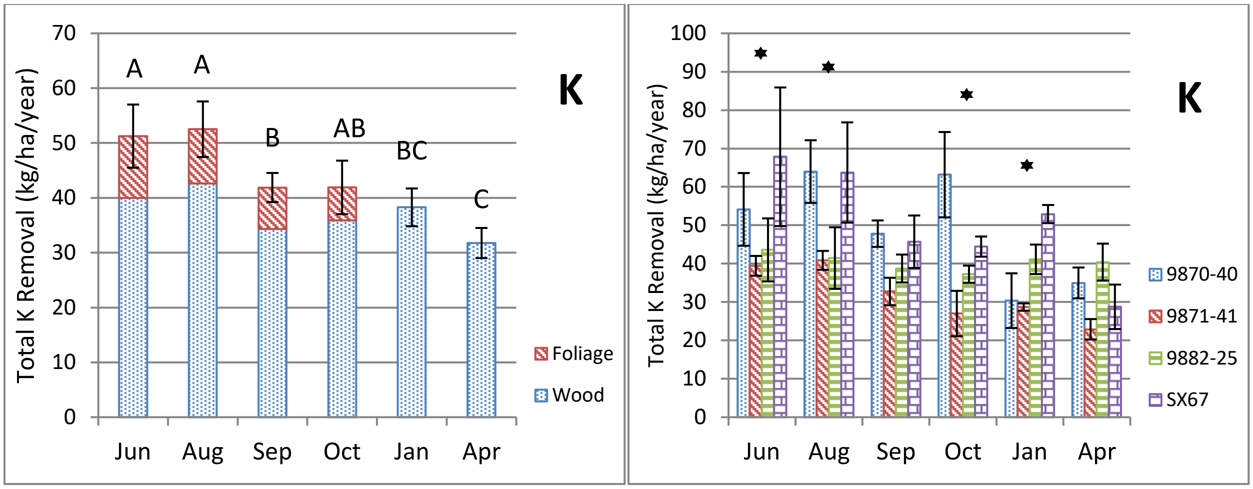


Figure 3.6. Removal of K (mean ± SE) in shrub willow wood and foliage in the first rotation for different harvest dates (left). Total K removal for treatments with similar letters (determined by Tukey HSD) are not significantly different (α=0.05). Right figure shows interaction of six harvest dates and four willow cultivars on total K removal. Significant differences among cultivars within a treatment are indicated by asterisks (*).

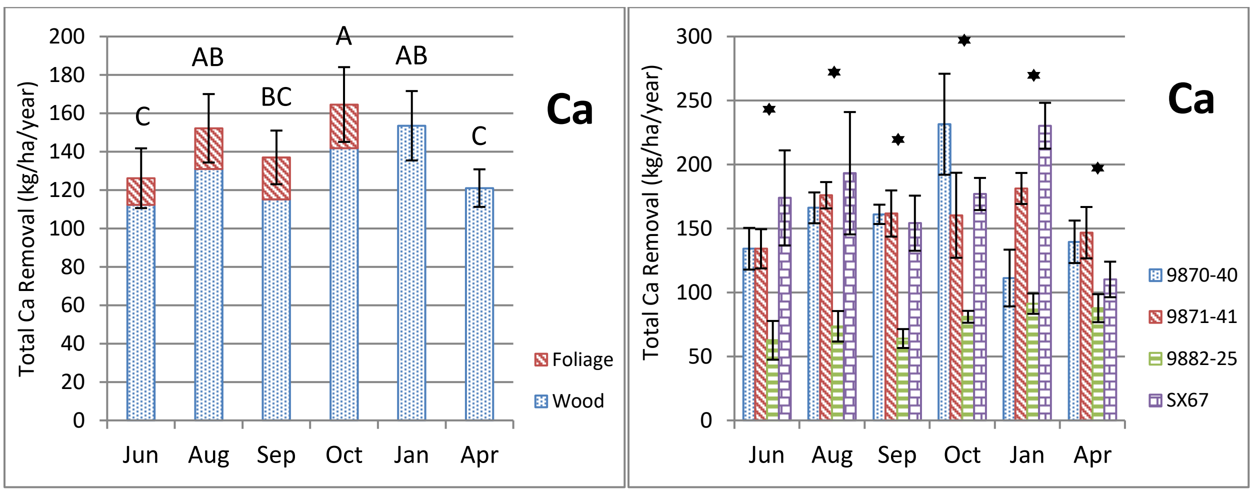


Figure 3.7. Removal of Ca (mean ± SE) in shrub willow wood and foliage in the first rotation for different harvest dates (left). Total Ca removal for treatments with similar letters (determined by Tukey HSD) are not significantly different (α=0.05). Right figure shows interaction of six harvest dates and four willow cultivars on total Ca removal. Significant differences among cultivars within a treatment are indicated by asterisks (*).

3.3.3.5. Magnesium

Total removals of Mg were significantly affected by harvest date, cultivar, and TxC (Table 3.4), with cultivar SX67 removing significantly more Mg in Jun (10.1 kg Mg ha^{-1} year^{-1}) and Jan (12.2 kg Mg ha^{-1} year^{-1}), while cultivar 9882-25 had the highest removal in Apr (10.8 kg Mg ha^{-1} year^{-1}) (Figure 3.8). Also, no significant differences among cultivars in treatments Aug, Sep, and Oct, were observed. A significant harvest date effect showed that total removals of Mg were lower in Apr (7.1 kg Mg ha^{-1} year^{-1}) and Jun (7.9 kg Mg ha^{-1} year^{-1}) and higher in Oct (10.4 kg Mg ha^{-1} year^{-1}) and August (9.6 kg Mg ha^{-1} year^{-1}). Wood only removal of Mg showed an increasing pattern until the crop reached the dormancy stage followed by a decrease in Apr (Figure 3.8), with the lowest removal occurring in Jun (5.1 kg Mg ha^{-1} year^{-1}) and the highest in Jan (8.5 kg Mg ha^{-1} year^{-1}). Foliar only Mg removal, did not show differences among harvest dates (Figure 3.8), but resulted higher in Aug (3.2 kg Mg ha^{-1} year^{-1}) and lower in Oct (2.5 kg Mg ha^{-1} year^{-1}).

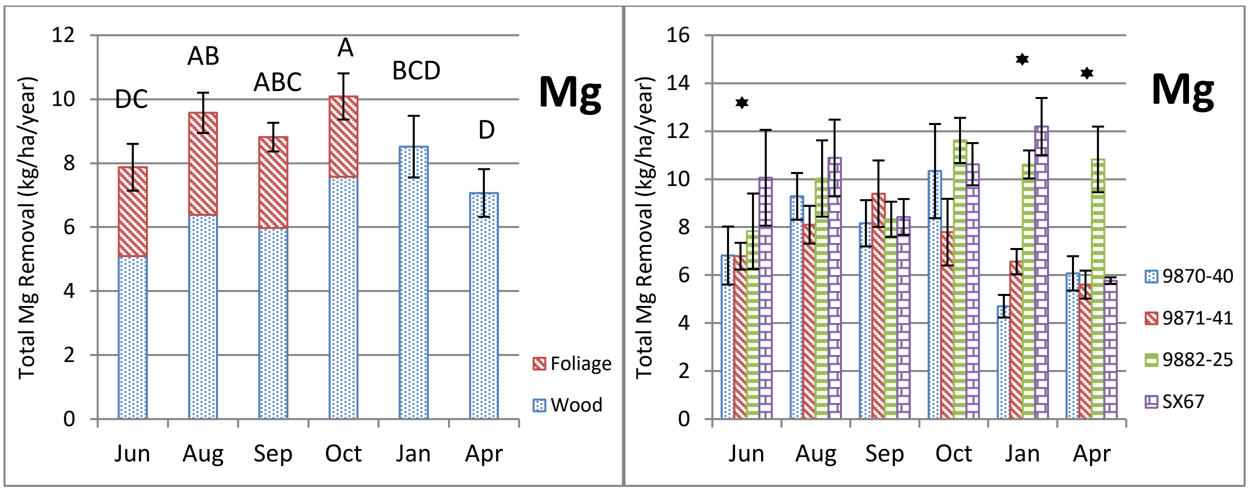


Figure 3.8. Removal of Mg (mean ± SE) in shrub willow wood and foliage in the first rotation for different harvest dates (left). Total Mg removal for treatments with similar letters (determined by Tukey HSD) are not significantly different (α=0.05). Right figure shows interaction of six harvest dates and four willow cultivars on total Mg removal. Significant differences among cultivars within a treatment are indicated by asterisks (*).

3.3.3.6. Sulfur

Significant effects of harvest date, cultivar, and TxC were observed in the total removal of S (Table 3.4), where cultivar SX67 had the highest removal in Jun (11.1 kg S ha^{-1} year^{-1}), Aug, and Jan (10.8 kg S ha^{-1} year^{-1}), while cultivar 9870-40 had the highest removal in Oct (12.4 kg S ha^{-1} year^{-1}) (Figure 3.9). There were no significant differences in total S removal among cultivars in Sep and Apr. The main significant harvest date effect on total S removal indicated that total removals were significantly lower in Apr (5.6 kg S ha^{-1} year^{-1}) than all other dates except Jan and significantly higher in Oct (9.4 kg S ha^{-1} year^{-1}) compared to Jun, Sep, Jan and

Apr (Figure 3.9). The removal of S via wood was significantly higher in Jan (7.1 kg S ha^{-1} $year^{-1}$) and significantly lower in Apr (5.6 kg S ha^{-1} $year^{-1}$), Aug (5.0 kg S ha^{-1} $year^{-1}$), Jun (4.9 kg S ha^{-1} $year^{-1}$), and Sep (4.8 kg S ha^{-1} $year^{-1}$). On the other hand, foliar removal of S was not significantly affected by harvest date, showing no pattern or differences among harvest dates (Figure 3.9).

3.3.3.7. Aluminum

The total removal of Al was significantly impacted by harvest date and the interaction TxC, while no effect was observed for cultivar (Table 3.4). The TxC interaction showed that total Al removals by cultivar 9870-40 were significantly higher in Oct (0.17 kg Al ha^{-1} $year^{-1}$) compared to the other cultivars, and that removal by SX67 in Apr (0.08 kg Al ha^{-1} $year^{-1}$) was significantly lower than by the other cultivars (Figure 3.10). The significant harvest date effect on total Al removal indicates that removals were significantly higher in Jan (0.14 kg Al ha^{-1} $year^{-1}$) and Apr (0.15 kg Al ha^{-1} $year^{-1}$) compared to the other harvest dates. A pattern of constant increase in total removal was observed from the crop's growing stage (Jun, Aug, and Sep) until the crop's dormancy stage (Oct, Jan) and late spring (Apr) (Figure 3.10). A similar pattern was observed for foliar only and wood only Al removal, in which lower removals increased from early season harvests as the crop approached dormancy stage (foliar) and during the crop's dormancy stage (wood) (Figure 3.10).

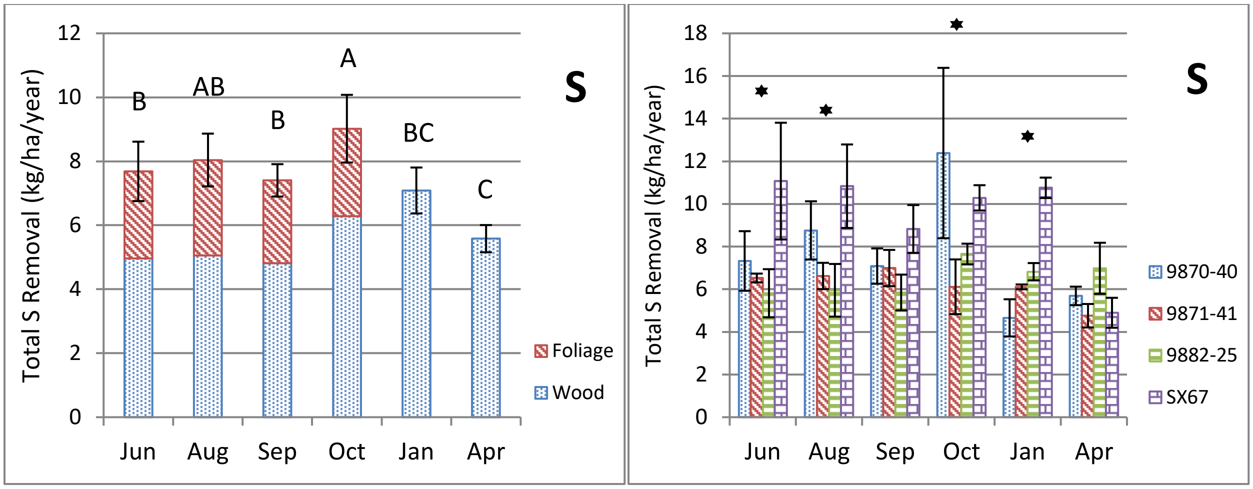


Figure 3.9. Removal of S (mean ± SE) in shrub willow wood and foliage in the first rotation for different harvest dates (left). Total S removal for treatments with similar letters (determined by Tukey HSD) are not significantly different (α=0.05). Right figure shows interaction of six harvest dates and four willow cultivars on total S removal. Significant differences among cultivars within a treatment are indicated by asterisks (*).

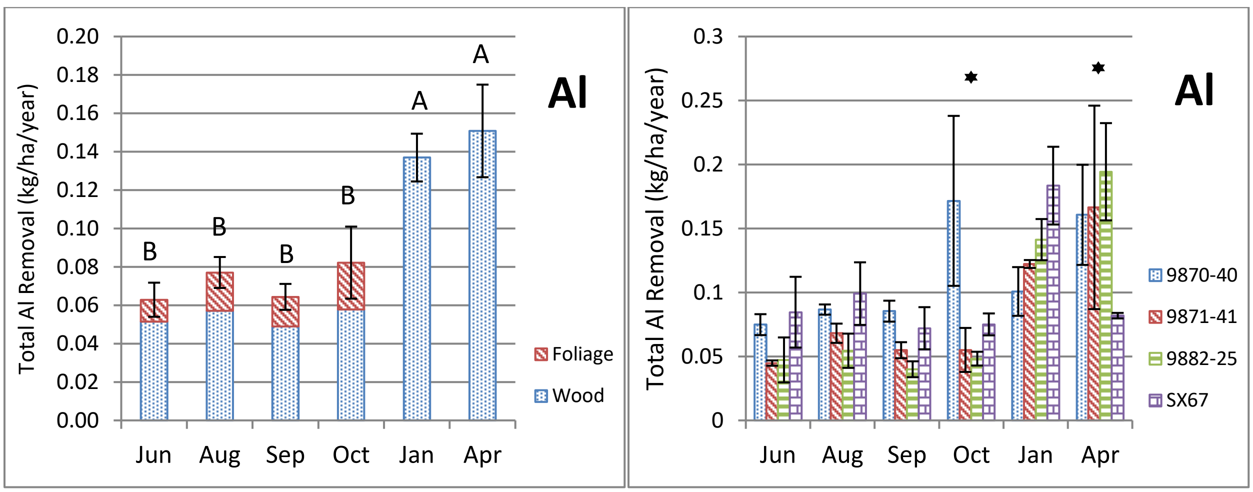


Figure 3.10. Removal of Al (mean ± SE) in shrub willow wood and foliage in the first rotation for different harvest dates (left). Total Al removal for treatments with similar letters (determined by Tukey HSD) are not significantly different (α=0.05). Right figure shows interaction of six harvest dates and four willow cultivars on total Al removal. Significant differences among cultivars within a treatment are indicated by asterisks (*).

3.4. Discussion

3.4.1. Effect of timing of harvest on biomass production and yield

Timing of harvest had a significant impact on both the total biomass production over two rotations and second rotation yield. This study indicated that harvesting during the growing season results in significantly lower total biomass production compared to dormant season harvests, and consequently, lower yield as well. However, it was observed that second rotation biomass production was significantly higher than first rotation production for harvests in Jun and

Aug. Second rotation yields for a number of shrub willow cultivars across a range of sites were higher than first rotation yields when first rotation yields were low and harvest occurred during the dormant season [26]. In our study, first rotation yield was 7.3 Mg ha^{-1} $year^{-1}$ in Jun and 7.6 Mg ha^{-1} $year^{-1}$ in Aug, and a significant increase in second rotation yield was observed (+5.8 Mg ha^{-1} $year^{-1}$ in Jun and +5.6 Mg ha^{-1} $year^{-1}$ in Aug). On the other hand, harvests in Sep, Oct, Jan, and Apr did not present significant changes in second rotation biomass production; however, they did show significant increases in their yield, given the shorter length of the second rotation (~4 years) compared to the first (~5 years). Sleight et al. [26] reported that when first rotation yield is between 9.4-12.9 Mg ha^{-1} $year^{-1}$, the probability of increasing yield in the second rotation is <50%. First rotation yields for Sep, Oct, Jan, and Apr ranged from 9.5 – 10.6 Mg ha^{-1} $year^{-1}$. Second rotation yields in our study increased by 78% for Jun and Aug, 28% for Sep, 52% for Oct, 87% for Jan, and 43% for Apr. Still, despite significant increases, the willow harvested during the growing season (Jun, Aug, and Sep) did not match the yields obtained by the plants harvested during the dormant season (Oct, Jan, and Apr), even though they had the equivalent of an extra growing season (in growing degree days [GDD]; Table 3.2), in comparison to dormant season harvests.

The effects of harvest timing on shrub willow, and other coppice species, growth have been studied [17]–[21], [27]; despite this, gaps in understanding the impact of harvesting season on the plants' growth remain. Certain commonalities exist among previous results; lower plant growth and development were observed when harvested during the growing season. The uncertainties about the harvest season effect are attributed to a variety of reasons: e.g. (1) possible lower root carbohydrate reserves when harvesting during summer, (2) frost damage of newly regenerated and immature shoots harvested late in the growing season, or (3) a limited nitrogen reserve supply for regrowth [17], [21], [28]. Our results showed similar growth response to previous studies. However, we cannot confirm any of these previous attributions.

Woody nitrogen concentration in this study showed a significant increase from the growing season until the dormancy season, with a significant decrease in early spring, during bud burst (Appendix 3.1), similarly to the observations made by other studies [28], [29]. Although we did not study the dynamics and translocation of nitrogen, carbohydrates or other compounds in the plant, or evaluate the plants for frost damage, significant reductions and increases in leaf and woody nitrogen, respectively, were observed as the plants approached the dormancy stage. Nonetheless, the reduced nutrient reserves observed during the growing season harvests (Jun, Aug, and Sep) seems like a plausible reason for the effect of harvest date on biomass production and yield.

3.4.2. Effect of timing of harvest on nutrient removal via harvested biomass

Major concerns of short rotations and frequent harvests of shrub willow are the amount of nutrients removed, the potential impact on soil nutrient content, and the long-term productivity of the crop. Although this issue has been studied for dormant season harvests [30]–[33], the study of how timing of harvest affects nutrient removal has been limited, with most of the research performed on nutrient concentration, allocation, and translocation in the shrub willow biomass [28], [29], [34]–[36]. Our results indicate significant effects of harvest date on total removals (woody+foliage) of N, K, Ca, Mg, S, and Al. Removal of total P was not significantly affected by harvest date, but still showed differences between Oct and Sep. Plants harvested in Oct had the highest N, P, Ca, Mg, and S removals, while Al removals were higher in Apr, and K removals higher in Jun and Aug. During the growing season, a high proportion of the nutrients are located in the foliage tissue; when the foliage starts to shed, a portion of these nutrients are translocated into the stems and root system [29], [34] (Figures 3.4 – 3.10), which explains the higher removals observed during Oct and Jan, when limited to no foliage is present and most of the nutrients are concentrated in the stems. During spring (Apr), the nutrients are translocated into growing parts (tips of twigs and branches) to support leaf production and branch growth, but given the lower proportion of tips compared to stem biomass, the higher concentration present in the tips are diluted and shadowed by the lower concentration in the stem biomass.

Nutrient removal was impacted by the interaction of the timing of the harvest and the different cultivars in this trial, which has potentially interesting implications for the deployment and management of willow crops. Among the cultivars studied, SX67 resulted in consistent high total biomass and annual yield, but variable and high nutrient removal across the harvest dates. Fabio et al., 2017 [37], studied the contributions of genotype and environment on shrub willow biomass composition, observing high influence of environment as well as genotype*environment interaction in yield, and concluded that the selection of genotypes and growing environment could be implemented to increase biomass production. Their results can help explain the significant differences observed between SX67 and the other cultivars in our study, where environmental conditions could have been favorable for SX67 growth, compared to the other cultivars and regardless of the harvest date. Fabio et al. [37] also found two SX cultivars (SX61 and SX64), which belongs to the same diversity group as SX67 (Table 3.1), to be stable and high yielding across a range of environmental conditions.

Considering harvest date effects on biomass production and nutrient removal the selection and deployment of different cultivars could be decided depending on site conditions and characteristics; however, this is based on a set of four cultivars and one site only. Cultivar SX67 would be a strong candidate to be deployed in marginal sites where leaf-on harvests might be required for some portion of the life of the crop, ensuring high yield in the following rotation; however, SX67 also showed a variable and high nutrient removal across harvest dates. Hence, deploying a combination of SX67 and 9871-41 (which resulted in variable yielding and low and variable nutrient removal across the harvest dates) could be beneficial both for the overall yield and nutrient removal rates on the site. Assuming harvests to occur from Aug to Oct (as observed in commercial sites in NY given poor site conditions in fall and winter seasons) we could assume average yield of 13.8 Mg ha^{-1} year^{-1} and removals of 69.7 kg N ha^{-1} year^{-1}, 9.9 kg P ha^{-1} year^{-1}, 42.4 kg K ha^{-1} year^{-1}, 170.4 kg Ca ha^{-1} year^{-1}, 9.2 kg Mg ha^{-1} year^{-1}, 8.3 kg S ha^{-1} year^{-1}, and 0.07 kg Al ha^{-1} year^{-1}. In contrast, a deployment of cultivars with characteristics similar to SX67 only, will ensure higher yields (15.5 Mg ha^{-1} year^{-1}), but will likely result in higher removal rates (80.5 kg N ha^{-1} year^{-1}, 12.0 kg P ha^{-1} year^{-1}, 51.3 kg K ha^{-1} year^{-1}, 174.7 kg Ca ha^{-1} year^{-1}, 10.0 kg Mg ha^{-1} year^{-1}, 10.0 kg S ha^{-1} year^{-1}, 0.08 kg Al ha^{-1} year^{-1}).

Commercial mechanized harvest operations in shrub willow have shown to leave between 7 – 15% of the total standing biomass as dropped material on the site [38] (See Chapter 4 for more information and data). In Chapter 4, the nutrient content in the dropped biomass represented on average 20 – 35% of the total nutrient content in above-ground shrub willow plants. Additionally, it has been noted that a high proportion of the foliage (when harvesting during the growing season) will remain on site (Figure 3.11; no data available), as a result of being knocked off as stems are pulled into the harvester or because of the lower density foliage is dropped on the ground and not blown into collection vehicles. Hence, although a significant effect of harvest date on nutrient removal was observed, the data for this study was collected by hand harvest and the foliage, as well as all the woody biomass parts, were carefully collected and sampled (See materials and methods section). In contrast, between 7 – 15% of the total standing woody biomass, as well as a proportion of the foliage, may be left behind. This could represent up to 35% (not considering the foliar nutrient content) of the nutrient content in a plant remaining on site.

Figure 3.11. Dropped biomass (woody and foliage) after a leaf-on mechanized harvest operation in a marginal shrub willow site in New York State. The green material within the double row is leaves and branch tips that were not pulled into the harvester.

3.4.3. Total biomass production, yield, and nutrient removal excluding cultivar SX67

The significant differences observed between cultivar SX67 on total biomass production, yield, and nutrient removal and the other cultivars impacted the overall results. Additionally, some of the significant TxC interactions observed on the studied parameters were highly influenced by SX67. Removing SX67 from the analyses might be useful in understanding patterns among other cultivars and their interaction with harvesting dates. Analyses were performed again on total biomass, annual yield, and removals of N, P, K, Ca, Mg, and S. The most visible change after removing cultivar SX67 from the analyses is that the TxC interaction is no longer significant for annual yield, indicating that cultivars 9870-40, 9871-41, and 9882-25 are affected in a similar way by harvest date.

The results of total biomass production remained similar, with the exception of the response of Jun and Aug (Figure 3.12), which resulted lower than the previous analysis, but still significantly differently from the other cultivars. Similarly, annual yield shows a similar pattern to the previous analysis, but instead of three groups with similar yields, two groups are now visible and significantly different from each other (Figure 3.12). The observations indicate that harvesting during fall, winter, and spring seasons (Oct, Jan, and Apr) will result in significantly higher yield, while the previous result showed similar yields in Aug, Jan, and Apr (Figure 3).

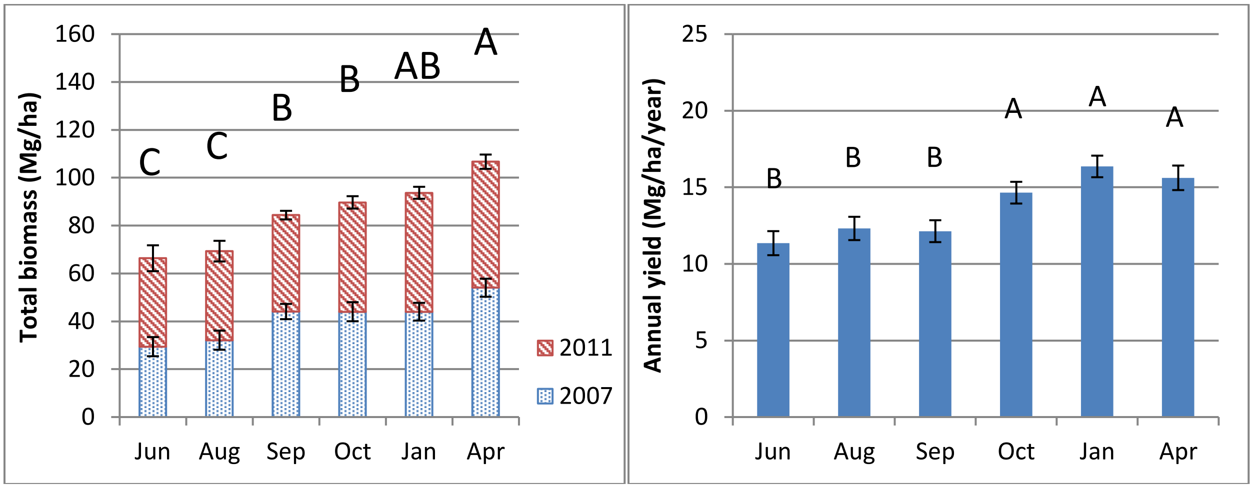


Figure 3.12. Total biomass production (mean ± SE) of two rotations (left graph) and average annual yield after 3 - 3.9 growing seasons (end of rotation) (right graph) across cultivars (excluding SX67) for different harvest dates. Harvest dates with similar letters (determined by Tukey HSD) are not signifficantly different from each other.

The exclusion of SX67 from the analyses had little impact on the nutrient removal pattern of K, Ca, Mg, and Al. On the other hand, removals of N, P, and S are now significantly higher in Oct compared to the other harvest dates, except Jun for P which is similar to Oct (Figure 3.13); however, the overall pattern of nutrient removal remains the same, with higher removals in Oct. The interaction TxC is still significant after removing SX67, but mostly because of cultivar 9870-40 (Figures 3.4 – 3.10). Two distinct cultivar groups can be observed, with one showing more consistent nutrient removals across harvest dates (9871-41 and 9882-25) and one showing more variation across harvest dates (SX67 and 9870-40). On the other hand, the yield of 9882-25 was highly variable across harvest dates (Figure 3.3), while lower variation was observed for the other cultivars. A careful selection of cultivar and growing environment, as explained by Fabio et al. [37], could ensure higher yields over rotations, as well as similar results when harvesting in different dates and seasons of the year. Hence, by considering cultivars with lower variation both in yield and nutrient removal, a wider harvesting window could be supported, ensuring the biomass production of subsequent rotations and facilitating the nutrient management practices. However, a wider array of cultivars and sites should be explored to confirm the patterns observed, since a limited suite of cultivars and only one site were used at this study.

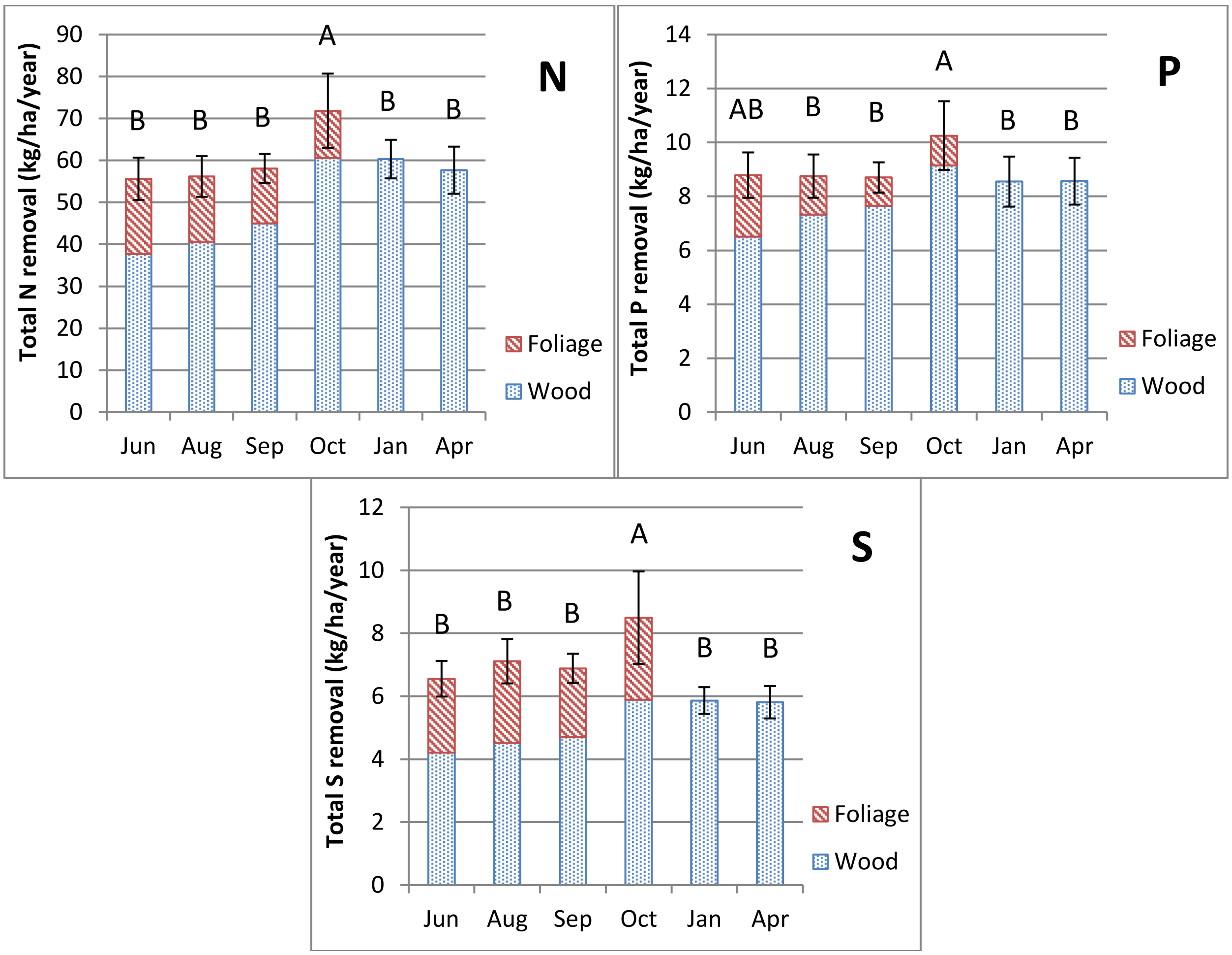


Figure 3.13. Removal (mean ± SE) of N, P, and S in wood and foliage for different harvest dates across cultivars (excluding SX67). Harvest dates with similar letter (determined by Tukey HSD) are not significantly different (α=0.05)

3.4.4. Implications of harvest dates for commercial operations

Our results indicate that harvesting during the plant's dormancy stage (late fall, winter, and early spring) will ensure higher biomass production as well as minimal nutrient export from the site via harvested biomass. Shrub willow best management practices indicate that harvesting during winter months, after leaf fall has already occurred, is preferred [14]. Additionally, our results coincide with other studies, indicating higher biomass production and shrub willow growth when the harvest is performed during the plant's dormancy stage [17], [19]–[21]. However, given the reality of commercial shrub willow harvest operations in NY, it might be challenging to precisely follow the harvesting guidelines and recommendations indicated by these results.

Shrub willow crops are commonly planted on marginal agricultural land in NYS [6]. The term "marginal land" refers to land at the margins of profit, where potential economic returns are at a breakeven point with production costs [39]. These lands generally have use restrictions, caused by slope, elevation, depth, soil texture, internal drainage, fertility, and/or remoteness. In the northeast US the limitations for this land are most often related to hydrology, which results in seasonal saturation or near saturation [6]. Hence, it has been observed that the operation of heavy machinery on these lands during winter time, when the soil might be too wet given snowfall before ground freezing has occurred, or simply as a result of excessive precipitation, is compromised, either by hindered access to the site or increased operating costs. For this reason, commercial shrub willow harvests in NY have started as early as mid-August and continued on into the winter in recent years.

According to our results, harvesting during August will result in significantly lower total biomass production and yield compared to fall or winter harvests. Total biomass production for the Aug harvest date resulted in 77.5 Mg ha^{-1}, while in Oct the total biomass production was 94.3 Mg ha^{-1}. Considering a wet biomass price at plant gate of $30.5 Mg^{-1} [40], we could estimate a gross revenue of $4,584.2 ha^{-1} after two rotations if harvesting during Aug and $5,236.9 ha^{-1} if harvested during Oct (Table 3.5). Still, if the results of this research are considered, and the harvest is performed during Apr, it would result in a total of $5,731 ha^{-1} after two rotations. These results however, do not consider other costs or incomes in the system, only the economic return generated by selling the biomass. As already mentioned harvesting during rainy or snowy periods could increase the harvesting costs or prohibit the harvest from happening. In addition to differences in biomass production recent analysis of willow harvests has shown that throughput from leaf-on harvests on dry ground conditions (29.7 Mg hr^{-1}) are 59% lower than leaf-off harvests on dry ground (71.8 Mg hr^{-1}). This will increase harvesting costs and reduce the profitability of leaf-on harvests [41]. While leaf-off harvest throughput in wet conditions (42.4 Mg hr^{-1}) was 41% lower than when in dry conditions. Despite higher biomass production and gross return generated in Apr, spring snow melt could contribute to soil water saturation, resulting in site conditions not ideal for operating harvesting equipment and increasing harvesting costs, which could lead to lower net revenue compared to other months, when harvesting conditions are ideal.

Table 3.5.Gross revenue from willow biomass depending on total biomass production for each harvest date. Price of biomass at gate is considered at $30.5/Mg [40]. No additional costs or incomes are considered.

Harvest date	1st rotation		2nd rotation		Gross Revenue Over Two Rotations
	Biomass wet	Gross Revenue	Biomass wet	Gross Revenue	
	Mg ha^{-1}	$/ha	Mg ha^{-1}	$/ha	$/ha
Jun	65.8	2,006.9	82.6	2,519.3	4,526.2
Aug	71.2	2,171.6	79.1	2,412.6	4,584.2
Sep	76.3	2,327.2	76.0	2,318.0	4,645.2
Oct	82.5	2,516.3	89.2	2,720.6	5,236.9
Jan	80.2	2,446.1	93.0	2,836.5	5,282.6
Apr	91.7	2,796.9	96.2	2,934.1	5,731.0

Gross revenue = Biomass wet (Mg) * $30.5/Mg

On the other hand, nutrient removal presented a pattern inverse to biomass production, but similar to annual yield, in which higher removals were observed for harvest dates during fall (Oct), followed by summer (Jun, Aug, and Sep) or winter (Jan) harvests, and generally lower in spring (Apr), especially for N and P, which are probably the ones that most often limit plant's growth and receive attention [42], [43]. Additionally, soil N and P levels have shown to decrease significantly after several shrub willow rotations (See Chapter 2). Our results indicate that the ideal season to perform harvest would be early spring prior to leaf out. Harvesting during early spring then, would ensure higher yields with nutrient export that is lower than harvesting at other times of the year. However, a considerable amount of the nutrients removed during summer and fall harvest dates are present in the leaves, while no leaves are removed during the winter and spring harvests. Considering only nutrients and Al removed in the woody biomass, we observe that summer harvest (Jun, Aug, and Sep) removed similar amounts as spring harvest (Apr), with exception of Al, which was considerably larger in Apr. We assumed that all leaf material (entire crown of the plant) was harvested during leaf-on stages; however, as previously mentioned, a high proportion of the foliage (data not available) remains on the site to decompose after a commercial mechanized harvest.

Another consideration is the potential to improve existing single pass cut and chip harvesting system through modifications to facilitate the separation of leaves and increase the amount of this material returned to the site or to increase the harvester's flotation to operate during wet soil conditions and avoid leaf-on harvests. This will reduce nutrient removals and improve soil conditions and the quality of the biomass that is collected for conversion to renewable energy products [44].

Commonly, results of nutrient removal are obtained from hand harvests and field trials. Observations of commercial shrub willow harvesting operations have shown that nutrient rich woody and leaf biomass is left on the site. Soil N and P levels have been noted to decrease after several rotations (See chapter 2), which could possibly have impacts on the crop's long-term productivity. These results, however, were observed in a research site that was hand harvested with all the aboveground biomass removed. It has been shown that 7 – 15% of the total standing biomass is not harvested during commercial harvesting operations [38], representing 20 – 35% of the total nutrient content in the woody above-ground willow biomass (See chapter 4 for more information). This biomass will remain on site, contributing to nutrient cycling through biomass decomposition. The biomass dropped after the mechanized harvest will likely contribute to maintain soil nutrient levels and help support the production of the following rotations. More research is needed in commercial harvest operations to determine the amount of dropped biomass for harvests at different times of the years (both woody and leaf) and the nutrient content in this biomass, as well as to observe how these operations impact the soil's nutrient levels and the crop's long-term productivity.

3.5. Conclusions

The total biomass production and nutrient removal results from this study support the common recommendation to harvest willow after leaf drop whenever possible. In order to ensure higher biomass production in subsequent rotations, shrub willow crops in NY should be harvested during leaf-off stage when possible. However, site and climatic limitations have forced harvesting operations to occur during the growing season in NY, which could reduce the following rotation's biomass production and possibly remove higher amounts of nutrients from the site. The selection and deployment of cultivars whose biomass production is not compromised by leaf-on harvests could ensure higher yields across rotations. Additionally, the development of methods to separate foliage from woody biomass or to facilitate the harvester's operability during wet soil conditions can contribute to the retention of the foliage on site and/or a wider harvesting window.

The different responses observed by different cultivars depending on harvest date demonstrate that overall shrub willow nutrient management and harvesting methods recommendations will not be effective for all cultivars and sites. Influences of environment and genotype*environment interaction on yield have been observed before, and our results indicate a similar effect of the interaction between cultivar and harvest date on both biomass production and nutrient removal. Hence, harvesting and nutrient management guidelines should be recommended by considering site and cultivar characteristics to ensure high yields and maintain soil conditions over multiple rotations. This will contribute to consistent generation of high quality biomass for conversion to renewable energy and potentially provide more gross revenue for the grower.

Further research on the importance of dropped biomass material (both leaf and woody) after mechanical harvest is required. Improvements in the system should focus on increasing harvesting throughput by collecting all merchantable biomass; however, the nutrient content in the dropped biomass might support the growth of future rotations and contribute to the soil nutrient levels and conditions. Harvesting operations should focus on separating foliage and woody biomass, and retaining the nutrient rich foliage and non-merchantable biomass (small twigs and tops of plants) on the site in order to reduce nutrient removal impacts of both leaf-on and leaf-off harvests. Hence new harvesting guidelines and recommendations should be developed based on research and the reality of commercial harvesting operations in the region.

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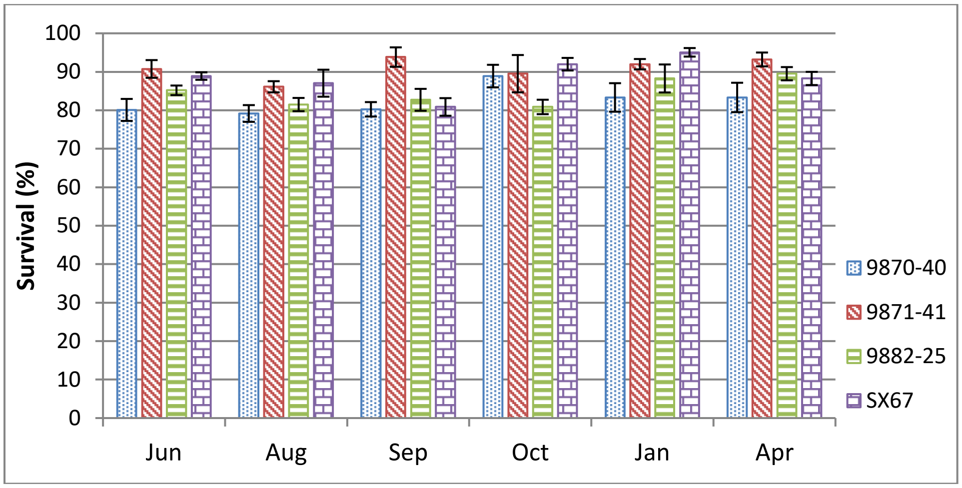
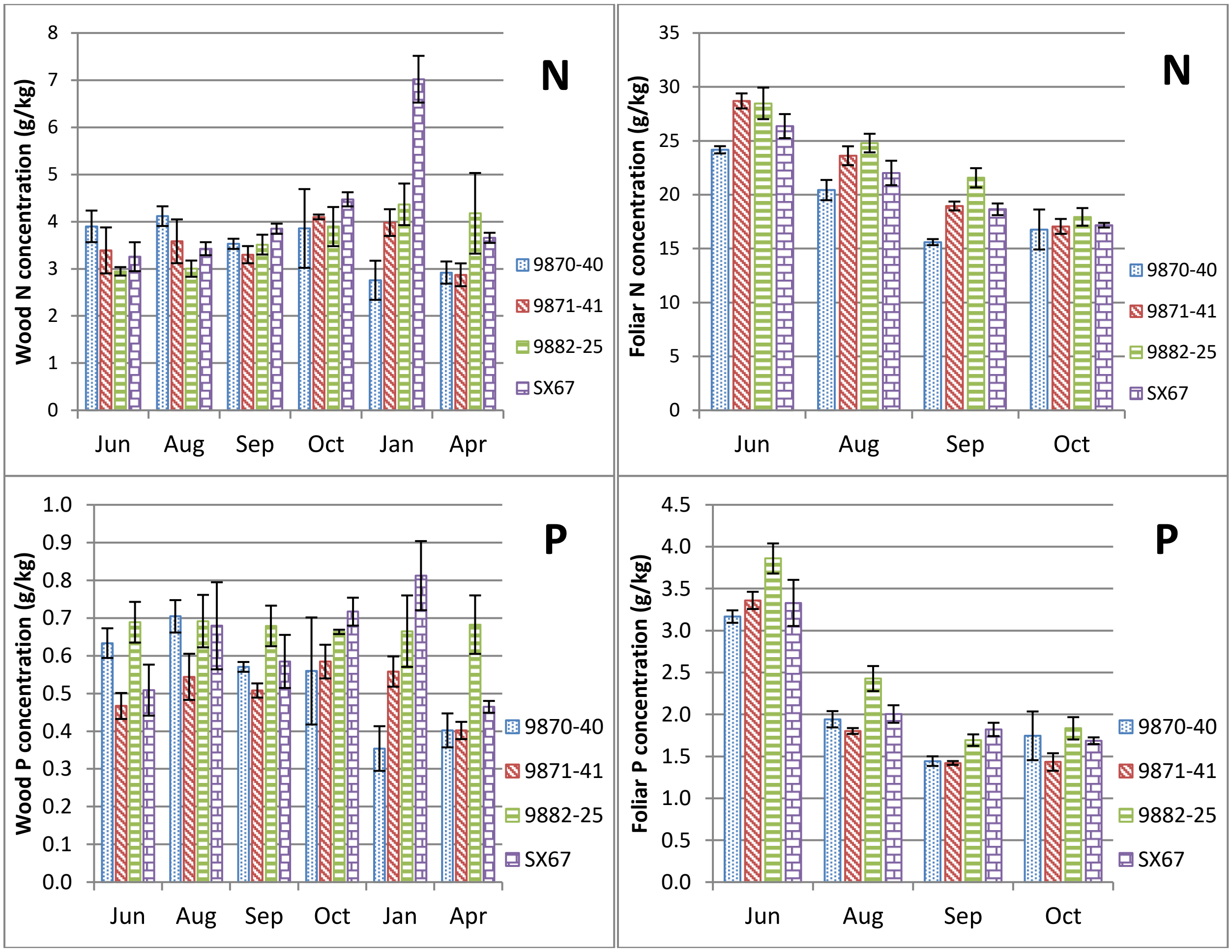
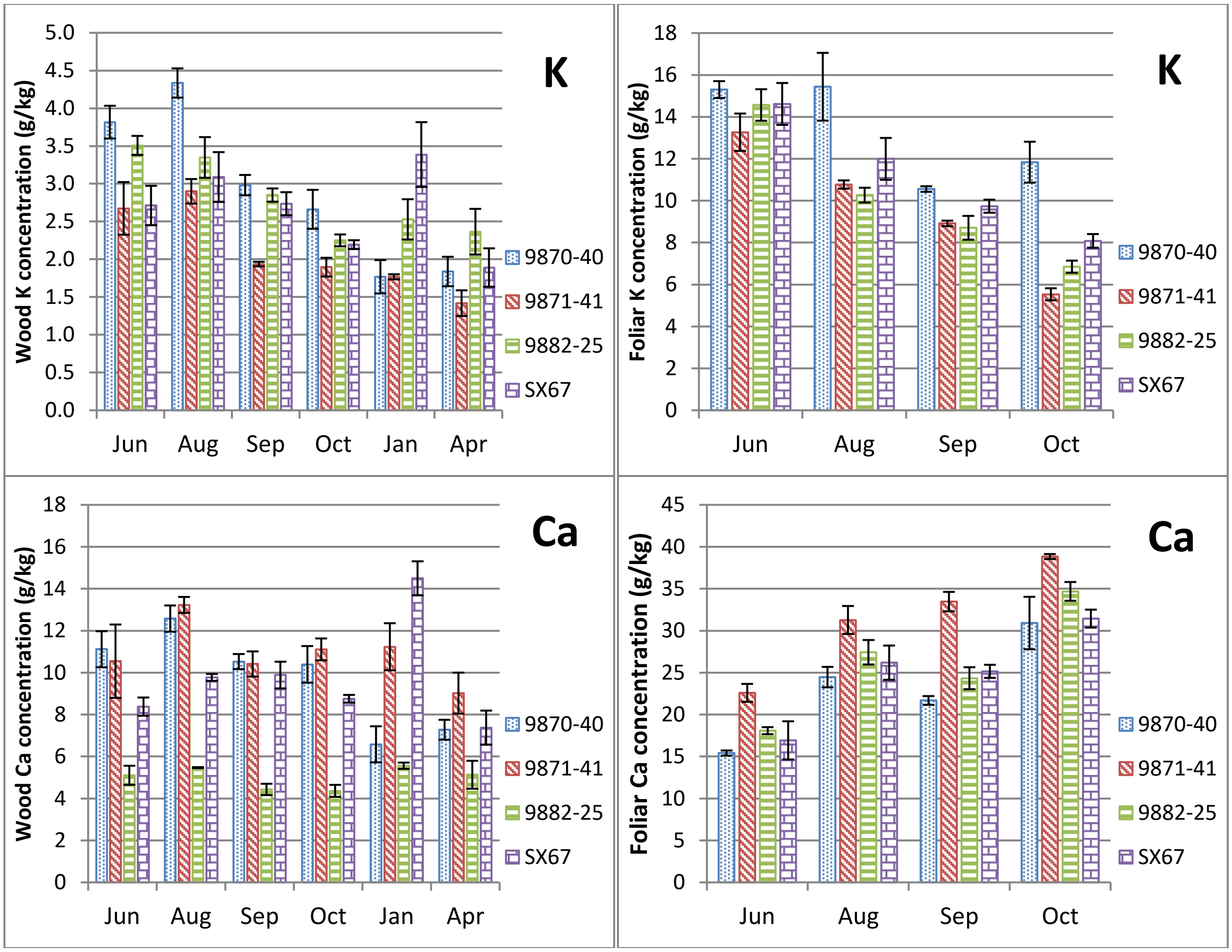
Supplemental material



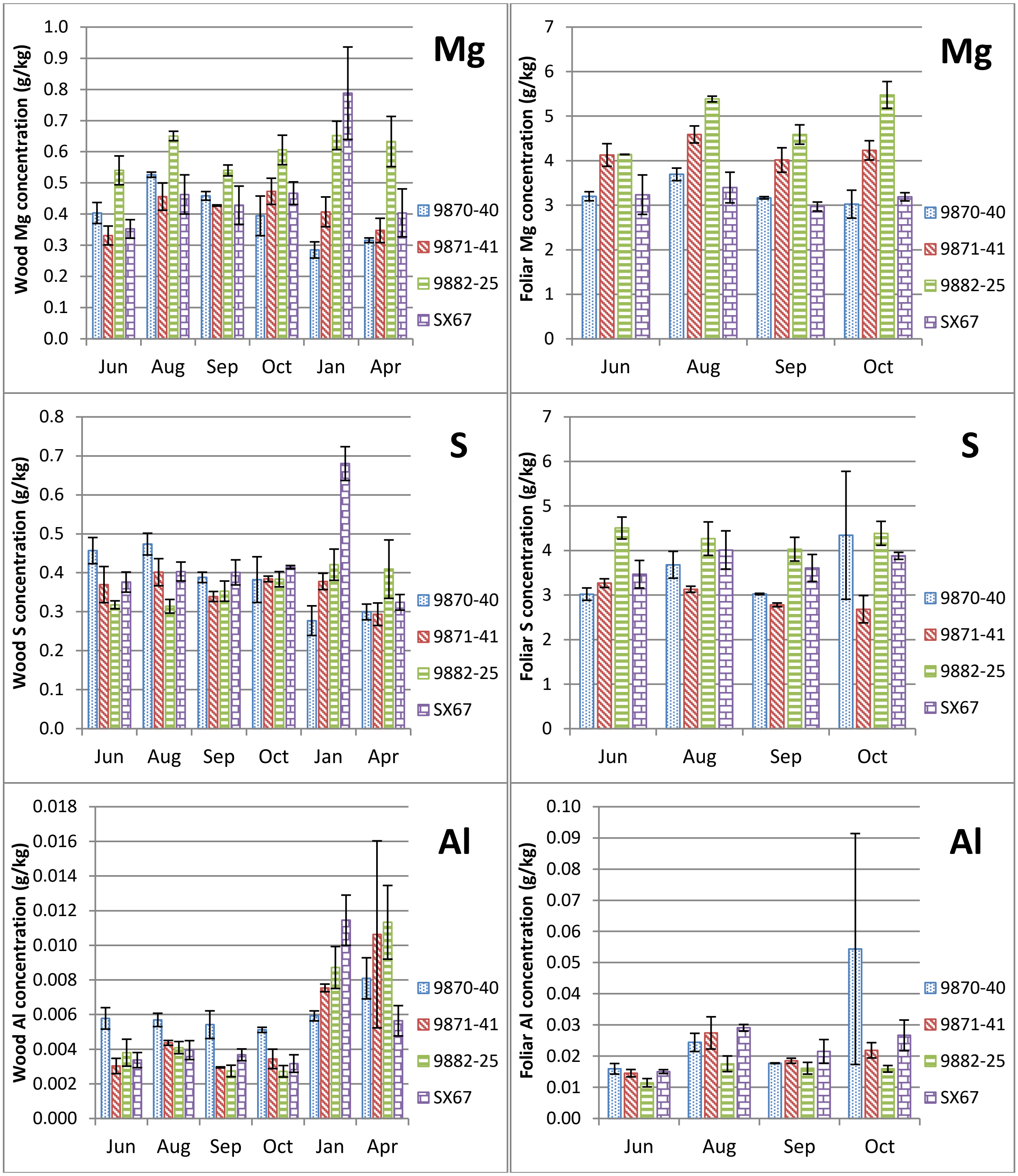
Appendix 3.1. Survival of four shrub willow cultivars at the end of the second rotation for different harvest dates (mean ± SE).



Appendix 3.2. N and P concentrations in wood and foliage for different harvest dates and four cultivars (mean ± SE).



Appendix 3.3. K and Ca concentrations in wood and foliage for different harvest dates and four cultivars (mean ± SE).



Appendix 3.4. Mg, S, and Al concentrations in wood and foliage for different harvest dates and four cultivars (mean ± SE).

CHAPTER IV: NUTRIENT REMOVAL VIA HARVESTED BIOMASS IN SHRUB WILLOW CROPS DIFFERS BETWEEN COMMERCIAL SCALE AND RESEARCH SCALE HARVESTING OPERATIONS

Abstract

The utilization of shrub willow biomass to produce bioenergy and bioproducts has been spurred by concerns about climate change and greenhouse gases emissions (GHG). Recent deployment of commercial shrub willow sites in the northeastern region of the US, specifically in NY, has raised questions about nutrient removal calculations in hand-harvested willow biomass at research fields. Observations have found that a proportion of the harvestable biomass remains on site as dropped material possibly with a high nutrient content that could be returned to the soil and support following rotations. This study compared the nutrient concentration and removal using hand-harvested and mechanized-harvested shrub willow biomass and estimated the amount of biomass left behind after a mechanized harvest and the nutrient content in these drops. Nutrient concentration in hand- and mechanized-harvested biomass were similar for N, P, Mg, and Al, but higher in mechanized-harvested biomass for K (+12%), Ca (+10%), and S (+9%). Total dropped biomass after the mechanized harvest was 1.6 – 4.5 Mg ha^{-1}, consisting mainly of merchantable biomass (cuts, 86%), and representing 7 – 15% of the total standing biomass. The nutrient content in the dropped biomass was 5.2-17.4 kg N ha^{-1}, 1.0-3.3 kg P ha^{-1}, 3.3-9.6 kg K ha^{-1}, 7.8-57.1 kg Ca ha^{-1}, 0.5-1.6 kg Mg ha^{-1}, 0.5-1.6 kg S ha^{-1}, and 0.02-0.05 kg Al ha^{-1}. These results indicate that dropped material contains approximately 20-35% of the total nutrient content in the harvested aboveground biomass, contributing to the growth of subsequent rotations and soil nutrient levels. Additionally, despite biomass loss, which could translate into revenue loss, it is necessary to assess the advantages and disadvantages of the

dropped biomass on the total system revenue to determine whether to improve the harvesting system or to adjust nutrient management practices.

Keywords: *Salix*, short rotation woody crops, nutrient management, nutrient concentration, soil fertility

4.1. Introduction

Despite the dominance of fossil and non-renewable sources for energy and materials production worldwide the interest in biomass as an alternative has been increasing over the last few years (see [1], [2] for more information of the role of biomass and bioenergy in climate change mitigation strategies). Short rotation woody crops (SRWC), including shrub willow, are considered a promising source of biomass in temperate climates, demonstrating great potential to serve as feedstock for a variety of end-products, provide environmental services, and offer social, economic, and environmental benefits [3]–[7]

Management of shrub willow crops is more intense than traditional forest plantations with its short harvest cycles of 3-4 years, fertilization each rotation, high planting density, and whole-plant harvest system, but less intense than most annual agronomic systems. The current recommended management practices for shrub willow [8]–[11] raise some concerns about potential negative impacts that may occur. The high growth rates obtained with these management techniques and frequent whole plant harvesting raise concerns about nutrient removals, long term site conditions and willow productivity over multiple rotations [12]. For this reason, nutrient management and removal in shrub willow crops have been areas of interest and research for many years, focusing on different aspects (such as fertilization rates, fertilizer types, and rotation length), regions of the world, and species/cultivars [8]–[12]. With the recent need and interest in developing a bioeconomy in the United States [7], [17] and the increasing interest in establishing shrub willow crops [18], [19], nutrient management guidelines specific to the region and modern cultivars will be needed to accommodate the nutritional needs of the crop and ensure high yield production. The utilization of data from research and large-scale harvests should provide a basis to develop guidelines for commercial shrub willow growers in order to perform appropriate nutrient management and ensure high yields over rotations.

Despite the relatively recent interest in shrub willow in the United States and the absence of established and consistent markets for the biomass, harvesting equipment and methodology dedicated to SRWC are being developed and studied [17]–[21], but no single dominant system exists [20], [23]. In 2008, Case New Holland (CNH) began the development of a short-rotation single pass, cut and chip header (130FB) that can be attached to their FR9000 series of forage harvester, and newer series as well, to operate in SRWC. Studies evaluating the performance of the harvester observed that a certain amount of woody biomass, referred to as “drops”, is not collected by the harvester, remaining on site as residues after the harvesting operation ends [23], [25]. The results showed that between 8 - 28% of the standing biomass is dropped in the field by the harvester, which will remain on site and return nutrients to the soil. Up to 88% of this material left behind consists of tips and ends of branches [23] that have a high bark to wood ratio and presumably a higher nutrient content than stem wood.

Nutrient removal in shrub willow research field trials is commonly evaluated following specific and strict guidelines and methodologies (see materials and methods section and Chapter 2 for more information). Commonly, small cultivar/species study plots are installed with effective measurement and buffer areas, a specific number of plants/stems are selected given desired characteristics (height, diameter, form, etc.), hand-harvested and carefully removed from the field, weighed, and finally the desired parts of the plant (stem, bark, branches, twigs, and leaf) are dried, split and ground before being analyzed for nutrient concentration (g kg^{-1}). Conversely, commercial scale shrub willow crop fields are planted with single cultivar blocks or mixed plantings where several cultivars are randomly mixed in each field. Additionally, it appears that commercial harvest operations leave a higher amount of drops to decompose on site than typical hand harvests [23], [25]. Hence, it is reasonable to assume that total nutrient removal by shrub willow crops calculated from hand-harvested biomass in research trials might differ from total nutrient removal calculated from commercial field harvested biomass, where drop losses occur. These differences in harvesting techniques and scale of operations for hand harvested research plots and commercial scale fields harvested with a forage harvester have raised questions about nutrient removal estimates that are used to determine fertilizer needs and long-term productivity of a site. Therefore, the objectives for this research are to (1) determine if nutrient concentration in a hand harvested willow trial site using research methods differs from a mechanized harvested commercial willow site and (2) to estimate how the drop losses from a mechanized harvest affect the nutrient removal.

4.2. Materials and methods

4.2.1. Site description

A willow demonstration site located in State College, PA (40°51'31"N, 077°47'45"W) was used to perform hand-harvest and mechanized-harvest operations. The soil at this site is defined as a well-drained deep Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalfs), with a depth to water table of more than 203 cm, and depth to restrictive layer (bedrock) ranging from 276 to 632 cm [26], [27]. The climate in the region according to Köppen classification is a temperate climate defined as a warm summer subtype humid continental climate, with annual precipitation ranging from 985 to 1041 mm.

The site was established in the spring of 2012, planted in double-rows with six shrub willow cultivars (Figure 4.1). The six cultivars are planted in blocks following a North-South orientation, with two separate blocks for each cultivar, totaling 12 blocks, and with a spacing of 1.5 m between double-rows, 0.8 m within double-rows, and 0.6 m between plants, for a density of 14,400 plants ha^{-1}. Each cultivar block had at least 15 double rows. The crop was coppiced after the first growing season to stimulate the regrowth of more stems and increase biomass production. The first harvest occurred after the 2015 growing season, when the plants were three years old; followed by a second harvest during the winter of 2018, where the samples and data for this project were collected.

4.2.2. Pre-harvest activities

An area of the field with longer north-south rows was selected for taking samples and measurements (see marked blocks on Figure 4.1), in order to facilitate the mechanized harvesting operations. Avoiding the edge rows, as well as edge rows in each cultivar block, five double-rows for each of the six cultivars were randomly selected (30 rows in total). Measurement plots, referred to as “drops plots”, were installed in each double row at set distances down each double row (Figures 4.1 and 4.2). The distances along the length of the rows were spaced out and then the double rows that the plots were allocated to were randomly selected. The drops plots consisted of one double-row (2.3 m wide) with 3 m in length (along the double-rows) for a total area of 6.9 m^2

Figure 4.1. Outline of the selected area at the site (yellow line) where hand harvesting and machine harvesting data were collected to assess biomass and nutrient losses. Measurement plots (red lines) were installed in each randomly selected double row and were spaced along the length of the rows for each cultivar (delineated by white lines).

Data collection occurred in three steps: (1) prior to mechanical harvesting, (2) during mechanical harvesting and (3) after mechanical harvesting. The pre-harvest activities consisted of hand-harvests in February of 2019, a week before the mechanized harvest. Three stems (small, medium and large) representing the range of diameters observed at each drops plot were selected in each of the measurement plots and cut with a brush saw at approximately 10-15 cm above the soil surface, to align with the expected height of the stumps cut by the harvester. The stems were removed from the field, chipped, and a 1–2 kg sample of the three chipped stems from each drops plot was collected in a paper bag, dried at 60°C to a constant weight, and its moisture content determined. This material was retained to determine nutrient concentrations of the standing biomass, which is described in further detail below.

4.2.3. Mechanized harvest

The mechanized harvest was completed with a CNH FR9080 forage harvester equipped with a 130FB coppice header, on February 28, March 2 and 4 of 2019. The operation consisted of a single-pass cut-and-chip operation, where the harvester cuts and chips the shrub-willow and blows the chipped biomass on a collection vehicle riding beside the harvester [23]. After harvesting an entire double-row, the collection vehicle was weighed full and after it was emptied using a platform scale to obtain the weight of the biomass harvested on the double-row. A 1-2 kg sample of chipped biomass was taken from the collection vehicle for each of the double rows where a drops plot was installed. The samples were collected in paper bags, weighed in the field, and later dried in the oven at 60ºC to a constant weight, and its moisture content determined. These samples were used to determine nutrient concentration of the harvested materials. Time-motion methods were utilized to study the harvest operation; with the utilization of hand-held Trimble GPS devices (one on board of each equipment) the location of the equipment was followed each second, resulting in geolocation and time data [23]. With the time-motion data and the weighed biomass, it was possible to calculate the harvested area (ha) and yield (Mg ha^{-1}) corresponding to each double-row/load.

4.2.4. Post-harvest activities

These activities consisted of collecting drops losses, material not collected by the harvester and left on the site. To determine whether or not to include stems that were only partially in the plot (crossing through the plot) in the drops data the following principle was used. The entire stem/branch/twig was included in the drops data if the cut end was located inside the plot. If the cut end was outside the plot them the entire stem/branch/twig was excluded from the drops data, regardless of the proportion of the drop inside or outside the plot. All the drops losses in the drops plots were collected and classified as follows [23]:

- Cuts: parts of the willow stems that were cut by the harvester's saw blades but did not feed into harvester.
- Chips: remaining biomass that was processed and chipped by the harvester, but was not loaded on the collection vehicle.
- Shakes: small twigs and branches, generally from the top of the plants that were not collected by the harvester and remain on the site.

Due to their small size, chips and shakes were only collected in two smaller subplots (2.3 x 0.3 m) randomly placed inside each of the measurement drops plot (Figure 4.2). The drops plots were visually divided in ten segments and two random numbers between 1-10 were randomly chosen. One subplot was installed at each segment of the plot corresponding to the chosen random numbers. All biomass belonging to each drop category was collected and no drops were left in the plot and subplots. Each drop category was collected in separate paper bags, placed in a drying oven at 60^{o}C, and dried to a constant weight.

4.2.5. Laboratory procedures

All samples collected during the study followed similar processing to determine nutrient concentrations. After being dried to a constant weight, the samples were split using a Gilson SP1 universal sample splitter (Gilson Company, Lewis Center, Ohio) in two subsamples to ensure the subsample composition was representative, then they were ground with a Willey Mill using a 40-mesh screen to produce a 300-400 g representative sub-sample, and 5 g of each sub-sample was sent to the Agricultural Analytical Services Lab at the Pennsylvania State University in order to determine nutrient concentration in the harvested biomass (the amount of nutrient in the biomass, expressed as grams of nutrient per kilogram of biomass [g kg^{-1}]) and to calculate nutrient content in the harvested biomass (the amount of nutrient present in the biomass per unit of area, expressed as kilograms of nutrient removed per hectare [kg ha^{-1}]) using the nutrient concentration, the mass of willow collected down the row and the area of the harvested row. Determination of N was done through the micro-Kjeldahl method, while the determination of P, K, Ca, Mg, Al, and S was performed through the microwave acid digestion method and the inductively coupled plasma atomic emission spectrometry (ICP-AES).

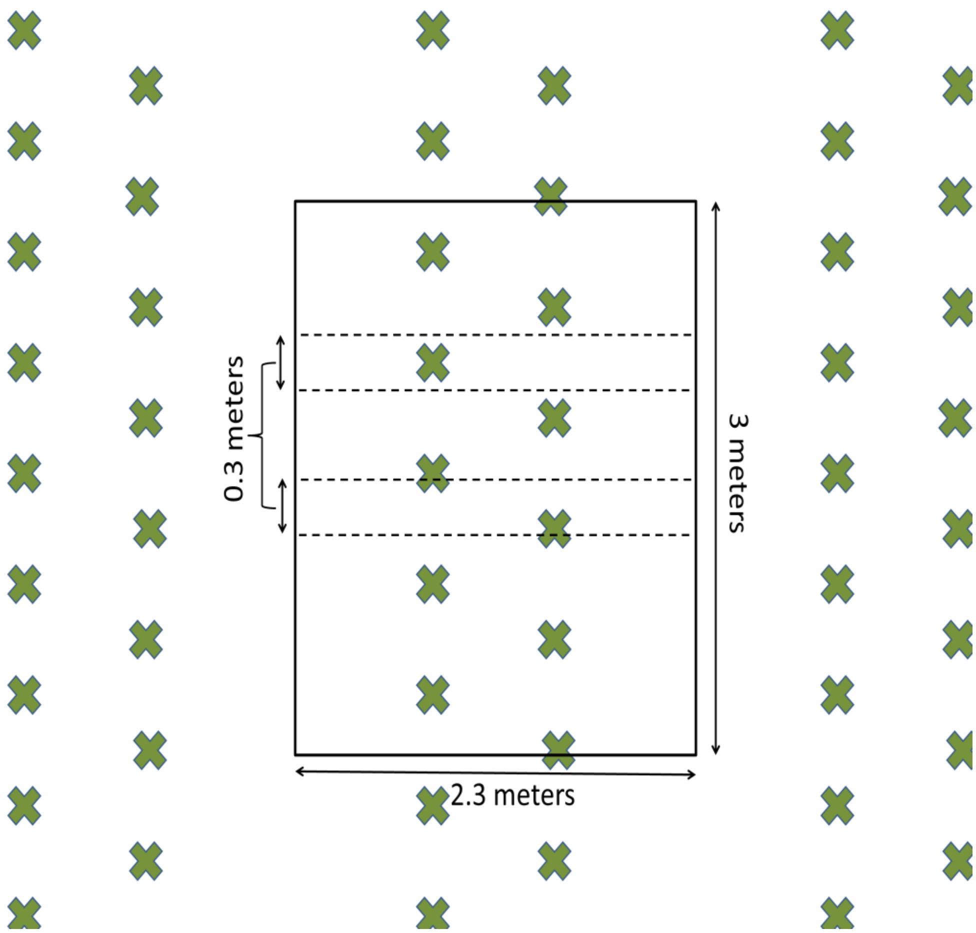


Figure 4.2. Plots used to collect shrub willow drops material after mechanical harvesting with a single pass cut and chip harvester. Crosses represent plants/stools. The drops plot (solid line) is placed in a double row for collection of cuts. Two 30 cm wide sub-plots (doted-line) are randomly placed across **each** drops plot to collect chips and shakes.

4.2.6. *Statistical Analyses*

The harvesting method study consisted of a split-plot design (harvesting method) with whole plots (row) and a randomized complete block design (RCBD; cultivars); while the drops study consisted of a RCBD with six blocks (cultivars) and five drops plots per block (Figure 4.1). Both studies present a random effect of plot nested within cultivar block. The experimental unit in both studies were the plots (rows). Statistical analyses were made in SAS 9.4 (SAS Institute) using the PROC GLIMMIX procedure for Generalized Linear Mixed Models (GLMM), since it allows for random effects in the model. Mixed models were built to estimate the effects of harvesting method, cultivar, and the interaction harvesting method*cultivar (HxC) on nutrient concentration and removal of hand- and mechanized harvested biomass as well as to estimate the effects of drop type, cultivar, and the interaction drop type*cultivar (DxC) on total weight of and nutrient content in dropped biomass after the mechanized harvest. The main effects significance were determined at a critical level of $\alpha=0.05$ while the interaction terms were determined at $\alpha=0.15$, in order to reduce the chances of committing type I error [28]. Significant differences between the studied factors were determined with the use of least square means (LS Means) instead of actual means.

4.3. Results

4.3.1. *Nutrient concentration in hand- and mechanized-harvested biomass*

Significant effects of harvesting method, cultivar, and HxC were observed in the biomass nutrient concentration depending on the nutritional element considered (Table 4.1). A significant interaction between harvesting method and cultivar (HxC) was observed for the concentration of P, K, Ca, and Mg. In the case of P, the concentration of mechanically harvested biomass was significantly higher in Preble and Fish Creek and higher for Ca for Preble and Fabius. For all other cultivars there was no difference between hand and harvester biomass for P and Ca. For K the concentration was significantly greater for harvester material for five of the six cultivars, the exception being SX61. Mg was the only element where a cultivar (Otisco) had higher concentration in the hand harvested material compared to the machine harvested. There was also one cultivar (Fish Creek) where harvester material Mg concentration was significantly greater than hand harvested material (Figure 4.3). No significant effect of harvesting method was observed in the harvested biomass nutrient concentration of N, P, Ca, Mg, and Al. The significant harvesting method effect was observed for the concentration of S, showing higher concentration in the hand harvested biomass (0.31 g S kg^{-1}) compared to the mechanized harvested biomass (0.29 g S kg^{-1}) (Figure 4.3). A significant cultivar effect was observed for the concentration of N and S (Table 4.1), in which Otisco (N and S) showed higher nutrient concentration compared to the other cultivars. Additionally, a significant effect of cultivar was observed in yield (Table 4.1), indicating higher yield by cultivar SX61, and significantly lower yield by cultivars Preble and Otisco (Figure 4.3).

Table 4.1. Analyses of variance results for the effects of harvesting method (hand versus machine harvested), cultivar, and the interaction HxC on yield and nutrient concentration of shrub willow biomass. Main effects significance determined using α=0.05 and the interaction with α=0.15. Significant differences between treatments are presented in bold format.

	Harvesting method (H)	Cultivar (C)	HxC
df	1	5	5
Yield	-	**<0.0001**	-
N	0.8584	**0.001**	0.9635
P	0.3798	**<0.0001**	**0.0484**
K	**<0.0001**	**<0.0001**	**0.05**
Ca	0.0616	**<0.0001**	**0.0708**
Mg	0.6959	0.0644	**0.0576**
S	**0.0015**	**0.0002**	0.3743
Al	0.2052	0.493	0.3868

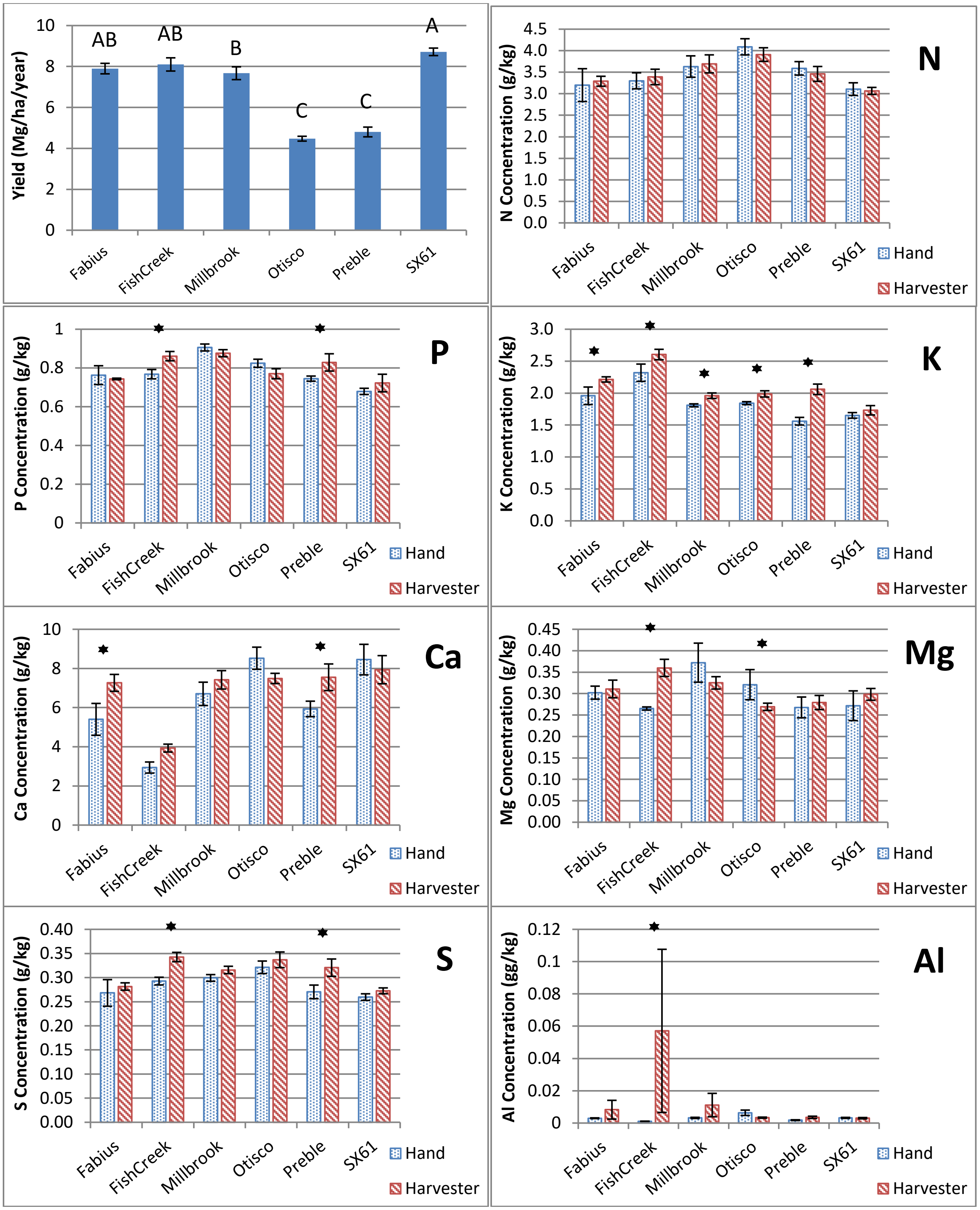


Figure 4.3. Yield (mean ± SE) of six willow cultivars and concentrations of N, P, K, Ca, Mg, S, and Al of hand- and mechanized-harvested willow biomass. Yield of cultivars with similar letters (determined by Tukey HSD) were not significantly different. Significant differences ($p < 0.05$) between nutrient concentration in hand- and mechanized harvested biomass are indicated by asterisks (*).

4.3.2. Drops losses biomass

The total amount of biomass left behind in the field following harvesting operations varied considerably depending on interaction of the type of dropped material and cultivar (DxC) (Table 4.2). Total drops biomass (chips + shakes + cuts) of cultivar SX61 (4.5 Mg ha^{-1}) was significantly higher than other cultivars, except Fabius (3.0 Mg ha^{-1}), which was statistically similar to SX61 (Table 4.3). The percentage of standing biomass that was left behind as drops ranged from 6.5% for Fish Creek to 14.8% for SX61. Cuts represented approximately 90-95% of total drops biomass for all cultivars, except for Fish Creek were cuts represented ~55% and chips made up another 39% (Table 4.3). Across all cultivars drop type effect indicated that total cuts (2.12 Mg ha^{-1}) biomass was significantly higher than shakes (0.15 Mg ha^{-1}) and chips (0.17 Mg ha^{-1}).

Table 4.2. Analyses of variance results for the effects of drops type, cultivar, and the interaction (DxC) on the amount and nutrient content of willow biomass dropped by a single pass cut and chip harvester. Main effects significance determined using α=0.05 and the interaction with α=0.15. Significant differences between treatments are presented in bold format.

	Drop type (D)	Cultivar (C)	DxC
df	2	5	10
Biomass	**<0.0001**	**0.0346**	**0.0008**
N	**<0.0001**	0.2257	**0.09**
P	**<0.0001**	0.1898	**0.0521**
K	**<0.0001**	0.153	**0.0447**
Ca	**<0.0001**	**0.0074**	**0.0003**
Mg	**<0.0001**	0.1898	**0.0393**
S	**<0.0001**	0.1437	**0.0369**
Al	**<0.0001**	0.1208	0.7283

Table 4.3. Amounts of three different categories (chips, cuts and shakes) of willow biomass (mean and standard error) left in the field following a harvesting operation with a single pass cut and chip harvester for six cultivars. Significant differences among cultivars are indicated by different letters (determined by Tukey HSD) and were determined at α=0.05.

Cultivar	Chips	Cuts	Shakes	Total drops	Standing biomass	% Drops*
	Mg_{dry} ha^{-1}					
Fabius	0.07 (0.02)a	2.65 (0.31)ab	0.30 (0.10)a	3.00 (0.42)ab	23.7 (0.8)	11.2
Fish Creek	0.66 (0.56)a	0.95 (0.19)b	0.08 (0.02)bc	1.70 (0.67)b	24.3 (1.0)	6.5
Millbrook	0.10 (0.03)a	1.66 (0.27)b	0.13 (0.02)abc	1.86 (0.23)b	23.0 (0.9)	7.5
Otisco	0.01 (0.00)a	1.82 (0.56)b	0.04 (0.00)c	1.86 (0.57)b	13.4 (0.4)	12.2
Preble	0.07 (0.04)a	1.43 (0.34)b	0.08 (0.04)bc	1.58 (0.38)b	14.4 (0.7)	9.9
SX61	0.09 (0.02)a	4.21 (1.19)a	0.26 (0.10)ab	4.54 (1.27)a	26.1 (1.7)	14.8

*The proportion of drops compared to total harvested biomass calculated as: % Drops = (Total drops + Standing biomass) / Total drops

4.3.3. Drops losses nutrient concentration

Nutrient concentration in the dropped biomass varied slightly depending on the nutritional element. Concentration of Ca was the only element with a significant DxC effect (Table 4.4), due to the significantly higher concentration in SX61 and significantly lower in Fish Creek. Additionally, concentration of Ca in shakes was higher for all cultivars with exception of Preble, and similar in chips and cuts for cultivars Fabius, Fish Creek, and Preble (Figure 4.4). A significant effect of drop type was observed for N, K, Ca, Mg, and S, but not for P and Al (Table 4.4). Concentrations of N, K, Ca, Mg, and S were higher in shakes compared to chips and cuts, P concentration in chips were statistically similar to shakes but higher compared to cuts, and Al concentration was statistically similar in the three drops types (Figure 4.4).

Table 4.4. Analyses of variance results for the effects of drops type, cultivar, and interaction DxC on nutrient concentration on willow biomass dropped by a single pass cut and chip harvester. Significance was determined using α=0.05. Significant differences among treatments are presented in bold.

	Drop type	Cultivar	DxC
df	2	5	10
N	**<0.0001**	0.4291	0.2728
P	0.1679	0.6244	0.9689
K	**<0.0001**	0.4348	0.3324
Ca	**<0.0001**	**<0.0001**	**0.0143**
Mg	**<0.0001**	0.1073	0.9209
S	**<0.0001**	0.3788	0.4485
Al	0.2247	0.3176	0.7052

4.3.4. Drops losses nutrient content

The nutrient content of drops varied slightly depending on nutritional element considered, but significantly depending on cultivar and drops type. A significant DxC interaction was observed for all the studied nutritional elements except Al, a significant drops type effect was observed for the content of all studied elements, and cultivar effect was significant for Ca content (Table 4.2). The significant DxC interaction was driven by the larger amount of nutrient content in chips in Fish Creek for all elements, which is driven by the higher chip biomass for this cultivar. Also, the proportion of the nutrient content in shakes was higher in Fabius and SX61 than in other cultivars (Figure 4.5). Among drops type, contents were significantly higher in cuts compared to chips and shakes for all elements. Drops nutrient content was strongly determined by drops biomass, where a positive linear relationship was observed, indicating that larger drop biomass translated into larger nutrient content (Table 4.3 and Figure 4.5). The significant cultivar effect on total Ca content indicated that the content of cultivar SX61 was larger than the other cultivars. This pattern of SX61 having a higher nutrient content was present for other elements, but due to the amount of variation in the date the differences were not statistically significant (Figure 4.5).

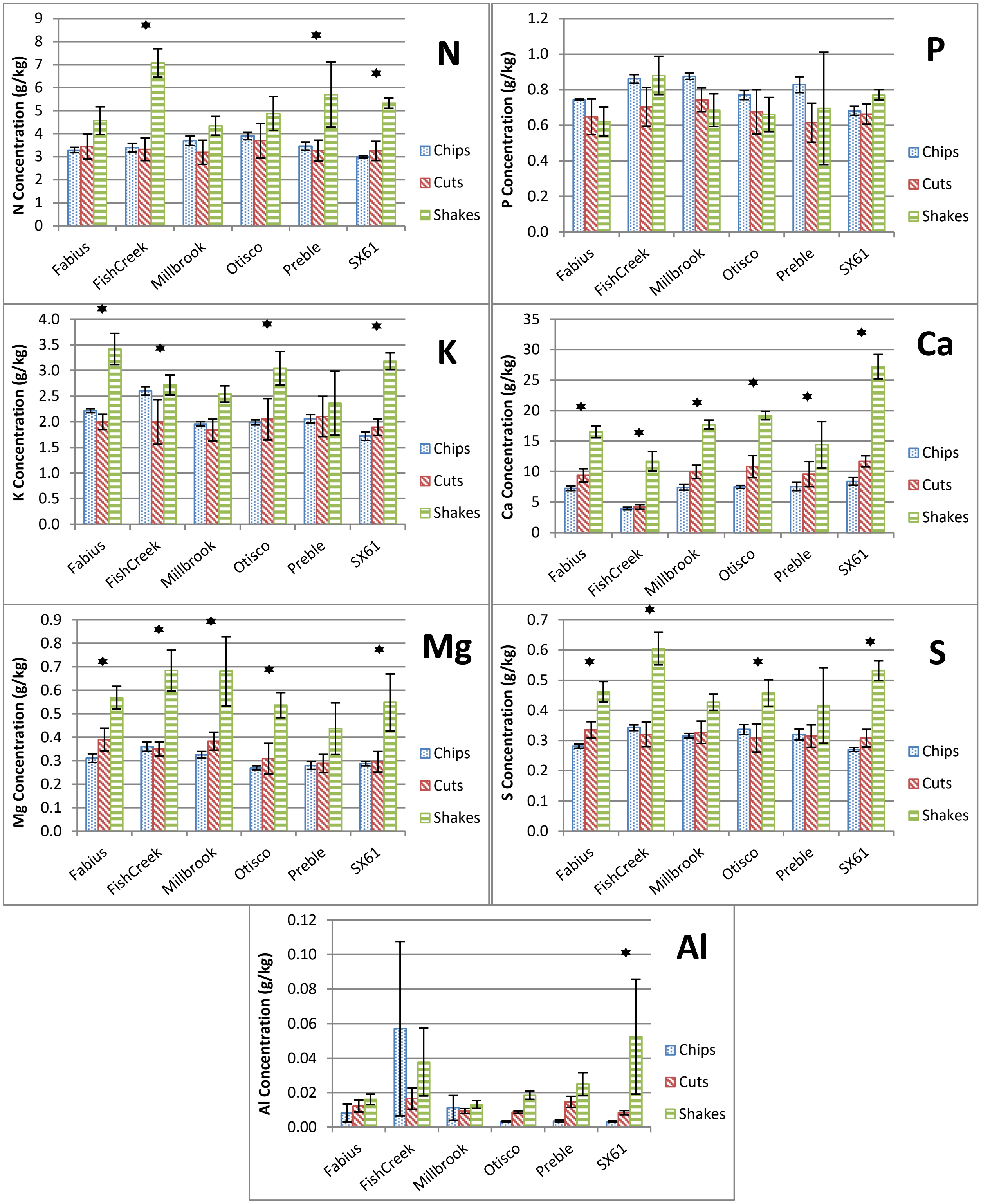


Figure 4.4. Nutrient concentration (N, P, K, Ca, Mg, S, and Al) on different types of material (cuts, shakes, and chips) left on the field after mechanical harvesting for six willow cultivars (mean ± SE). Asterisks (*) indicate significant difference among drops type within cultivars. Significant terms determined with α=0.05.

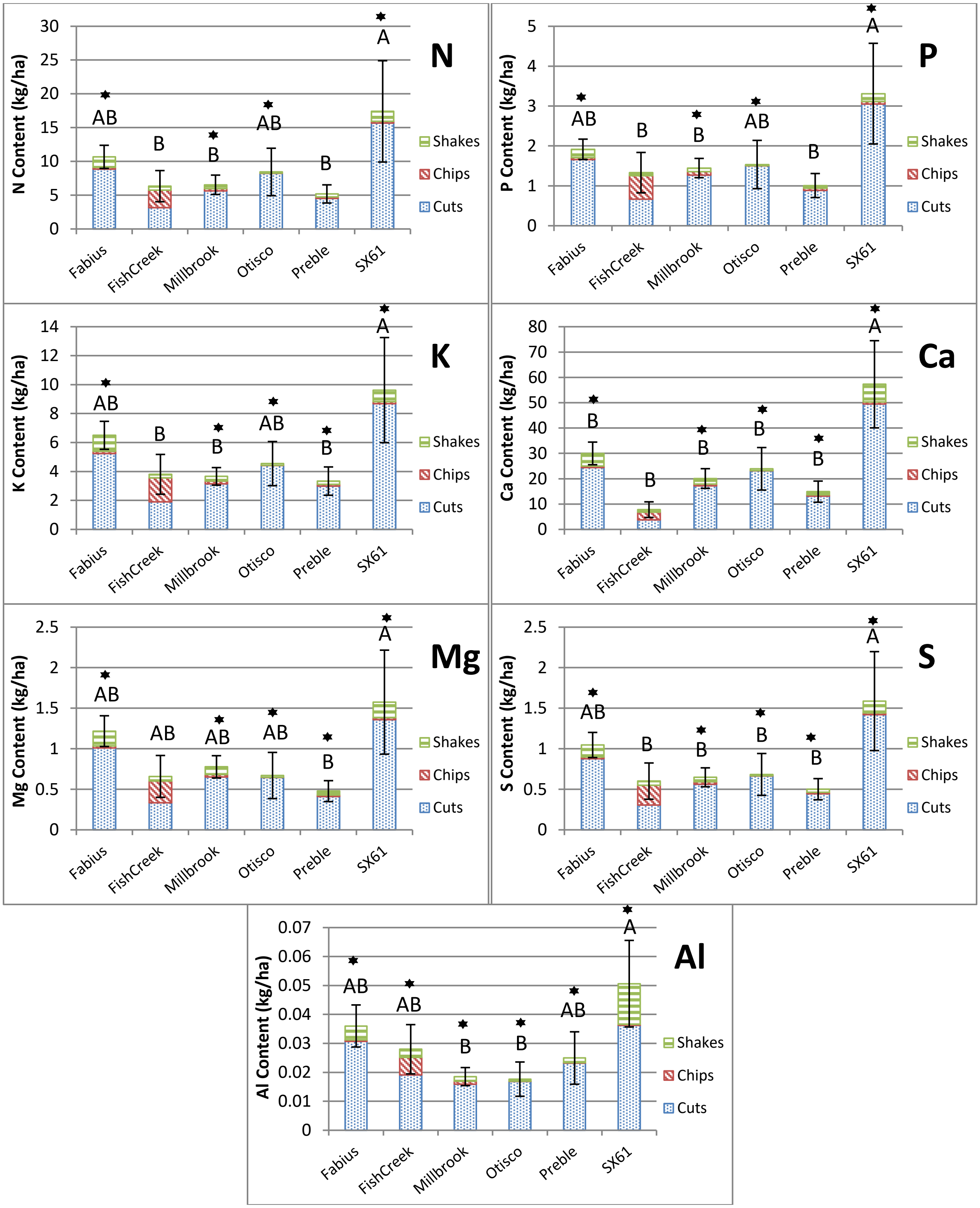


Figure 4.5. Nutrient content (N, P, K, Ca, Mg, S, and Al) of different types of material (cuts, shakes, and chips) for six willow cultivars left on the field after mechanical harvesting (mean ± SE). Total nutrient content left behind is not significantly different for cultivars with similar letters (determined by Tukey HSD). Asterisks (*) indicate significant difference among drops type within cultivars. Significant terms determined with α=0.05.

4.4. Discussion

4.4.1. Nutrient concentration in hand- and mechanized-harvested biomass

The HxC for nutrient concentrations indicated that whenever a significant difference occurred between hand- and mechanized harvested biomass, the higher concentration would be in the mechanized-harvested biomass, except in the case of Mg concentration in cultivar Otisco, which resulted higher in hand-harvested biomass (Figure 4.3). Our initial hypothesis indicated that there would be higher nutrient concentrations in hand-harvested biomass, given the detailed harvesting methodology in which the plants are handled carefully and all the plant's parts (stem, bark, branches, and twigs) are collected and analyzed for nutrient concentration. Furthermore, previous reports indicated a high amount of dropped material following mechanized harvests [29], which was confirmed in this project (Table 4.3), including nutrient rich parts of the plants such as small twigs and branches (Figure 4.5). These differences in nutrient concentration could be a product of biomass samples taken at only one or two planes (locations) in the collection vehicle [30] during the mechanized harvest, instead of sampling a greater number of planes and having a more representative sample of the entire collection vehicle. Additionally, the mechanized-harvested biomass could present some soil contamination collected by the harvester when pulling the plants into the header, which could contribute to the slightly higher nutrient concentration observed.

Nutrient concentration and removal in shrub willow crops have been extensively studied [12], [13], [15], [31] (see Chapter 2 for more information on nutrient removal studies in shrub willow crops), but the research on nutrient removal by large-scale commercial shrub willow harvest operations is scarce. A previous study [22] also compared nutrient concentration in mechanized- and hand-harvested willow biomass, finding that concentrations of Ca, Cu, K, Mg, P, S, and Zn were higher in mechanized-harvested biomass. Similarly, our results indicated a significant HxC interaction, in which concentrations of P, K, Ca, and Mg resulted higher in mechanized-harvested biomass, with exception of cultivar Otisco and Mg concentration, which was higher in hand-harvested biomass, and a significant harvesting method effect for S indicating higher concentration in mechanized-harvested biomass.

Despite the statistically significantly higher nutrient concentration observed in mechanized-harvested biomass, some of these differences between the two harvesting methods are not that important from a practical point of view and will not represent significantly different nutrient removal. For example, the concentration of K in Millbrook was 8% significantly higher in mechanized-harvested biomass, while P in Fish Creek was 11% higher in mechanized-harvested biomass (Figure 4.3). These values correspond to additional 1.2 kg K ha^{-1} and 0.8 kg P ha^{-1} (See supplementary material) removed by mechanized-harvested biomass compared to hand-harvested biomass, which were not statistically different. Hence, although statistical differences were observed between nutrient concentrations in hand- and mechanized-harvested biomass, these differences might not represent significantly or practical higher removals.

Research methods used to collect biomass and estimate nutrient removal in hand- and mechanized harvests do not take into consideration the nutrient content of the dropped material left on site after a mechanical harvest. Our results indicated an important amount of dropped biomass after the mechanical harvest (Table 4.3) containing 20 – 35% of the total nutrient content that was present in the total above-ground woody biomass (Figure 4.5). Hence, it is important to account for drops losses and the nutrient content of these losses when estimating nutrient removal through nutrient concentration in biomass that has been harvested and removed from the field. Few studies in shrub willow nutrient removal have accounted for several sources of nutrient inputs and outputs [15], [32], [33], and most studies consider only nutrient removal via harvested biomass to estimate and recommend nutrient management in shrub willow crops (see chapter 2 for more information); however, previous observations have shown little impact of repeated harvests of shrub willow field trials on long-term nutrient levels in soil (see chapter 2) and the drops losses observed in our study could indicate an even smaller impact in commercial shrub willow crops mechanically harvested. Considering above ground activity and nutrient inputs and transfers within the soil-willow system (foliage nutrients, atmospheric deposition, and canopy exchange) presented by Amichev et al., 2014, [33], we could estimate an additional 5-16% of N, 4-13% of P, 2-6% of K, 2-18% of Ca, 1-3% of Mg, and 1-3% of S inputs provided by the dropped biomass after the mechanized harvest.

4.4.2. Drops losses biomass

The development of harvesting equipment and methodology for SRWC have been slow but constant, and an apparent preference is the use of single-pass cut-and-chip forage harvesters [20], [29]. The CNH FR9080 equipped with the 130FB header used in this study has been evaluated recently in the US, including a collection and evaluation of dropped biomass [29]. Dropped biomass in our study ranged from 1.6 to 4.5 Mg ha^{-1}, with cuts representing approximately 87% of total dropped biomass (Table 4.3). Similar values of dropped biomass were observed in other studies (Table 4.5). Eisenbies et al., 2014 [29] collected between 1.5 and 2.1 Mg ha^{-1} of dropped biomass after harvest, representing 8% of the total harvested biomass. Although similar results observed in both studies, the highest proportion of drop type in their study was represented by shakes (average of 44%), contrasting to our results. A possible explanation for the larger amount of cuts in our study could be the phenotypic characteristics of the plants harvested. We observed that cultivars with plants with larger stem diameter at cut height (Figure 4.6) produced a higher amount of dropped material, especially cuts (Table 4.3), while plants with smaller diameter produced lower amount of dropped material. Cultivar SX61 had ~28% of stems with diameter >30 mm and dropped 4.2 Mg ha^{-1} of cuts while cultivar Fish Creek had only ~7% of stems with diameter >30 mm and dropped 0.9 Mg ha^{-1} of cuts (Table 4.3). Nonetheless, a large variation was observed in the dropped biomass between plots and within cultivars, especially SX61. The use of larger plots covering a larger area of the site in future studies would help reduce the variability observed.

Table 4.5. Results from studies focusing on dropped biomass after mechanized harvest of SRWC species using different harvesting equipment

Source	Harvesting system	SRWC species	Total dropped biomass	Total harvested biomass	Relative loss
			Mg_{dry} ha^{-1}		%
[29]	CNH FR forage harvester with 130FB header	Shrub willow	1.5 – 2.1	19 – 25.5	8
[11]	CNH FR forage harvester with 130FB header	Shrub willow and poplar	1.1 – 3.2	9.8 – 11.7	11 – 28
[25]	Nordic Biomass Stemster MKIII	Shrub willow and poplar	0.02 – 0.4	4.7 – 9.8	0.3 – 4
[34]	Anderson Biobaler WB-55	Shrub willow	1-3 – 3.6	12.3 – 28.4	6 – 16
This study	CNH FR forage harvester with 130FB header	Shrub willow	1.6 – 4.5	13.4 – 26.1	7 – 15

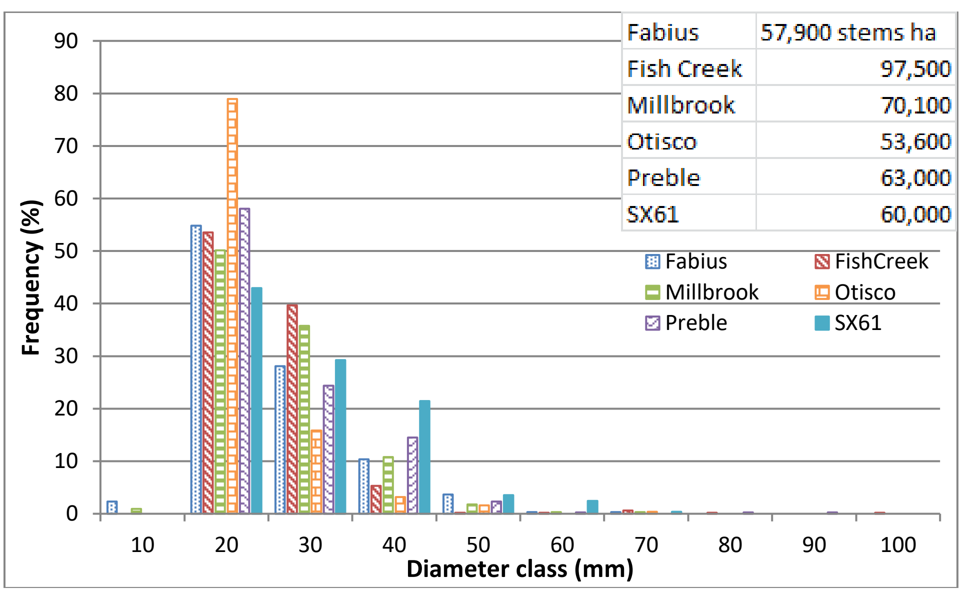


Figure 4.6. Stem diameter distribution (at cut height) as a proportion of the total number of stems for six shrub willow cultivars harvested with a single pass cut and chip system.

The harvest system using the CNH with the 130FB header have shown similar dropped biomass results in three different studies (Table 4.5), with slight differences probably as a result of higher standing biomass, diameter, harvester and collection vehicle operator, and different harvested species. Hence, considering the values observed in our study, in which the average total dropped biomass was 10% of total harvested biomass and the merchantable dropped biomass (cuts) represented 87% of total dropped biomass, we could assume a loss of 9% of the total merchantable and harvestable biomass (Table 4.3). More research and development of harvesting systems might be necessary in order to avoid losses and improve revenue. However, despite apparent economic loss, this dropped material could play an important role supplying key nutrients for the following rotation and supporting the upcoming rotation's yield.

Table 4.6. Total harvestable biomass, proportion of dropped biomass, total possible revenue, and revenue left on site as dropped biomass for six cultivars after the mechanized harvest assuming a biomass price at plant gate of \$30.5 Mg^{-1} of wet biomass [35].

	Total harvestable biomass	Proportion of dropped biomass	Total revenue	Revenue lost on site as dropped biomass
	Mg_{wet} ha^{-1}	%	\$ ha^{-1}	\$ ha^{-1}
Fabius	44.3	11.2	1,351	151
Fish Creek	42.3	6.5	1,290	84
Millbrook	45.1	7.5	1,376	103
Otisco	25.9	12.2	790	96
Preble	29.0	9.9	885	88
SX61	49.5	14.8	1,510	224

4.4.3. Drops losses nutrient concentration and content

The observed nutrient content in the dropped biomass was mostly influenced by the proportion of biomass left on the site rather than by the nutrient concentration in the biomass. Nutrient concentration in the biomass followed the order shakes > cuts = chips, while the nutrient content in the biomass was cuts > shakes > chips, although some variation was observed in chips and shakes depending on cultivar. As expected, the nutrient concentration of shakes was higher, given the higher bark proportion compared to wood and the higher nutrient concentration of the bark [36], [37]; however, the amount of shakes present was one to two orders of magnitude lower than cuts, hence the higher nutrient content in the biomass of cuts.

The dropped biomass can increase the previously considered inputs into shrub willow system [33] with up to additional 16% of N, 13% of P, 6% of K, 18% of Ca, 3% of Mg, and 3% of S. The initial concern about the use of whole-tree harvesting impacting the crop's long-term productivity or even causing soil nutrient depletion might be out of place in commercial shrub willow sites. Our results showed that 20 – 35% of the total nutrient present in above–ground standing willow biomass will actually stay on site in the form of dropped biomass (Table 4.7). It has been observed that soil nutrient levels under shrub willow experimental sites are slightly altered after several rotations (see chapter 2). Nutrient removal on these sites have been determined using hand-harvested biomass from measuring plots; and final soil nutrient levels were higher than expected, if only nutrient removal via harvested biomass was considered. A mechanized harvest is also used in these sites to harvest the remaining plants additional to the plants in the measuring plots, which most likely dropped biomass that is also contributing to maintain the soil nutrient levels in these sites, additionally to the other inputs and exchanges already known and accounted for [15], [33]. Furthermore, given the observed differences between nutrient concentration and nutrient content in dropped biomass presented by different cultivars (Table 4.7), it could be expected that the selection and deployment of cultivars will require different nutrient management practices depending on the combination of cultivars used.

Table 4.7. Nutrient content (kg ha^{-1}) in hand- and mechanized-harvested and dropped biomass and dropped biomass nutrient content as a proportion of the total nutrient content in above-ground shrub willow standing biomass

	Present in harvested biomass		Present in dropped biomass	Proportion of total nutrient in dropped biomass	
	kg ha^{-1}		kg ha^{-1}	%	
	Mechanized harvest	Hand harvest		Mechanized Harvest	Hand Harvest
N					
Fabius	25.93	25.23	10.63	29	30
Fish Creek	27.46	26.76	6.34	19	19
Millbrook	28.32	27.74	6.43	19	19
Otisco	17.52	18.25	8.40	32	32
Preble	16.71	17.02	5.19	24	23
SX61	26.67	26.98	17.35	39	39
P					
Fabius	5.87	6.02	1.90	24	24
Fish Creek	6.99	6.22	1.33	16	18
Millbrook	6.71	6.93	1.43	18	17
Otisco	3.45	3.69	1.53	31	29
Preble	3.98	3.57	1.01	20	22
SX61	6.28	5.92	3.30	34	36
K					
Fabius	17.46	15.51	6.47	27	29
Fish Creek	21.06	18.67	3.80	15	17
Millbrook	15.02	13.87	3.60	19	21
Otisco	8.89	8.24	4.52	34	35
Preble	10.00	7.45	3.34	25	31
SX61	15.13	14.40	9.59	39	40
Ca					
Fabius	57.09	42.95	29.89	34	41
Fish Creek	31.96	23.74	7.80	20	25
Millbrook	56.72	51.14	19.65	26	28
Otisco	33.43	38.38	23.78	42	38
Preble	37.15	28.43	14.91	29	34
SX61	69.84	74.22	57.13	45	43
Mg					
Fabius	2.47	2.38	1.21	33	34
Fish Creek	2.92	2.15	0.66	18	23
Millbrook	2.48	2.83	0.76	23	21
Otisco	1.20	1.44	0.67	36	32
Preble	1.35	1.29	0.48	26	27
SX61	2.60	2.37	1.57	38	40
S					
Fabius	2.22	2.12	1.04	32	33
Fish Creek	2.78	2.37	0.60	18	20
Millbrook	2.42	2.29	0.64	21	22
Otisco	1.51	1.44	0.68	31	32
Preble	1.56	1.28	0.50	24	28
SX61	2.38	2.26	1.58	40	41

Al					
Fabius	0.068	0.024	0.036	34	60
Fish Creek	0.519	0.009	0.028	5	76
Millbrook	0.077	0.024	0.018	19	43
Otisco	0.015	0.029	0.018	54	38
Preble	0.018	0.008	0.025	59	75
SX61	0.026	0.028	0.051	66	64

Among macronutrients, N and P are probably the ones to receive more attention, given their importance on plants growth rate [38], [39]. The content of these elements in dropped material represents approximately a fourth of the total content in the woody part of a willow plant (Table 4.6). Current nutrient management practices in shrub willow crops in NY recommend the addition of 100 kg ha^{-1} of nitrogen [40], [41] in the spring following a harvest, with limited concern on P given past land uses, management, and soil types. The results observed in this study, combined with the results observed in Chapter 2, can potentially change these recommendations, either by reducing the amount of fertilizer applied or the recurrence of the practice.

Finally, our results indicate that the amount of dropped biomass, and as a result the nutrient content of drops, varied considerably depending on cultivar, probably because of the plant's phenotypic characteristics. A proper selection of high yielding cultivars, with smaller diameter stems, could limit the amount of dropped biomass, increase the harvested biomass, and ensure higher revenues. On the other hand, reducing dropped biomass by improving harvester operations would increase nutrient removals, which may impact nutrient management and long term productivity. Future research should focus on the economic advantages of collecting more biomass and, possibly, applying fertilizers against maintaining the drops as residues but reducing the fertilization needs.

4.5. Conclusion

Nutrient concentration in hand- and mechanized harvested shrub willow biomass were similar in this trial. The determination of nutrient concentrations using hand-harvesting methods provided a reasonable estimate of nutrient removal compared to machine harvested estimates, annulling our previous hypothesis of higher nutrient concentration in hand-harvested biomass. However, when determining nutrient removal using data from hand-harvested trials in order to recommend nutrient management for shrub willow commercial sites, it is important to take into consideration the high amount of dropped biomass after a commercial harvest operation and the nutrient content in this biomass that is left on the site.

Several mechanized shrub willow harvesting systems are available in the market, depending on the region considered. Previous research on these systems indicate that they all leave some biomass behind and our results are in general agreement with the limited available literature [25], [29], [34]. An average of 10% of the total standing biomass, out of which 9% could be considered merchantable biomass (cuts), is left on the site as residues or dropped material. In order to maximize revenue, it is important to develop a system that could capture more of the available merchantable biomass. However, the occurrence of dropped material could also play an important role to support the crop's long-term productivity by retaining a considerable amount of key nutrients on site, and hence, contributing to the great nutrient cycling capacity of commercial shrub willow crops, even when harvested as whole-plants.

The high nutrient content observed in the drops, especially in cuts, will likely supply an extra 16% of N, 13% of P, 6% of K, 18% of Ca, 3% of Mg, and 3% of S for the following rotations. Non-merchantable parts of the plants (shakes) had higher nutrient concentration compared to merchantable parts, but lower biomass weight. Cultivars with larger stem diameters presented higher amounts of dropped material, especially cuts. Previous studies [29] reported shakes accounting for 15 – 88% of total dropped biomass, contrasting to our results. A selection of high yielding cultivars, with smaller stem diameter, could be beneficial both for the throughput of the harvester (collecting a higher proportion of the standing biomass) and for the nutrient management, since a higher proportion of non-merchantable biomass, with higher nutrient concentration, could remain on site as drops. Nonetheless, future research should focus on the advantages and disadvantages of the dropped biomass on the soil's nutrient budget, growth of shrub willow in the next rotation, and the system's total revenue, looking at the shrub willow as an integrated system and weighing the different tradeoffs.

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CHAPTER V: CONCLUSIONS AND CONSIDERATIONS FOR FUTURE RESEARCH

5.1. Conclusions

Evaluations of nutrient concentration and nutrient removal in harvested shrub willow biomass was conducted under different scenarios. First, 18 willow cultivars planted at two sites in NY showed different patterns in nutrient removal over three three-year rotations; furthermore, the soil N (at Belleville) and P (at both sites) concentrations were significantly reduced over the three three-year rotations. Second, timing of harvest had significant effects on second rotation aboveground biomass production and nutrient removal rates of four cultivars planted in NY, with higher biomass production occurring on plots harvested during leaf-off season and higher nutrient removal on plots harvested during leaf-on season. Third, nutrient concentration was similar in hand-harvested and mechanized-harvested shrub willow biomass, however, dropped biomass after a mechanized harvest was 7 – 15% of the total standing biomass. The nutrients in this material could then be available to the shrub willow system via biomass decomposition and should be considered when determining nutrient removal rates in commercial shrub willow sites.

The observed differences in nutrient removal by five top yielding commercial cultivars over three-rotations indicated that N and P removals were similar across rotations while removals in later rotations (third and fourth) were 1.5-2x (K), 1.2-2x (Ca), and 1.3-1.8x (Mg) the removals observed of the second rotation. No significant differences in the removals of K, Ca, and Mg were observed between third and fourth rotations, although considerable variability was observed among cultivars and sites. Nutrient management guidelines for shrub willow crops in NY recommend the application of 100 kg N ha^{-1} in the spring following each harvest, regardless of the rotation, cultivars planted, or site. The findings of this dissertation indicate that nutrient management guidelines should be developed specifically depending on the site and soil characteristics, the combination of cultivars deployed, past land-uses, soil nutrient levels, and could even vary depending on the rotation. Furthermore, the relationship between nutrient removals via harvested biomass and soil nutrient levels confirm the importance of site and cultivar specific nutrient management. Soil N and P levels at Belleville were significantly lower after three rotations, soil P was the only significant reduction observed at Tully, soil K levels increased at Tully, and the other elements at both sites remained constant. Previous land use at Belleville (corn production) may have contributed to high initial N and P concentrations, which is likely the cause of the reductions to similar levels observed at Tully. These results indicate the great capacity that shrub willow has to use and cycle nutrients within the plant-soil system and self-supply a high amount of nutrients required to produce high yields. Adequate nutrient management guidelines for commercial willow sites should consider site and soil nutritional status prior to crop establishment and cultivar selection and deployment.

The effects of timing of harvest on shrub willow biomass production and nutrient removal confirmed our initial hypothesis. Overall, plants harvested during leaf-off stages presented significantly higher total biomass production compared to plants harvested during leaf-on stage. Additionally, nutrient removal in leaf-on harvests, especially in late fall (October), resulted significantly higher than in leaf-off harvests, especially in late spring (April). However, significant differences were observed between the studied cultivars and how they responded to timing of harvest and it was possible to separate them into two distinct groups. The group composed by cultivars SX67 and 9870-40 showed variable nutrient removal across harvest dates (higher in leaf-on harvests) and little variation in biomass production; while the group composed by cultivars 9871-41 and 9882-25 showed similar nutrient removals across harvest dates but higher biomass production (higher in leaf-off harvests). A deployment of a combination of cultivars with different responses to harvest date could be beneficial for total biomass production and nutrient removal, should the harvest occur at different times of the year; however, it would present difficulties to design an adequate nutrient management plan. On the other hand, assuming harvests occur during the growing season until fall (August until October/November), which is becoming more common in NY, the utilization of cultivars similar to SX67 could result beneficial to maintain higher long-term biomass production rates, but could possibly require higher fertilization rates compared to current practices in order to ensure a sufficient nutrient supply to support the crop's requirements and growth.

The concentration of N, P, Mg and Al in hand-harvested and machine harvested willow biomass was similar but hand-harvested concentrations of K, Ca and S were lower. These differences, however, might be insignificant from a practical point of view, resulting in small changes in nutrient removal, which would not have significance from a nutrient management point of view. Nevertheless, the high amount of biomass dropped after the harvest (up to 4.5 Mg ha^{-1}) contains an important proportion of the total nutrient content in a willow site (19-39% of N, 16-34% of P, 15-40% of K, 20-45% of Ca, 18-40% of Mg, 18-41% of S, and 5-76% of Al) and serve as nutrient input and source for the upcoming rotations. The use of hand-harvested biomass and research methodology might provide useful nutrient concentrations; however, calculations of nutrient removal in commercial settings will have to consider the nutrient content in the dropped biomass in order to be accurate and suggest nutrient management plans. The selection of cultivars with phenotypic attributes that improve harvester efficiency (increased throughput [Mg ha^{-1}]) could reduce the amount of dropped biomass, and increase total revenue. However, the advantages and disadvantages of the dropped biomass for the soil's nutrient levels, crop long-term productivity, and the system's total revenue should be weighed to determine the different tradeoffs.

5.2. Considerations for future research

Despite addressing several issues and concerns about nutrient removal in shrub willow research and commercial fields, there still remain several knowledge gaps in the subject:

1. The study on willow long-term nutrient removal should continue. Few studies have followed productivity over two or three rotations, and even fewer have followed nutrient removal. This dissertation observed differences in nutrient removal across three rotations, however it is unclear if future rotations will continue a similar trend to the observed, if it will change, or how site can continue to impact these trends.
2. Monitoring in soil nutrient levels at the end of each rotation could provide invaluable information. The changes in soil nutrient levels observed in this dissertation are a product of ~10 years of soil-plant nutrient dynamics, and no knowledge of the gap between the two sampling dates are provided. Understanding how soil nutrient levels are impacted on a rotation basis could be crucial to understand nutrient dynamics in this system and to develop long-term nutrient management plans.
3. Nutrient removal during leaf-on season is mostly affected by the high nutrient content of the foliage; however, this study was conducted on small research plots that included the careful removal of all the foliage. There has not been a study that assesses the amount of biomass that is left behind after a leaf-on harvest. Additional studies should focus on the amount of foliage removed from and left on site after a mechanized harvest and the nutrient content in this biomass.
4. Commercial shrub willow harvest practices in NY are operating during leaf-on stages for reasons already mentioned (Chapter 3 and 4); hence, methods to reduce nutrient removal in harvested biomass, especially from foliage, should be studied and developed (e.g. harvester modifications to separate foliage and other non-merchantable biomass from the woody and merchantable biomass).
5. Since both biomass production and nutrient removal by cultivars already commercially deployed were impacted by timing of harvest, the development of equipment or modifications in current equipment should focus on increasing the harvester’s flotation and operability on wet soils with minimal or reduced disturbance. Adapting current

equipment to operate in marginal sites might result simpler than adapting the crop or the site to allow equipment access.