Biomass Production and Nutrient Removal by Perennial Energy Grasses Produced on a Wet Marginal Land

Danielle R. Cooney[1] · Nictor Namoi[1] · Colleen Zumpf[1] · Soo-Hyun Lim · Maria Villamil[1] · Robert Mitchell[2] · D. K. Lee[1]

Abstract

Growing dedicated bioenergy crops on marginal land can provide beneficial outcomes including biomass production and energy, resource management, and ecosystem services. We investigated the effects of harvest timing (peak standing crop [PEAK] or after killing frost [KF]) and nitrogen (N) fertilizer rates (0, 56, and 112 kg N ha^{-1}) on yield, nutrient concentrations, and nutrient removal rates of perennial grasses on a wet marginal land. We evaluated three monocultures, including switchgrass (*Panicum virgatum* L., SW), *Miscanthus x giganteus* (MG), prairie cordgrass (*Spartina pectinata* Link, PCG), and a polyculture mixture of big bluestem (*Andropogon gerardii* Vitman), Indiangrass (*Sorghastrum nutans* (L.) Nash), and sideoats grama (*Bouteloua curtipendula* Torr., MIX). Increasing the application of N did correlate with increased biomass, concentration, and subsequent removal of nutrients across almost all treatment combinations. In all grass treatments except MG, PEAK harvesting increased yield and nutrient removal. At PEAK harvest, switchgrass is ideal for optimizing both biomass production and nutrient removal. While our results also suggest short-term plasticity for farmers when selecting harvest timing for optimal nutrient removal, KF harvest is recommended to ensure long-term stand longevity and adequate nutrient removal. If the KF harvest is adopted, MG would be the ideal option for optimizing biomass yield potential. Additionally, we found that the yield of polyculture did not vary much with harvest timing, suggesting better yield stability. Future studies should give consideration for long-term evaluation of polyculture mixtures to assess their biomass yields and nutrient removal capacities.

Keywords Nitrogen · Removal · Harvest timing · Perennial grass · Vegetative buffer · Bioenergy crops · Marginal land

Introduction

As of 2017, farmland accounted for approximately 40% (364.2 million hectares), with 160.4 million hectares used for crop production (USDA, NASS, 2017). Cropland expansion has aligned with potential economic gains and has encouraged cultivation on marginal land despite the need to apply more fertilizer to maintain yields. Marginal lands are often subject to erosion, drought, flooding, and nutrient leaching, which present challenges for annual row crop production systems [1]. Yet, marginal land may present an opportunity for production of perennial grasses, since perennial grasses have been shown to be adaptable to land deemed unsuitable for crop production, especially when these marginal areas are utilized as buffer strips [2, 3].

When installed at the edge of fields, buffers slow down movement of water and sediment and intercept subsurface nitrates, thus providing important ecosystem services including pollution reduction and nutrient recovery [4–6]. In addition to ecosystem service benefits, perennial grasses grown as buffers can be harvested to provide a feedstock for bioenergy production [5, 7]. Biomass feedstock production using perennial grasses on marginal lands can have significant contributions to the nation's bioenergy goals with minimum adverse effects on existing agricultural land [4, 8, 9].

Perennial energy grasses can be productive with minimum management, including fertilization [10–12]. Yet,

✉ D. K. Lee
leedk@illinois.edu

1 Department of Crop Sciences, University of II at Urbana-Champaign, 1102 S. Goodwin Avenue, Urbana, IL 61801, USA

2 Sorghum and Forage Research Unit, USDA-ARS, University of Nebraska-Lincoln, Wheat251 Filley HallEast Campus, Lincoln, NE 68583-0937, USA

studies also show that biomass yields can be enhanced with applications of fertilizer. For example, Lee et al. [13] demonstrated that nitrogen (N) fertilizer at 112 kg N ha^{-1} and 224 kg N ha^{-1} increased switchgrass yield by 27% and 13%, respectively, whereas Lee et al. [14] observed a linear increase in yield of *Miscanthus x giganteus* with N application (60 kg N ha^{-1} and 120 kg N ha^{-1}). Thus, installing perennial grasses as a buffer strip at the edges of row crop fields could prove useful for in situ recovery of nutrients lost from row crop fields, reducing the cost associated with direct applications of fertilizer to the stand of perennial grasses [6, 15]. Perennial grasses as buffer strips can therefore create a positive opportunity for better management of resources for row crops and ecosystem services and provide the potential for increased biomass for renewable energy production.

However, the productivity and ecosystems service benefits of perennial grass buffers are not always straightforward; the outcomes may be confounded by the growth environment, management practices, and the species used [11]. For example, biomass production following nitrogen fertilization will vary with application rates and grass species. Furthermore, results may change with time depending on timing and frequency of harvest, along with diversity of represented species [16–19]. Similarly, the ecosystem service of nutrient uptake and removal may also vary depending on the aforementioned factors. Thus, the impacts of utilizing buffer strips are not ubiquitous across environments and systems.

Numerous studies have examined the removal of nutrients from monoculture buffer strips, but little research has examined production and nutrient removal potential of perennial grass species, including mixtures, in marginal lands, which is particularly important considering the site-specific nature of these systems. In the current study, we aim to determine the best management practices for sustainable perennial energy grass production on a wet, marginally productive piece of land that was previously under crop production in a watershed with a high priority for nutrient loss reduction [15]. Specifically, the study evaluated (i) the nutrient uptake and removal by perennial warm-season grasses, including three monocultures of switchgrass (*Panicum virgatum* L.), *Miscanthus x giganteus*, prairie cordgrass (*Spartina pectinata* Link), and a polyculture mixture of big bluestem (*Andropogon gerardii* Vitman), Indiangrass (*Sorghastrum nutans* (L.) Nash), and sideoats grama (*Bouteloua curtipendula* Torr.), and (ii) the impact of harvest timing and N management on nutrient uptake and removal. These findings were part of a larger 4-year study that focused solely on the effects of harvest regime (harvest timing and frequency) and nitrogen management on warm-season grasses' biomass production potential [17].

Materials and Methods

Location and Site Characteristics

Study was conducted from 2012 to 2015 at University of Illinois research farm in Urbana, Illinois (40°07′20.4″ N, − 88°22′09.0″ W) on a footslope landscape position located in the Embarras River Watershed, an area of high priority for nutrients (N and P) loss reduction [15]. The soil is a Drummer silty clay loam (fine-silty, mixed, super-active, mesic Typic Endoaquolls) with land capability classification (LCC) 5 W (USDA NRCS, 1197). Baseline soil characteristics were soil organic matter of 5%, pH 6.7, NH_4-N 6.0 mg kg^{-1}, NO_3-N 1.0 mg kg^{-1}, P 72 mg kg^{-1}, and K 157 mg kg^{-1}. Further details about the site are described by Zumpf et al. [17].

Temperature and precipitation for the two growing seasons reported (2014 and 2015), along with their 30-year averages (1981–2010), are shown in Fig. 1. Annual precipitation in 2014 was comparable to the long-term average (1008 mm), while 2015 received an additional 104 mm of precipitation.

Crop Management and Experimental Design

Crop establishment and management are detailed by Zumpf et al. [17]. In May 2012, switchgrass, prairie cordgrass, and the polyculture mixture were established by drilling pre-weighed seeds at a rate of 325 pure live seed (PLS) per m^{-2} at 19 cm row spacing and 1.3 cm depth using a no-till drill (Great Plains Plot planter, Salina, KS). *Miscanthus x giganteus* (MG) was plug planted in June with 60 cm row spacing and 90 cm spacing between plants. Drought conditions in 2012 lead to poor stand establishment, as identified by frequency stand counts [20], warranting a replant in May of 2013. A second replication of the experiment was also established in May of 2013 in an area adjacent to the plots planted in 2012. All treatments reported in this paper include both replications.

The experimental design was arranged as a split-split plot within a randomized complete block design (RCBD). The whole plot factor of harvest timing consisted of two regimes either a late summer harvest at peak standing crop (PEAK) or harvest that was conducted after a killing frost (KF). Four different treatments of grass species were assigned to the subplots including the monocultures: (i) switchgrass "liberty" variety, a lowland cultivar (SG), (ii) prairie cordgrass "Savoy" variety (PCG), (iii) *Miscanthus x giganteus* (MG), or (iv) a Midwest adapted polyculture mixture consisting of "Goldmine" big bluestem, "Warrior" Indiangrass, and "Butte" sideoats grama (MIX).

Fig. 1 Monthly values for average precipitation and temperature in Urbana, IL (Willard airport: collected from Weather Underground 2020), and the 30-year monthly averages (1981–2010; data – Angel, n.d). Colored lines represent 2014, 2015, and 30-year average monthly temperature (secondary *y*-axis). Bars and dark line represent total monthly and 30-year average precipitation (primary *y*-axis), respectively
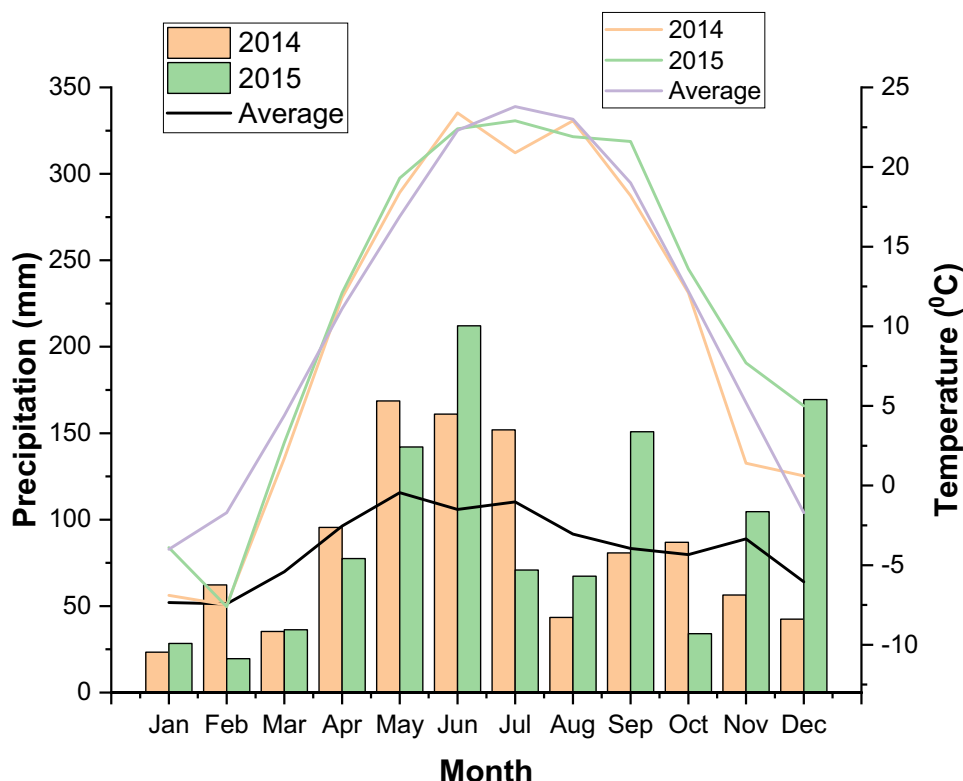
Sub-subplots (1.5 m × 4.6 m) received spring applied nitrogen (N) fertilizer, urea (46–0-0) at rates of 0, 56, and 112 kg N ha^{-1}. Weed control was accomplished in the SW and PCG plots using a pre-emergence application of atrazine (2-chloro-4ethylamine-6-isopropylamino-s-triazine) at 2 kg a.i. ha^{-1}. Plots containing MIX were sprayed with imazapic (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid) at 70 g a.i. ha^{-1}.

Biomass Harvesting and Nutrient Concentration Analysis

During the designated time (PEAK or KF), the biomass was harvested using a biomass plot harvester (Wintersteiger Cibus S, Salt Lake City, UT) from a 1.2 m × 4.0 m area in the middle of each sub-subplot. In 2014 and 2015, PEAK harvesting was carried out on August 28 and September 15 and KF harvesting on December 1 and December 18, respectively, at a 10 cm cutting height. Fresh weight was recorded, and a subsample (~ 1.0 kg) was obtained from the sampling chopper unit of the plot harvester for dry matter (DM) calculation. The subsamples were weighed, oven dried for 3 days at 60 °C, weighed again, and the moisture content determined. The moisture content was then used to adjust the fresh matter of the 1.2 m × 4.0 m harvest area to derive DM yields, which were then reported per ha.

To determine the nutrient [nitrogen (N), phosphorous (P), and potassium (K)] concentration of the harvested biomass, the oven dried subsample was ground using a cutting mill to pass a 1-mm screen. Nitrogen content was determined by dry combustion method using a LECO FP-528 N/Protein Determinator (LECO Inc., St. Joseph, MI), while P and K were measured using PerkinElmer 8300 inductively coupled plasma optical (ISP) emission spectrometer (PerkinElmer, Inc., Waltham, MA). Nutrient removal (kg ha^{-1}) through harvested biomass was calculated by multiplying DM yield (Mg ha^{-1}) by plant tissue concentration (g kg^{-1}).

Data Analysis

The DM, tissue nutrient (NPK) concentration, and nutrient removal were averaged over the 2012 and 2013 planting seasons for statistical analysis. Effects of the imposed treatments on DM, tissues nutrient (NPK) concentration, and nutrient removal was determined using the generalized linear mixed model approach from the R package "lme4" [21]. Fixed factors were harvest year (year), harvest timing (HT), grass species (species) and rates of nitrogen application (N rate), while random factors were the blocks. The *p* values were obtained by the type II Wald chi-square tests using the "Anova" function from the "Car" package [22]. Where treatment effects were statistically significant ($p < 0.05$), we conducted a Tukey post hoc test and extracted pairwise

comparisons using the "CLD" function from the "emmeans" package [23]. All analyses were carried out using R version 4.0.0 [24]

Results

Dry Biomass Yields

Our results showed significant interaction effects of year × HT × species ($p < 0.001$) and year × HT × species × N rate ($p = 0.048$) on DM yield (Table 1). DM yields after PEAK harvest were higher than KF across all species except MG, but this advantage over KF declined over time. The KF harvest did not result in any yield decline between the 2 years over time (Fig. 2). Ultimately, MG yielded the overall highest DM compared to all other species when harvested at KF. Nitrogen application tended to increase DM yield. However, appreciable increases over the unfertilized control were only observed when 112 kg N ha^{-1} was applied to SW and MG at PEAK and KF harvest, respectively ($p = 0.001$) (Fig. 3). Further detailed discussions related to the DM yield response to treatments are reported by Zumpf et al. [17].

Nutrient Content in Biomass Tissue

Three-way interaction effects of harvest year × HT × species ($p < 0.001$) affected tissue N content (Table 1). Tissue P and K contents were both affected by the interaction effects of year × species ($p = 0.004$ and $p < 0.001$, respectively) and HT × species (both $p < 0.001$). Tissue N in MG generally

trended higher, although not always significant, relative to all other species studied at PEAK (Fig. 2). At KF in 2015, however, the trend was reversed with a marked > 40% lower tissue N in MG relative to other species. Overall, tissue N was higher at PEAK harvest compared to KF harvest for all species. Similar to tissue N, the P and K tissue content was higher at PEAK harvest compared to KF for all species ($p < 0.001$). Unlike N, however, the higher concentration of tissue P and K in MG at PEAK was significant compared to SW ($p < 0.001$) and PCG ($p < 0.001$). Furthermore, concentrations of both P ($p = 0.004$) and K ($p < 0.001$) were lower in 2015 compared to 2014 for all grass species.

The main effect of N application affected both N ($p = 0.005$) and P ($p = 0.001$) concentration in plant tissues, but not K content (Table 1). Tissue N concentration generally exhibited an increasing trend with increased amounts of N applied. Yet, only the 112 kg ha^{-1} N application rate increased the tissue N concentration relative to the 0 kg ha^{-1} N control ($p = 0.004$). With tissue P concentration, the trend was reversed, as tissue P content decreased with increasing rates of N application. Similar to N tissue concentration, significant differences in tissue P content were only observed between 112 kg ha^{-1} N and 0 kg ha^{-1} N rates ($p = 0.001$) (Table 2).

N, P, and K Removal

The main effect of N application has a significant impact on the removal of the macro nutrients N ($p < 0.001$), P ($p = 0.009$), and K ($p < 0.001$). The HT × species two-way interaction affected N ($p = 0.004$), P ($p < 0.001$), and K

Table 1 ANOVA for biomass yields, NPK tissue concentration, and removal of warm-season grasses species grown on a buffer strip at the University of Illinois research farm in Urbana, Illinois. Significant main effects and their interactions ($p = 0.05$) are marked with an asterisk

Source of variation	Yield	Nutrient Concentration			Nutrient Removal		
		N	P	K	N	P	K
Year	< 0.001*	0.01*	< 0.001*	< 0.001*	< 0.001*	0.03*	< 0.001*
Timing	0.05*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Species	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
N rate	< 0.001*	0.01*	0.00*	0.42	< 0.001*	0.01*	< 0.001*
Year × timing	< 0.001*	< 0.001*	0.92	< 0.001*	< 0.001*	0.01*	< 0.001*
Year × species	< 0.001*	< 0.001*	0.00*	< 0.001*	0.02*	0.21	0.80
Timing × species	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.004*	< 0.001*	< 0.001*
Year × N rate	0.41	0.93	0.21	0.11	0.62	0.33	0.07
Timing × N rate	0.62	0.93	0.39	0.22	0.87	0.68	0.35
Species × N rate	0.31	0.60	0.30	0.97	0.14	0.11	0.63
Year × timing × species	< 0.001*	< 0.001*	0.17	0.26	0.19	0.89	0.93
Year × timing × N rate	0.59	0.37	0.29	0.45	0.28	0.46	0.41
Year × species × N rate	0.89	0.89	0.86	0.84	0.41	0.30	0.44
Timing × species × N rate	0.05*	0.83	0.24	0.624	0.40	0.36	0.54
Year × timing × species × N rate	0.25	0.92	0.71	0.74	0.69	0.81	0.43
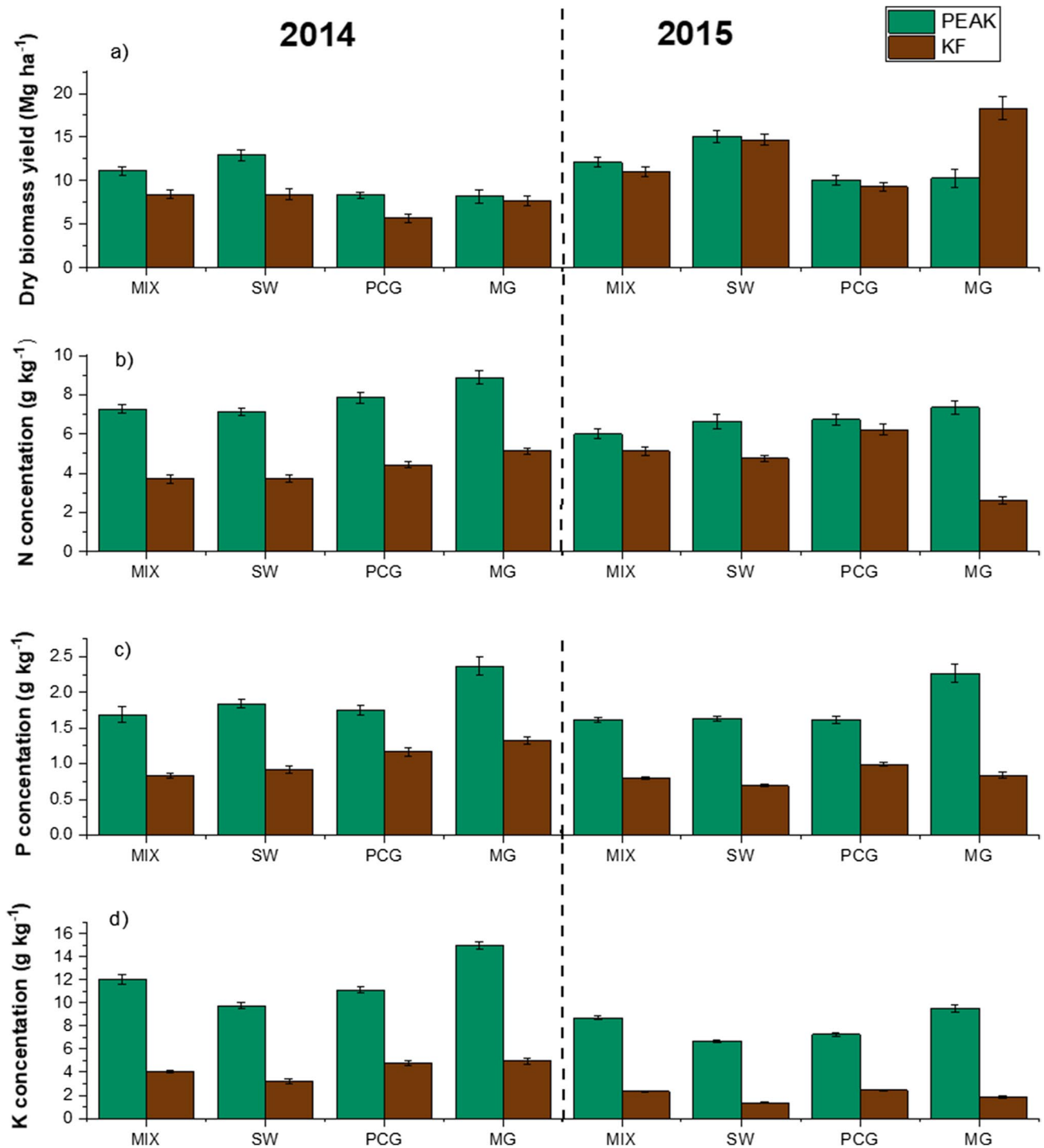
Fig. 2 **a** Biomass yield Mg ha^{-1}, and (**b**) N, (**c**) P, and (**d**) K tissue concentrations (g kg^{-1}) averaged across N rate for big bluestem, Indiangrass, and sideoats grama polyculture mixture (MIX), monocultures of switchgrass (SW), prairie cordgrass (PCG), and *Miscanthus x* *giganteus* (MG). Harvest occurred at peak standing crop (PEAK) and after killing frost (KF) across two years. Error bars indicate standard error of the means

($p < 0.001$) removals. All three nutrient removals were also affected by year $\times$ HT (N, $p < 0.001$; P, $p = 0.007$; and K, $p < 0.001$), while year $\times$ species interactions only impacted N removal ($p = 0.02$) (Table 1). Averaged across species,

harvesting at PEAK resulted in a P removal increase of more than 100%, an approximate 300% increase in K removal, and an N removal increase of greater than 70% (Fig. 4). At KF harvest, only MG removed more P ($> 40\%$) relative

Fig. 3 Nitrogen rates (0, 56 and 112 kg ha⁻¹) effect on biomass yield (Mg ha⁻¹) and N removal (kg ha⁻¹) from big bluestem, Indiangrass, and sideoats grama polyculture mixture (MIX), monocultures of switchgrass (SW), prairie cordgrass (PCG), and *Miscanthus x giganteus* (MG) when harvested at peak standing crop (PEAK) and after killing frost (KF) across 2 years. Error bars indicate standard error of the means
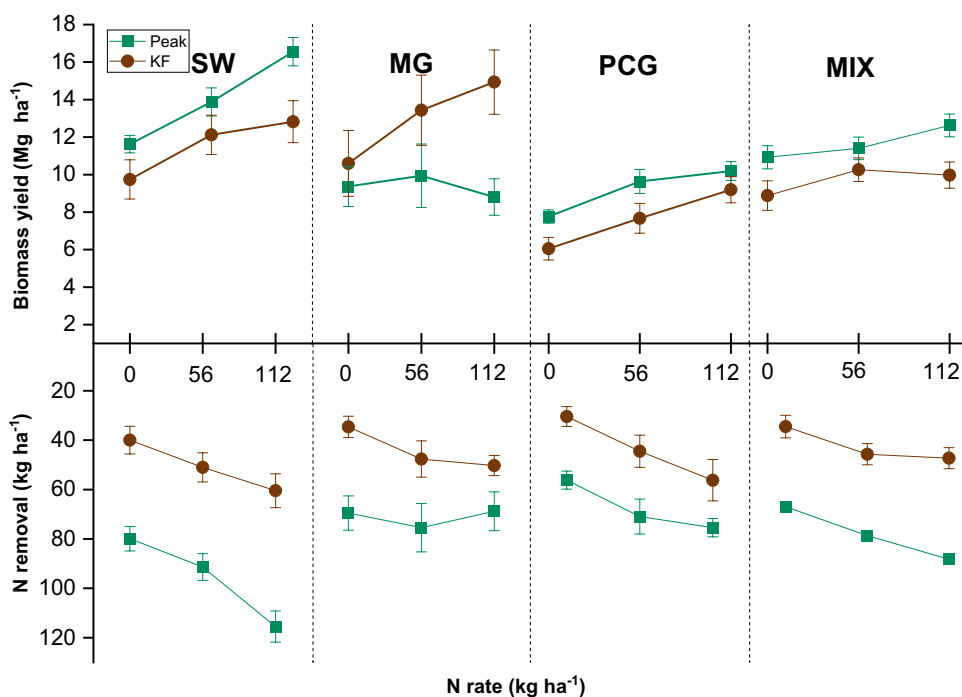

Table 2 Nutrient concentrations and removal of N, P, and K following application of nitrogen (no interactions were noted between N rate and other factors). Means followed by different letters in super-script are significantly different at $p < 0.05$. Means were separated based on Tukey's honest significant difference (HSD) test. Numbers in brackets indicate standard error

| N rate	Nutrient concentration (g kg⁻¹)			Nutrient removal (kg ha⁻¹)		
	N	P	K	N	P	K
0	5.56 (0.189)[a]	1.38 (0.05)[a]	6.50 (0.36)[a]	51.52 (2.32)[a]	12.70 (0.61)[a]	60.39 (3.92)[a]
56	5.73 (0.18)[ab]	1.31 (0.06)[a]	6.41 (0.39)[a]	62.84 (2.75)[b]	14.01 (0.71)[b]	67.87 (4.73)[b]
112	6.06 (0.19)[b]	1.25 (0.05)[b]	6.33 (0.36)[a]	70.24 (2.89)[c]	14.23 (0.64)[b]	71.53 (4.43)[b]
Mean	5.78	1.31	6.41	61.53	13.65	66.6

to other species; besides MG, all other species had similar removals of N, P, and K. At PEAK harvest, SW resulted in higher removal of N and P in both years (average removals of 115 kg ha⁻¹ and 26 kg ha⁻¹, respectively) relative to other species evaluated, although the difference was not significant for K. At KF, removals of N, P, and K averaged across species were 45 kg ha⁻¹, 9 kg ha⁻¹, and 28 kg ha⁻¹, respectively. Applying N increased the removal of N, P, and K. Removal of N increased linearly with increasing application rates, while removal of P and K was similar at 56 kg ha⁻¹ N and 112 kg ha⁻¹ N (Table 2).

Discussion

Effect of N and Harvest Management on Dry Matter Yields

Unfertilized MG yields vary throughout Illinois and can range from 8.46 to 28 Mg ha⁻¹ [11, 25, 26]. Our results averaged about 7 Mg ha⁻¹ in 2014 and 13 Mg ha⁻¹ in 2015, which are lower than the Illinois average of 23 Mg ha⁻¹ [27] but are not unexpected given the relative youth of the stand. Switchgrass without the addition of fertilizer tend

Fig. 4 **a** Nitrogen, **b** P, and **c** K removal (kg ha^{-1}) from (i) big bluestem, Indiangrass, and sideoats grama polyculture mixture (MIX), monocultures of switchgrass (SW), prairie cordgrass (PCG), and *Miscanthus x giganteus* (MG) and (ii) removals across all species at peak standing crop (PEAK) and after killing frost (KF) harvests. All results are given across 2 years. Error bars indicate standard error of the means
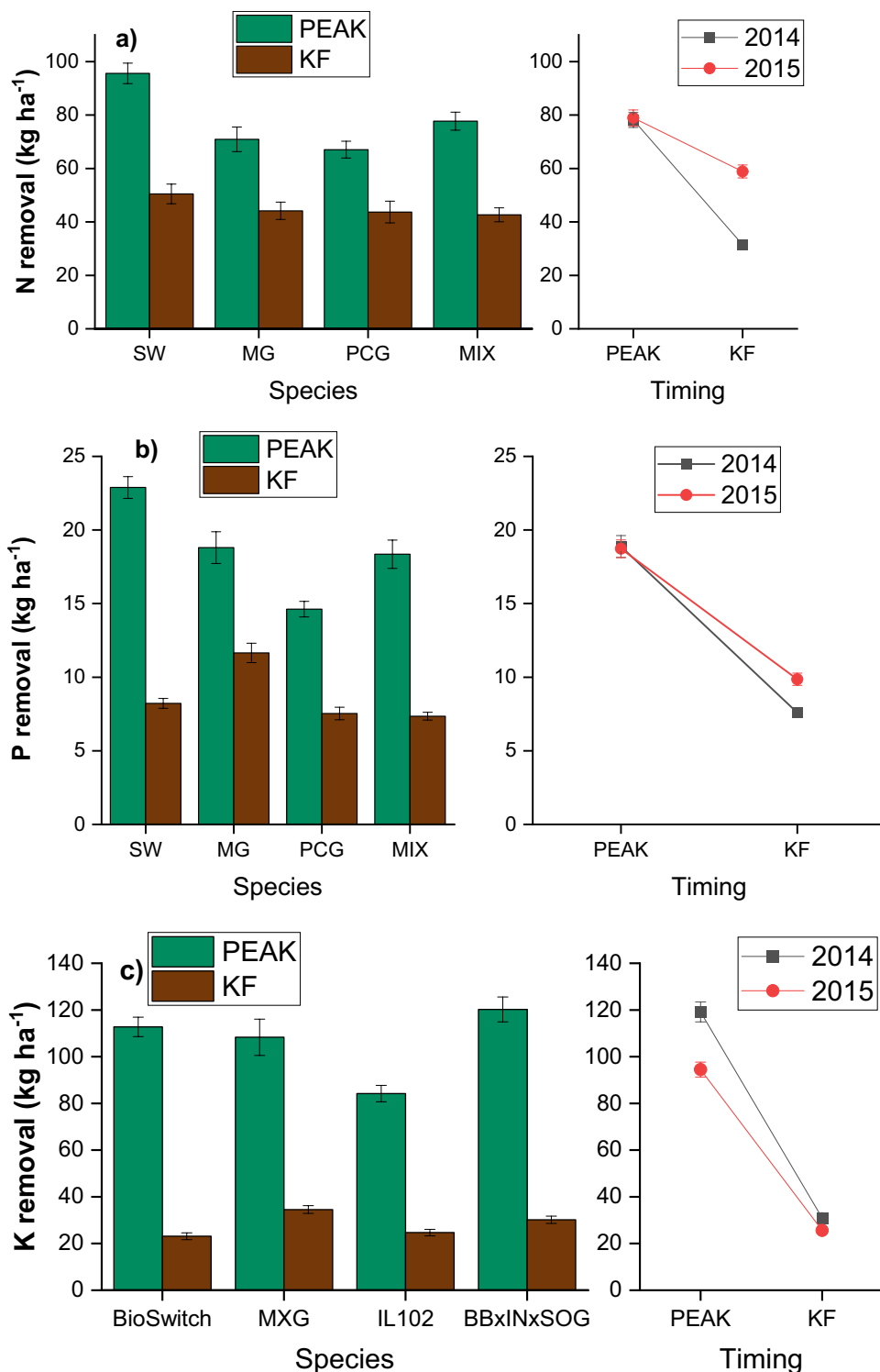
to produce DM yield approximately two to three times lower than MG. "Cave-in-Rock," another Midwestern-adapted lowland variety, has a potential yield range of 6 to 13 Mg ha^{-1} in Illinois, while Liberty has a yield range of 9 to 16.4 Mg ha^{-1} [11, 28, 29], which is consistent with the results of our experiment producing 9 Mg ha^{-1} in 2014

and 13 Mg ha^{-1} in 2015. Guo et al. [30] reported yields of Savoy varying from 6 to 10 Mg ha^{-1} across 3 years, complementing our results of about 7 Mg ha^{-1} and 13 Mg ha^{-1} in 2014 and 2015, respectively.

The lower DM yield at KF harvest for all species except MG might be leaf and panicle loss related senescence.

Anderson et al. [31] reported biomass reductions of up to 25% under delayed harvest. The DM decline in 2015 at PEAK harvest can be attributed to a loss in growth vigor [17] although the observations and conclusions from this study are limited to short-range insights. However, Tejera et al. [32] proved that 2- to 3-year-old MG can show signs of aging, which can be alleviated by adequate with N application. In addition, Rushing et al. [33] demonstrated that frequent harvesting of warm-season perennials can lead to a decline in biomass yield within 2 years. Harvesting at PEAK may reduce the amount of nutrients available for remobilization into the underground storage organs, thus negatively impacting winter dormancy and survival. Findings by Dierking et al. [34] confirmed contrasting trends in shoot and rhizome N, while shoot N declined from summer to winter, and rhizome N had a marked increase in the same period. Harvesting at KF allows the perennial grass time to translocate nutrients to the underground organs, which enhances over-wintering success, vigorous regrowth, and resilience to drought [35–38].

We found that this specific MIX provided no advantage in biomass yield over any of the monocultures tested. Furthermore, unlike the monocultures, harvesting the MIX at KF did not result in any noticeable increases in yield over time (Fig. 2). These findings contradict findings by Dhakal and Islam [39] and Tilman et al. [40, 41] who found that polycultures consistently achieved greater productivity than monocultures. Other studies, however, found that biomass yield declined with increasing grass species [42, 43]. A mixture's DM yield is determined by whether or not the species that are best adapted to the site are present [18, 44]. We surmise that the tested mixture provided minimal advantages in producible biomass relative to monocultures since it lacked a "driver." Lee et al. [18] in their study also found that SW, as a driver species, increased the yields of big bluestem mixtures, while big bluestem mixtures containing sideoats grama and Indiangrass failed to show consistent results.

The consistent yield of MIX over time might be related to relative changes in species composition of mixtures. Harmoney et al. [45] showed that certain species tend to dominate polycultures over time, while others decline in proportion. Our study did not monitor changes in species composition, but we suggest that a change in proportion of one species might be compensated by other species, which helped maintain steady DM yields over time. Longer-term studies need to be undertaken to determine whether stability of yield is maintained over a longer time period. Nonetheless, our results imply that this polyculture was less likely to fluctuate with changes in weather. Thus, they may allow for better planning from a biomass production perspective.

The different DM responses due to N application rates are not uncommon (Fig. 3). While some studies show increasing DM yield with increasing application of N [14, 31, 46], others show no appreciable changes [46]. These differences have been attributed to site-specific differences, mostly related to precipitation, soil conditions, and management [47, 48]. This could explain why the N-rate of 56 kg ha^{-1} did not affect MG and SW yields in our study but influenced yield production in similar studies [31]. Furthermore, site-specific conditions could help explain the lack of yield response with PCG across all N rates in our study despite regional similarities of Guo et al. [30] that demonstrated improved DM yield. Fike et al. [49] SW work suggested drainage as a critical factor determining the response to N application and concluded that SW response to N in poorly drained soils (as is in this case) is limited. We also do not rule out the role of free-living and symbiotic nitrogen-fixing bacterial, along with arbuscular mycorrhizal fungi (AMF) associations; collectively, these organisms can be attributed to roles in providing the bulk of nitrogen needed by grasses. Studies show that N application suppresses these organisms and thus reduce the effectiveness of fertilizer application [10, 12, 50]. While our study did not specifically quantify the influence of micro-organisms in the rhizosphere, our results did indicate, however, that higher N application rates at 112 kg ha^{-1} increased the DM yield of SW species (at PEAK) and MG species (at KF). This observation aligns with meta-analyses showing that where response is obtainable, the maximal response occurs at around 100 kg N ha^{-1} [11, 51]. One reason for the high SW yields at PEAK but not at KF could be attributed to leaf senescence onto the ground unable to be collected, depending on the timing of when the harvest is conducted. In contrast, the higher DM yield of MG at KF is likely correlated to the later flowering of MG. Miscanthus matures much later than other species, allowing an increased vegetative growth window, which contributes heavily to biomass yield accumulation [52, 53]. The late vegetative growth, combined with the higher N use efficiency of MG [54] and year-on-year maturity, could be the reason for the noticeably higher yield in 2015.

Effect of N and Harvest Management on Tissue N, P, and K Content

The higher N, P, and K concentration in tissues at PEAK compared to KF across all species (Fig. 2) can be related to the morphology of perennial grasses. Delayed harvesting allows recycling of nutrients to the below ground tissues for overwintering [35–38]. Studies have found that up to 50–60% of shoot N is remobilized to the underground tissues when biomass is harvested after KF [55, 56]. In our study, average decline in tissue N across all species at KF was 45% and 30% in 2014 and 2015, respectively; P declined by 44% and 53% and K by 64% and 75% in 2014 and 2015, respectively.

Remobilization of N to the underground storage organs may not be the only reason for the decline in tissue N of MG, as the decline in MG was striking compared to the other species. The contrast in N concentration of MG compared to other species at KF is related to the higher biomass produced by MG, especially at KF in 2015. This dilution effect of MG has been reported by Cadoux et al. [54] in their meta-analysis, concluding that the lower N dilution curve could be the main reason for the high nitrogen use efficiency of MG. Equally plausible is that considerable amount of N might have been lost in leaf drop, during senescence in the period between PEAK and KF. Amougou et al. [57] in northern France demonstrated that MG leaves contained substantial quantities N and could contribute to as much as 16 kg N ha^{-1} annually to the soil.

We observed an increase in tissue N with increasing N rate, but an inverse relationship between N rate and tissue P (Table 2). Coblentz et al. [58] also observed a similar trend in bermudagrass and attributed the decline in P concentration to the dilution effect related to increasing dry matter content. Further, our findings showing limited effect of N application on K concentration in tissues are similar to those obtained by Guretzky et al. [59], working with SW, and Evers et al. [60] who worked with a ryegrass–bermudagrass mixture.

Our results for P and K tissue concentrations are mostly driven by the relationship of harvest timing. When averaged across all species, two notable significant differences are observed between 2014 and 2015. The concentration of P is significantly different at KF, whereas K tissue concentration is significant for PEAK. Yet, differences from 2014 to 2015 could be attributed to delayed harvest in 2015 might have played a role in reduced P and K content, as nutrient content declines with harvest date [62]. Furthermore, significant differences of K at PEAK could also be a result of warm temperatures and below average rainfall in later summer months of July and August (Fig. 1). Given the scope of the evaluated treatment effects and no additions of P or K fertilization, an interest into the aspects of comparing these tissue concentrations to changes in the soil composition relative to baseline samples could have provided an additional dimension of discussion. However, we do not have any below-ground data available. We note the potential opportunity for continuing research into below-ground work with an increased focus on understanding interactions between perennial grass species, AMF, and the acquisition of nutrients. Currently, literature has attributed an enhanced P uptake to AMF, although it should be noted benefits have been dependent upon soil P levels and specific plant and fungal species-specific interactions [63–65]. The role of AMF in the context of K is much more limited in knowledge, but increased interest in the relationship of plants and AMF in K acquisition is growing [61].

Effect of N and Harvest Management on Removal of N, P, and K

To reduce losses of nutrients from agricultural fields, nutrient removal ability of perennials is of primary concern. Our results indicate that removal of all three nutrients is maximized when harvest is done at PEAK (Fig. 3 and Fig. 4). This response most likely correlates the higher concentration of nutrients in the biomass at PEAK compared to KF [37, 47, 62]. The higher concentrations coupled with the higher biomass yields observed at PEAK allows for increased removal of these nutrients. The noticeably low P and K concentration in MG at KF but higher removal of especially of P could however indicate that removal of these nutrients is mostly driven by biomass yields (Fig. 2). Evers [60] and Coblentz et al. [58] found that P and K removal was mostly reliant on biomass yield, observing only the N concentrations increased with increasing N rates. Further, these studies showed that concentration of P declined, yet removals still increased. Our study trends are better align with Evers [60] and Mohammed et al. [66] including the increasing N removals with increasing N rates (Table 2 and Fig. 3), which can be attributed to luxury consumption of N [11]. These results, however, conflict with those of [59] who concluded that switchgrass removes at least 4 k g N Mg^{-1} regardless of N application rates, and that the removals are uncorrelated with N application rates. We speculate that N removal is driven by both N concentration and biomass yield, while P and K removal is mostly driven by biomass yields.

Other than the higher P removal with MG that is likely related to high biomass in 2015, our results show that the removal of nutrients do not differ between species when harvesting at KF (Fig. 4). We suggest that the differential rates of nutrient remobilization to the roots coupled with the differential losses in biomass between species confounded nutrient removals from soil. Future research needs to investigate whether differences in nutrient removal between species become more apparent as the perennial grass stand ages. In contrast with KF, harvesting at PEAK showed a clear advantage for SW in removing N and P. Still, the average removals of N (96 kg ha^{-1}) and P (23 kg ha^{-1}) are consistent with results reported by Serapiglia et al. [55] and are driven by the high DM yields at PEAK.

Conclusion

Warm-season perennial grasses can play a critical role as bioenergy feedstocks, while potentially providing ecosystems benefits, especially downstream nutrient removal. Our

results indicate that nutrient removal by perennial grass species largely depends on their DM yield. Harvesting at PEAK might be considered effective in preventing nutrient movement downstream albeit results are limited to short-term implications. Switchgrass might be the candidate grass for short-term removal. In the long-term, however, PEAK harvesting might compromise the DM yield leading to a reduction in removal potential. Our study indicates that for this environment, MG harvested at KF may provide better DM yield and nutrient removal outcomes. Overall, high N application rates positively impact biomass yield and thus likely enhance nutrient removal. Finally, this study demonstrated that although the polyculture treatment was lower yielding relative to the evaluated monocultures, mixtures may not be prone to the wide fluctuations in productivity and might provide a good opportunity for a more reliable alternative crop. We recommend longer-term studies to continue to assess the removal capacities of the perennial grass monocultures and mixtures, with a focus on better understanding the influence of the surrounding and below ground environment.

Declarations

Ethics Approval There are no ethical issues associated with this manuscript.

Consent for Publication The authors have approval to publish from the Institutes.

Conflict of Interest The authors declare no competing interests.

References

1. Miyake S, Smith C, Peterson A, McAlpine C, Renouf M, Waters D (2015) Environmental implications of using 'underutilised agricultural land' for future bioenergy crop production. Agric Syst 139:180–195. https://doi.org/10.1016/j.agsy.2015.06.010
2. Pancaldi F, Trindade LM (2020) Marginal lands to grow novel bio-based crops: A plant breeding perspective. Front Plant Sci 11:227. https://doi.org/10.3389/fpls.2020.00227
3. Ferrarini A, Serra P, Almagro M, Trevisan M, Amaducci S (2017) Multiple ecosystem services provision and biomass logistics management in bioenergy buffers: a state-of-the-art review. Renew Sustain Energy Rev 73:277–290. https://doi.org/10.1016/j.rser.2017.01.052
4. Agostini A, Serra P, Giuntoli J, Martani E, Ferrarini A, Amaducci S (2021) Biofuels from perennial energy crops on buffer strips: a win-win strategy. J Clean Prod 297:126703. https://doi.org/10.1016/j.jclepro.2021.126703
5. Zumpf C, Ssegane H, Negri MC, Campbell P, Cacho J (2017) Yield and water quality impacts of field-scale integration of willow into a continuous corn rotation system. J Environ Qual 46(4):811–818. https://doi.org/10.2134/jeq2017.02.0082
6. Ssegane H, Zumpf C, Cristina Negri M, Campbell P, Heavey JP, Volk TA (2016) The economics of growing shrub willow as a bioenergy buffer on agricultural fields: a case study in the Midwest Corn Belt. Biofuels, Bioprod Biorefin 10(6):776–789. https://doi.org/10.1002/bbb.1679
7. Englund O, Dimitriou I, Dale VH, Kline KL, Mola-Yudego B, Murphy F, English B, Mcgrath J, Busch G, Negri MC, Brown M, Goss K, Jackson S, Parish ES, Cacho J, Zumpf C, Quinn J, Mishra SK 2020 Multifunctional perennial production systems for bioenergy: performance and progress. WIREs Energy and Environment 9(5). https://doi.org/10.1002/wene.375
8. Williams JD, Robertson DS, Long DS, Wuest SB, Kumar D, Juneja A, Murthy GS (2016) Ethanol production potential from conservation buffers in the inland Pacific Northwest. J Renew Sustain Energ 8(5):053102. https://doi.org/10.1063/1.4962414
9. Englund O, Börjesson P, Mola-Yudego B, Berndes G, Dimitriou I, Cederberg C, Scarlat N 2021 Strategic deployment of riparian buffers and windbreaks in Europe can co-deliver biomass and environmental benefits. Communications Earth & Environment 2 (1). https://doi.org/10.1038/s43247-021-00247-y
10. Jach-Smith LC, Jackson RD 2020 Inorganic N addition replaces N supplied to switchgrass (*Panicum virgatum*) by arbuscular mycorrhizal fungi. Ecological Applications 30 (2). https://doi.org/10.1002/eap.2047
11. Monti A, Zegada-Lizarazu W, Zanetti F, Casler M (2019) Nitrogen fertilization management of switchgrass miscanthus and giant reed: a review. Advanc Agronomy 153:87–119. https://doi.org/10.1016/bs.agron.2018.08.001
12. Casler MD (2022) Nitrogen fertilization and harvest management of switchgrass: impacts on biomass yield and nitrogen removal. BioEnergy Research. https://doi.org/10.1007/s12155-022-10435-z
13. Lee DK, Owens VN, Doolittle JJ (2007) Switchgrass and soil carbon sequestration response to ammonium nitrate, manure, and harvest frequency on conservation reserve program land. Agron J 99(2):462–468. https://doi.org/10.2134/agronj2006.0152

14. Lee M-S, Wycislo A, Guo J, Lee DK, Voigt T (2017) Nitrogen fertilization effects on biomass production and yield components of *Miscanthus ×giganteus*. Frontiers in Plant Science 8. https://doi.org/10.3389/fpls.2017.00544

15. IEPA, IDOA, and University of Illinois Extension (2017) Illinois Nutrient Loss Reduction Strategy Biennial Report 2015-2017. Illinois Environmental Protection Agency and Illinois Department of Agriculture; Springfield, Illinois. University of Illinois Extension; Urbana, Illinois. http://www2.illinois.gov/epa/Documents/iepa/water-quality/watershed-management/excess-nutrients/NLRS-Biennial-Report/NLRS%20Biennial%20Report.pdf

16. Sharma BP, Zhang N, Lee D, Heaton E, Delucia EH, Sacks EJ, Kantola IB, Boersma NN, Long SP, Voigt TB, Khanna M (2022) Responsiveness of miscanthus and switchgrass yields to stand age and nitrogen fertilization: A meta-regression analysis. GCB Bioenergy 14(5):539–557. https://doi.org/10.1111/gcbb.12929

17. Zumpf C, Lee MS, Thapa S, Guo J, Mitchell R, Volenec JJ, Lee D (2019) Impact of warm-season grass management on feedstock production on marginal farmland in Central Illinois. GCB Bioenergy 11(10):1202–1214. https://doi.org/10.1111/gcbb.12627

18. Lee M-S, Mitchell R, Heaton E, Zumpf C, Lee DK (2019) Warm-season grass monocultures and mixtures for sustainable bioenergy feedstock production in the midwest, USA. BioEnergy Research 12(1):43–54. https://doi.org/10.1007/s12155-018-9947-7

19. Mulkey VR, Owens VN, Lee DK (2006) Management of switchgrass-dominated conservation reserve program lands for biomass production in South Dakota. Crop Sci 46(2):712–720. https://doi.org/10.2135/cropsci2005.04-0007

20. Vogel KP, Masters RA (2001) Frequency grida simple tool for measuring grassland establishment Rangeland Ecology & Management. J Range Manag Arch 54(6):653–655. https://doi.org/10.2307/4003666

21. Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 65(1):1–48. https://doi.org/10.18637/jss.v067.i01

22. Fox J, Weisberg S (2019) An R Companion to applied regression, 3rd edn. Sage, Thousand Oaks CA

23. Lenth RV (2021) Emmeans: estimated marginal means, aka least-squares means. R package version 1.5.4. https://CRAN.R-project.org/package=emmeans

24. R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing

25. Parrish AS, Lee M-S, Voigt TB, Lee DK (2021) *Miscanthus × giganteus* responses to nitrogen fertilization and harvest timing in Illinois. BioEnergy Research, USA. https://doi.org/10.1007/s12155-021-10244-w

26. Davis MP, David MB, Voigt TB, Mitchell CA (2015) Effect of nitrogen addition on Miscanthus × giganteus yield, nitrogen losses, and soil organic matter across five sites. GCB Bioenergy 7(6):1222–1231. https://doi.org/10.1111/gcbb.12217

27. Arundale RA, Dohleman FG, Voigt TB, Long SP (2014) Nitrogen fertilization does significantly increase yields of stands of *Miscanthus x giganteus* and Panicum virgatum in multiyear trials in Illinois. Bioenergy Research 7(1):408–416. https://doi.org/10.1007/s12155-013-9385-5

28. Iqbal Y, Gauder M, Claupein W, Graeff-Hönninger S, Lewandowski I (2015) Yield and quality development comparison between miscanthus and switchgrass over a period of 10 years. Energy 89:268–276. https://doi.org/10.1016/j.energy.2015.05.134

29. Heaton EA, Dohleman FG, Long SP (2008) Meeting US biofuel goals with less land: the potential of Miscanthus. Glob Change Biol 14(9):2000–2014. https://doi.org/10.1111/j.1365-2486.2008.01662.x

30. Guo J, Thapa S, Voigt T, Owens V, Boe A, Lee DK (2017) Biomass yield and feedstock quality of prairie cordgrass in response to seeding rate, row spacing, and nitrogen fertilization. Agron J 109(6):2474–2485. https://doi.org/10.2134/agronj2017.03.0179

31. Anderson EK, Parrish AS, Voigt TB, Owens VN, Hong C-H, Lee DK (2013) Nitrogen fertility and harvest management of switchgrass for sustainable bioenergy feedstock production in Illinois. Ind Crops Prod 48:19–27. https://doi.org/10.1016/j.indcrop.2013.03.029

32. Tejera MD, Miguez FE, Heaton EA (2021) The older plant gets the sun: age-related changes in Miscanthus × giganteus phenology. GCB Bioenergy 13(1):4–20. https://doi.org/10.1111/gcbb.12745

33. Rushing JB, Lemus RW, White JA, Lyles JC, Thornton MT (2019) Yield of native warm-season grasses in response to nitrogen and harvest frequency. Agron J 111(1):193–199. https://doi.org/10.2134/agronj2018.01.0009

34. Dierking RM, Allen DJ, Cunningham SM, Brouder SM, Volenec JJ (2017) Nitrogen reserve pools in two Miscanthus x giganteus genotypes under contrasting N managements. Front Plant Sci 8:1618. https://doi.org/10.3389/fpls.2017.01618

35. Heaton EA, Dohleman FG, Long SP (2009) Seasonal nitrogen dynamics of Miscanthus x giganteus and Panicum virgatum. GCB Bioenergy 1(4):297–307. https://doi.org/10.1111/j.1757-1707.2009.01022.x

36. Dohleman FG, Heaton EA, Arundale RA, Long SP (2012) Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in *Miscanthus ×* giganteus and Panicum virgatum across three growing seasons. GCB Bioenergy 4(5):534–544. https://doi.org/10.1111/j.1757-1707.2011.01153.x

37. Lindsey K, Johnson A, Kim P, Jackson S, Labbé N (2013) Monitoring switchgrass composition to optimize harvesting periods for bioenergy and value-added products. Biomass Bioenerg 56:29–37. https://doi.org/10.1016/j.biombioe.2013.04.023

38. Wayman S, Bowden RD, Mitchell RB (2014) Seasonal changes in shoot and root nitrogen distribution in switchgrass (Panicum virgatum). BioEnergy Research 7(1):243–252. https://doi.org/10.1007/s12155-013-9365-9

39. Dhakal D, Islam M (2018) Grass-legume mixtures for improved soil health in cultivated agroecosystem. Sustainability 10(8):2718. https://doi.org/10.3390/su10082718

40. Tilman D, Reich PB, Knops J, Wedin D, Mielke T, Lehman C (2001) Diversity and productivity in a long-term grassland experiment. Science 294(5543):843–845. https://doi.org/10.1126/science.1060391

41. Tilman D, Reich PB, Knops JMH (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441(7093):629–632. https://doi.org/10.1038/nature04742

42. Adler PR, Sanderson MA, Weimer PJ, Vogel KP (2009) Plant species composition and biofuel yields of conservation grasslands. Ecol Appl 19(8):2202–2209. https://doi.org/10.1890/07-2094.1

43. Johnson MVV, Kiniry JR, Sanchez H, Polley HW, Fay PA (2010) Comparing biomass yields of low-input high-diversity communities with managed monocultures across the central United States. Bioenergy Research 3(4):353–361. https://doi.org/10.1007/s12155-010-9094-2

44. Picasso VD, Brummer EC, Liebman M, Dixon PM, Wilsey BJ (2008) Crop species diversity affects productivity and weed suppression in perennial polycultures under two management strategies. Crop Sci 48(1):331–342. https://doi.org/10.2135/cropsci2007.04.0225

45. Harmoney KR, Lee DK, Kallenbach RL, Aberle EZ (2016) Species composition changes in conservation reserve program (CRP) grassland when managed for biomass feedstock production. BioEnergy Research 9(4):1180–1188. https://doi.org/10.1007/s12155-016-9764-9

46. Namoi N, Jang C, Robins Z, Lin C-H, Lim S-H, Voigt T, Lee D (2022) Aerial imagery can detect nitrogen fertilizer effects on biomass and stand health of *Miscanthus × giganteus*. Remote Sens-Basel 14(6):1435. https://doi.org/10.3390/rs14061435

47. Kering MK, Butler TJ, Biermacher JT, Guretzky JA (2012) Biomass yield and nutrient removal rates of perennial grasses under nitrogen fertilization. Bioenergy Research 5(1):61–70. https://doi.org/10.1007/s12155-011-9167-x

48. Heaton E (2004) A quantitative review comparing the yields of two candidate C4 perennial biomass crops in relation to nitrogen, temperature and water. Biomass Bioenerg 27(1):21–30. https://doi.org/10.1016/j.biombioe.2003.10.005

49. Fike JH, Pease JW, Owens VN, Farris RL, Hansen JL, Heaton EA, Hong CO, Mayton HS, Mitchell RB, Viands DR (2017) Switchgrass nitrogen response and estimated production costs on diverse sites. GCB Bioenergy 9(10):1526–1542. https://doi.org/10.1111/gcbb.12444

50. Emery SM, Kinnetz ER, Bell-Dereske L, Stahlheber KA, Gross KL, Pennington D (2018) Low variation in arbuscular mycorrhizal fungal associations and effects on biomass among switchgrass cultivars. Biomass Bioenerg 119:503–508. https://doi.org/10.1016/j.biombioe.2018.10.012

51. Wullschleger SD, Davis EB, Borsuk ME, Gunderson CA, Lynd LR (2010) Biomass production in switchgrass across the United States: database description and determinants of yield. Agron J 102(4):1158–1168. https://doi.org/10.2134/agronj2010.0087

52. Williams M, Douglas J (2011) Planting and managing giant miscanthus as a biomass energy crop. USDA-NRCS Plant Materials Program, Washington, DC Technical Note 4:30

53. Anderson E, Arundale R, Maughan M, Oladeinde A, Wycislo A, Voigt T (2011) Growth and agronomy of Miscanthus x giganteus for biomass production. Biofuels 2(1):71–87. https://doi.org/10.4155/bfs.10.80

54. Cadoux S, Riche AB, Yates NE, Machet J-M (2012) Nutrient requirements of Miscanthus x giganteus: conclusions from a review of published studies. Biomass Bioenerg 38:14–22. https://doi.org/10.1016/j.biombioe.2011.01.015

55. Serapiglia MJ, Boateng AA, Lee DK, Casler MD (2016) Switchgrass harvest time management can impact biomass yield and nutrient content. Crop Sci 56(4):1970–1980. https://doi.org/10.2135/cropsci2015.08.0527

56. Sarath G, Baird LM, Mitchell RB (2014) Senescence, dormancy and tillering in perennial C-4 grasses. Plant Sci 217:140–151. https://doi.org/10.1016/j.plantsci.2013.12.012

57. Amougou N, Bertrand I, Cadoux S, Recous S (2012) Miscanthus x giganteus leaf senescence, decomposition and C and N inputs to soil. GCB Bioenergy 4(6):698–707. https://doi.org/10.1111/j.1757-1707.2012.01192.x

58. Coblentz WK, Turner JE, Scarbrough DA, Humphry JB, Coffey KP, Daniels MB, Gunsaulis JL, Teague KA, Speight JD, Moore PA (2004) Effects of nitrogen fertilization on phosphorus uptake in bermudagrass forage grown on high soil-test phosphorus sites. Prof Anim Sci 20 2:146–154. https://doi.org/10.15232/s1080-7446(15)31289-4

59. Guretzky JA, Biermacher JT, Cook BJ, Kering MK, Mosali J (2011) Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. Plant Soil 339(1–2):69–81. https://doi.org/10.1007/s11104-010-0376-4

60. Evers GW (2002) Ryegrass–bermudagrass production and nutrient uptake when combining nitrogen fertilizer with broiler litter. Agron J 94(4):905–910. https://doi.org/10.2134/agronj2002.9050

61. Garcia K, Zimmermann SD 2014 The role of mycorrhizal associations in plant potassium nutrition. Front Plant Sci 5:1–9. https://doi.org/10.3389/fpls.2014.00337

62. Gamble JD, Jungers JM, Wyse DL, Johnson GA, Lamb JA, Sheaffer CC (2015) Harvest date effects on biomass yield, moisture content, mineral concentration, and mineral export in switchgrass and native polycultures managed for bioenergy. BioEnergy Research 8(2):740–749. https://doi.org/10.1007/s12155-014-9555-0

63. Treseder KK (2013) The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. Plant Soil 371(1–2):1–13. https://doi.org/10.1007/s11104-013-1681-5

64. Worchel ER, Giauque HE, Kivlin SN (2013) Fungal symbionts alter plant drought response. Microb Ecol 65(3):671–678. https://doi.org/10.1007/s00248-012-0151-6

65. Delavaux CS, Smith-Ramesh LM, Kuebbing SE (2017) Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. Ecology 98(8):2111–2119. https://doi.org/10.1002/ecy.1892

66. Mohammed YA, Chen C, Lee DK (2014) Harvest time and nitrogen fertilization to improve bioenergy feedstock yield and quality. Agron J 106(1):57–65. https://doi.org/10.2134/agronj2013.0272