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A nutrient-based sustainability assessment of purpose-grown poplar and switchgrass biomass production systems established on marginal lands in Canada

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ABSTRACT

Sustainability of purpose-grown biomass production on marginal lands in Canada is uncertain. In this study, an assessment of biomass yield and sustainability was performed for two poplar clones (*Populus deltoides* x *P. nigra*, DN-34 – PDN, and *P. nigra* x *P. maximowiczii*, NM-6 – PNM) and two switchgrass cultivars (*Panicum virgatum* cv. Cave-in-Rock – SGC, and *Panicum virgatum* cv. Nebraska – SGN) on three marginal lands in Guelph (Ontario), Kemptville (Ontario) and Nappan (Nova Scotia) in Canada. The differences in stem biomass across sites were not significant however, differences in stem biomass among plants were statistically significant between poplar and switchgrass ($p < 0.0001$), and between poplar clones ($p < 0.0001$). Two-year stem biomass yield in PNM ($15.27 \pm 1.28 \text{ t ha}^{-1}$) was significantly higher than PDN ($7.02 \pm 0.54 \text{ t ha}^{-1}$), SGC ($2.57 \pm 0.28 \text{ t ha}^{-1}$) and SGN ($1.45 \pm 0.22 \text{ t ha}^{-1}$). Two sustainability indices, based on macro-nutrients (MBSI) and nitrogen (NBSI), were developed to assess sustainability. Both indices show that biomass production system of high yielding poplar clone PNM is depicting nutrient loss and may require external nutrient inputs via fertilization during the establishment phase. Higher index values for switchgrass, SGC (1.47 ± 0.22 , 1.11 ± 0.15) and SGN (1.37 ± 0.16 , 1.17 ± 0.12) for MBSI and NBSI, respectively, indicate that despite low stem biomass yields, switchgrass biomass production is sustainable. These findings suggest that, from a nutrient perspective, sustainable biomass production systems can be established on marginal lands in Canada. However, there is a trade-off between high yield and long-term sustainability in purpose-grown biomass production systems.

Short Title

Ashiq et al. - Sustainability of Biomass on Marginal Lands in Canada

42 **Key Words**

43 marginal lands, nutrient, poplar, sustainability, switchgrass

For Review Only

INTRODUCTION

The demand for biomass is increasing for renewable energy production. It is estimated that by 2030 biomass would be the single most important renewable energy resource with 30% utilization in biofuel production, and contribution of biomass from purpose-grown crops, within bioenergy mix, will increase up to 33-39% (Nakada et al. 2014). However, achievement of this potential biomass from purpose-grown crops mainly depends on two highly uncertain factors: land availability and biomass yields derived from available lands (Bauen et al. 2009). The modest nutrient requirement of purpose-grown biomass crops make them suitable to be grown on marginal lands: lands that are otherwise unsuited for sustained agriculture production due to various geo-physical and climatic limitations (CGIAR-TAC 2000; Aylott et al. 2010; Gelfand et al. 2013). In Canada, a Land Suitability Rating System (LSRS) is used to assess suitability of land for agriculture production. The LSRS uses soil, climate and landscape information to classify land into seven discrete classes (1-7): class 1 being highly suitable and class 7 unsuitable. The intermediate classes (3 to 4) have some moderate to severe limitations that make them as marginal lands for specific agriculture crop production (Agronomic Interpretation Working Group 1995). It is estimated that about 9.48 million hectares of marginal lands in Canada can be utilized to grow purpose-grown biomass (Liu et al. 2012).

The production of purpose-grown biomass heavily depends on continued availability of nutrients (Hangs et al. 2014a). Frequent harvestings can lead to enhanced nutrient removal, which can reduce site productivity over time (Adegbidi et al. 2001; Palviainen and Finer 2012; Ge et al. 2015). Therefore, it is pivotal for a sustainable biomass production that nutrient input should compensate for nutrient loss via biomass harvest. Nutrient input can come from a variety of internal, such as leaf litter decomposition and root turnover; and external sources such as dry

deposition (dust) and wet deposition (precipitation) (Reynolds et al. 2001; Schroth et al. 2001; Liu et al. 2002). The nutrient inputs from leaf litter decomposition and root turnover have important roles in maintaining nutrient budget for biomass growth and production. However, the type of nutrient input depends on biomass crops. For instance, leaf litter in herbaceous crops is minimal as these crops undergo total above-ground biomass removal annually whereas, in woody crops almost all leaves shed as leaf litter and add nutrients to the soil via decomposition and nutrient mineralization. Generally, leaf litter of woody biomass crops contribute more potassium (K), and roots contribute more nitrogen (N) and phosphorus (P) whereas, in herbaceous crops both leaf litter and roots provide N in addition to K nutrients (Holou et al. 2013; Amichev et al. 2014).

A complete understanding of nutrient cycling in terms of type and amount of nutrient input, and whether these nutrient inputs will offset nutrient removal via harvest is therefore, essential to assess long term sustainability of purpose-grown biomass. From a nutrient perspective, even though biomass crop productivity is mainly driven by the availability of N, P, and K nutrients (Brown and Driessche 2005; Parrish and Fike 2005; Guillemette and DesRochers 2008), the role of N nutrient alone has been emphasised in several studies (McLaughlin and Kszos 2005; Rennenberg et al. 2010; Qin et al. 2015). This nutrient cycling aspect is poorly understood for purpose-grown poplar (*Populus* spp.) and switchgrass (*Panicum virgatum*) production on marginal lands in Canada. Accordingly, a research network was established in 2014 to quantify biomass production and nutrient cycling in purpose-grown poplar and switchgrass systems on marginal lands in Canada. In this study, we focus on leaf litter and root turnover as nutrient input sources to assess potential sustainability of purpose-grown biomass crops on marginal lands. We developed two sustainability indices: one based on three macro nutrients i.e; N, P, and K

nutrients, and the other based on only N nutrients (to account for the enhanced role of N in productivity).

MATERIALS AND METHODS

Study Sites and Experimental Design

This research is based on data from three study sites in Canada: two sites at the University of Guelph research stations in Guelph and Kemptville (Ontario), and one site at Saint Mary's University research station in Nappan (Nova Scotia). According to LSRS, these sites are class 3 – 4 lands. Specifically, Class 3 lands have moderate limitations that reduce the choice of crops or require special conservation practice, and Class 4 lands have severe limitations that restricts the choice of crops or require special conservation practices and very careful management, or both (Agronomic Interpretation Working Group 1995). Geographic locations and baseline soil characteristics of the study sites are shown in Table 1. At each site, experimental plots of 200 m² (10 m x 20 m) were established in 2014 for two poplar clones (DN34 - *Populus deltoides* x *P. nigra*, and NM6 – *P. nigra* x *P. maximowiczii*; hereafter referred as PDN and PNM, respectively) and two switchgrass cultivars (*Panicum virgatum* cv. Cave-in-Rock, and *Panicum virgatum* cv. Nebraska; hereafter referred as SGC and SGN, respectively) in a Randomized Complete Block Design (RCBD) with four replications. A 2 m buffer was set between plots to prevent any shading effect. Following the European double row design, PDN and PNM cuttings were planted at a density of 16,500 cuttings per hectare in a double row configuration with 1.5 m between a set of double rows (Cardinael et al. 2012). Within a double row, rows were 0.75 m apart and within a row each cutting was 0.60 m apart (Figure 1). Plots of SGC and SGN were established by seeding in rows 0.18 m apart at the rate of 30 kg ha⁻¹ and 25 kg ha⁻¹ respectively. The higher seeding rate was applied to compensate for the lower germination rate (< 50%) and to cope with

the low site fertility. The difference in seeding rate between the cultivars was mainly to compensate for differences observed in their respective germination rates.

Biomass Yields

In this study 2-year (2015 and 2016) biomass yields are evaluated for leaf biomass, stem/woody biomass and root biomass of poplar and switchgrass. As the focus of this research is nutrient-based sustainability assessment, the biomass samplings were carried-out after senescence (late October – early November) to allow for nutrient translocation and to minimize nutrient harvest from the system (Gorlitsky et al. 2015; Ashworth et al. 2017). The nutrients in these biomass components were further analysed to assess potential sustainability of poplar and switchgrass biomass production across the study sites.

Stem/Woody Biomass

In energy crops, woody biomass is usually harvested once every 2-3 years whereas, herbaceous biomass is harvested annually. In this study, annual yields of switchgrass biomass for 2015 and 2016 were consolidated to do comparison with 2-year poplar woody biomass yield.

In 2016, eight poplar plants from a randomly selected 2.25 m x 2.25 m area in the middle double rows in each poplar plot were harvested, and weighed for the wet weight. A sub-sample was oven dried at 65°C until a constant mass was reached. After determining moisture contents (Eq 1), woody biomass dry weight was calculated on a hectare basis.

$$\text{Moisture content (\%)} = \frac{\text{Wet Weight} - \text{Dry Weight}}{\text{Wet Weight}} \times 100 \quad (\text{Eq 1})$$

Switchgrass biomass samples were collected after the killing frost in late October/early November in 2015 and 2016. In each switchgrass plot, a 1 m² (1 m x 1 m) quadrant was used to collect samples. After harvest, all leaves were removed from stems. Leaves and stems were weighed separately for wet weight. Then these samples were dried at 65°C to achieve a constant

weight. Moisture contents were determined using Eq 1 and dry weights for stem and leaf biomass were calculated on a hectare basis.

Leaf Biomass

Leaf samples for both poplar and switchgrass were collected in 2015 and 2016. Traditionally, poplar leaf biomass samples are collected using leaf litter traps. However, the windy conditions at Nappan site made it difficult to collect samples using litter traps as leaves were blown away from the traps. So, leaf plucking method was used to collect poplar leaves across all sites. In each poplar plot, all leaves from 10 randomly selected plants were picked, and weighed for wet weight. To determine moisture contents, collected samples were dried at 65°C for few days till a constant mass was achieved. Finally, dry weight of leaf biomass was calculated on a hectare basis based on plant density per hectare.

Switchgrass leaf biomass was measured from the leaves separated from the stems, and similar procedure executed for poplar leaves was applied to calculate dry weight on a hectare basis based on sample area harvested.

Root Biomass

Root biomass samples for both poplar and switchgrass were collected at the end of 2016 growing season. In each polar plot, two plants were randomly selected for root samples. Then, a quadrant of 60 cm x 60 cm was laid out around each selected plot in such a way that plant stump was in the centre of the quadrant (Figure 1). In each quadrant, soil with all roots was collected up to 20 cm depth. For switchgrass root sampling, a quadrant of 30 cm x 30 cm was used to collect soils with roots up to 20 cm depth. All soil samples were then rinsed on 2 mm mesh screen to separate roots. Washed root samples were weighted for wet weight, and then oven dried at 65°C until a

constant mass was achieved. After determining moisture contents (Eq 1), root biomass dry weights were calculated on a hectare basis.

Assessment of Sustainability

Sustainability of biomass crop production depends on continued availability of nutrients that can be made available through various internal and external sources. In this study, the potential total nutrient input through leaf litter and root turnover was used to assess sustainability of poplar and switchgrass biomass production on marginal lands.

Nutrient Analyses

Sub-samples of all biomass samples (stem, leaves and roots) of poplar (PDN and PNM) and switchgrass (SGC and SGN) for all plots were milled using a Wiley Mill, and analyzed for N, P, and K nutrients at SGS Agri-food Laboratories Inc., Guelph, Ontario, Canada. The nutrients in senescing leaves were used in all analyses to account for nutrient translocation during leaf senescence in poplar leaves (Mccoll 1980; Cooke and Weih 2005).

Sustainability Indices

Two nutrient input/output based sustainability indices were developed: macro-nutrient based sustainability index (MBSI) - ratio of total N, P, and K nutrients input (via leaf litter and root turnover) to total N, P, and K nutrients output (via woody/stem biomass harvest), and nitrogen based sustainability index (NBSI) - ratio of total N nutrient input (via leaf litter and root turnover) to total N output (via woody/stem biomass harvest). A value of less than 1 for the developed sustainability indices indicates the system is undergoing net loss of nutrients and will not be sustainable over the long term (Mohammadzade and Bazrgar 2014). However, the actual sustainability will depend on additional factors, such as rate of nutrient release, atmospheric

deposition and nutrient leaching etc. These additional factors are beyond the scope of this study, and are being investigated in a contemporary study.

Poplar is deciduous and shed all leaves by the end of growing season. Hence, in case of poplar 100% leaf biomass was considered as nutrient input source in MBSI (Eq 2) and NBSI (Eq 3).

$$MBSI_P = \frac{\sum_{i=0}^n (leaf_{N,P,K} + root_{N,P,K})}{\sum_{i=0}^n (stem_{N,P,K})} \quad (\text{Eq 2})$$

$$NBSI_P = \frac{\sum_{i=0}^n (leaf_N + root_N)}{\sum_{i=0}^n (stem_N)} \quad (\text{Eq 3})$$

Complete leaf shed does not occur in switchgrass, and leaves are harvested with stems and subsequently used for various purposes such as animal bedding and as mulch. In this study, it was observed that about 20% of leaves remained in plots after harvesting. Therefore, MBSI and NBSI indices for switchgrass were modified considering only 20% of leaf biomass as nutrient input source. The MBSI and NBSI for switchgrass are expressed in Eq 4 and Eq 5, respectively.

$$MBSI_S = \frac{\sum_{i=0}^n (0.2leaf_{N,P,K} + root_{N,P,K})}{\sum_{i=0}^n (0.8leaf_{N,P,K} + stem_{N,P,K})} \quad (\text{Eq 4})$$

$$NBSI_S = \frac{\sum_{i=0}^n (0.2leaf_N + root_N)}{\sum_{i=0}^n (0.8leaf_N + stem_N)} \quad (\text{Eq 5})$$

Statistical Analyses

All treatments were tested for statistical parameters using SAS 9.4 (SAS Institute Inc. Cary, NC). General Linear Procedures in SAS were used to perform statistical analyses. PROC GLM was used to perform partitioned analysis of the LS-means for site and plant interaction sliced by three experimental sites. To evaluate the planned comparison between plants, the treatment sum of square was portioned using orthogonal contrast approach. Combined analysis was conducted as a RCBD in places for 2-year data. A Shapiro–Wilk test ($\alpha = 0.05$) was used to test whether

residuals followed a normal distribution and, where necessary, log transformations were used to normalize residuals.

RESULTS

Biomass Yields

Results for 2-year leaf biomass, stem biomass and root biomass are presented in Table 2-5 and Figure 2. These results show that differences between overall leaf biomass and root biomass yields across sites are statistically significant whereas, stem biomass yields (woody and herbaceous) across sites are comparable (Table 2). Leaf biomass yield at Kemptville is significantly higher ($5.60 \pm 1.25 \text{ t ha}^{-1}$) than Guelph ($3.26 \pm 0.17 \text{ t ha}^{-1}$) and Nappan ($2.53 \pm 0.32 \text{ t ha}^{-1}$). The differences in combined (poplar and switchgrass) leaf biomass yields at Guelph ($3.26 \pm 0.17 \text{ t ha}^{-1}$) and Nappan ($2.53 \pm 0.32 \text{ t ha}^{-1}$) are non-significant. For root biomass, yields at Guelph ($2.72 \pm 0.42 \text{ t ha}^{-1}$) and Nappan ($2.81 \pm 0.63 \text{ t ha}^{-1}$) are significantly higher than Kemptville ($1.86 \pm 0.22 \text{ t ha}^{-1}$) (Table 3).

When compared among plants, the differences in leaf biomass, stem biomass and root biomass all are statistically significant (Table 2). In general, leaf and stem biomass yields are significantly higher in poplar clones whereas, root biomass yields are significantly higher in switchgrass. Leaf and stem biomass yields are significantly higher in PNM ($6.73 \pm 1.26 \text{ t ha}^{-1}$, $15.27 \pm 1.28 \text{ t ha}^{-1}$) than PDN ($4.06 \pm 0.80 \text{ t ha}^{-1}$, $7.02 \pm 0.54 \text{ t ha}^{-1}$). In case of switchgrass, the differences in leaf biomass and stem biomass between SGC ($2.65 \pm 0.27 \text{ t ha}^{-1}$, $2.57 \pm 0.28 \text{ t ha}^{-1}$) and SGN ($1.74 \pm 0.30 \text{ t ha}^{-1}$, $1.45 \pm 0.22 \text{ t ha}^{-1}$) are non-significant. Contrarily, root biomass yield in SGC ($4.34 \pm 0.61 \text{ t ha}^{-1}$) is significantly higher than SGN ($2.68 \pm 0.44 \text{ t ha}^{-1}$), and the differences between poplar clones (PNM – $1.41 \pm 0.21 \text{ t ha}^{-1}$, PDN – $1.38 \pm 0.25 \text{ t ha}^{-1}$) are non-significant (Table 4).

Comparison among plants shows that differences are highly significant (i) between poplar and switchgrass for leaf biomass ($5.40 \pm 0.80 \text{ t ha}^{-1}$, $2.20 \pm 0.22 \text{ t ha}^{-1}$), stem biomass ($11.14 \pm 1.16 \text{ t ha}^{-1}$, $2.01 \pm 0.21 \text{ t ha}^{-1}$) and root biomass ($1.40 \pm 0.17 \text{ t ha}^{-1}$, $3.51 \pm 0.41 \text{ t ha}^{-1}$); (ii) between PDN and PNM for leaf biomass ($4.06 \pm 0.80 \text{ t ha}^{-1}$, $6.73 \pm 1.26 \text{ t ha}^{-1}$) and stem biomass ($7.02 \pm 0.54 \text{ t ha}^{-1}$, $15.27 \pm 1.28 \text{ t ha}^{-1}$); and (iii) between SGC and SGN for root biomass ($4.34 \pm 0.61 \text{ t ha}^{-1}$, $2.68 \pm 0.44 \text{ t ha}^{-1}$) (Table 2). Results also show that sites exhibit different trends in producing leaf, stem and root biomass (Table 5, Figure 3). At Guelph, yield differences are significant (i) between poplar and switchgrass for root biomass ($1.13 \pm 0.19 \text{ t ha}^{-1}$, $4.30 \pm 0.22 \text{ t ha}^{-1}$) and stem biomass ($10.77 \pm 1.43 \text{ t ha}^{-1}$, $2.63 \pm 0.44 \text{ t ha}^{-1}$), and (ii) within poplar (PDN vs PNM) for stem biomass ($8.08 \pm 0.77 \text{ t ha}^{-1}$, $13.47 \pm 1.99 \text{ t ha}^{-1}$) (Figure 3). At Kemptville, yield differences are significant (i) between poplar and switchgrass for leaf biomass ($9.67 \pm 1.43 \text{ t ha}^{-1}$, $1.52 \pm 0.27 \text{ t ha}^{-1}$) and stem biomass ($12.78 \pm 3.08 \text{ t ha}^{-1}$, $1.15 \pm 0.22 \text{ t ha}^{-1}$), (ii) within poplar (PDN vs PNM) for leaf biomass ($7.24 \pm 1.37 \text{ t ha}^{-1}$, $12.09 \pm 1.83 \text{ t ha}^{-1}$) and stem biomass ($7.54 \pm 1.13 \text{ t ha}^{-1}$, $18.03 \pm 3.01 \text{ t ha}^{-1}$), and (iii) within switchgrass (SGC vs SGN) for root biomass ($2.10 \pm 0.32 \text{ t ha}^{-1}$, $0.66 \pm 0.10 \text{ t ha}^{-1}$). At Nappan, yield differences are significant (i) between poplar and switchgrass for root biomass ($0.77 \pm 0.09 \text{ t ha}^{-1}$, $4.85 \pm 0.73 \text{ t ha}^{-1}$) and stem biomass ($10.69 \pm 1.93 \text{ t ha}^{-1}$, $2.25 \pm 0.11 \text{ t ha}^{-1}$), (ii) within poplar (PDN vs PNM) for leaf biomass ($2.45 \pm 0.10 \text{ t ha}^{-1}$, $4.56 \pm 0.28 \text{ t ha}^{-1}$) and stem biomass ($5.69 \pm 0.42 \text{ t ha}^{-1}$, $15.68 \pm 1.49 \text{ t ha}^{-1}$), and (iii) within switchgrass (SGC vs SGN) for root biomass ($6.01 \pm 1.11 \text{ t ha}^{-1}$, $3.68 \pm 0.446 \text{ t ha}^{-1}$). Across all three sites, stem biomass yields are significantly higher in PNM (Guelph $13.47 \pm 1.99 \text{ t ha}^{-1}$, Kemptville $18.03 \pm 3.01 \text{ t ha}^{-1}$, Nappan $15.68 \pm 1.49 \text{ t ha}^{-1}$) than PDN (Guelph $8.08 \pm 0.77 \text{ t ha}^{-1}$, Kemptville $7.54 \pm 1.13 \text{ t ha}^{-1}$, Nappan $5.69 \pm 0.42 \text{ t ha}^{-1}$).

245 Sustainability

246 Results of nutrient analyses are presented in **Error! Reference source not found.** The N and P
 247 nutrient concentrations for both plants (poplar and switchgrass) are the highest in leaf biomass
 248 ($11.30 \pm 0.82 \text{ g kg}^{-1}$, $2.13 \pm 0.22 \text{ g kg}^{-1}$) followed by root biomass ($6.60 \pm 0.55 \text{ g kg}^{-1}$, $1.45 \pm 0.16 \text{ g}$
 249 kg^{-1}) and stem biomass ($5.87 \pm 0.67 \text{ g kg}^{-1}$, $1.08 \pm 0.06 \text{ g kg}^{-1}$), across all sites. Whereas, K nutrient
 250 concentration is the highest in root biomass ($7.25 \pm 0.55 \text{ g kg}^{-1}$) followed by leaf biomass
 251 (5.90 ± 1.31) and stem biomass ($4.24 \pm 0.51 \text{ g kg}^{-1}$). Within plants, N nutrient concentration across
 252 all sites is highest in PDN leaves ($15.38 \pm 0.60 \text{ g kg}^{-1}$) followed by PNM leaves $11.18 \pm 0.66 \text{ g kg}^{-1}$.
 253 ¹). At the site level, overall nutrient concentrations in stems and leaf biomass are higher at
 254 Kemptville whereas, nutrients in root biomass are higher at Guelph.

255 Results of MBSI and NBSI based on 2-year nutrient dynamics are presented in Table 2-5 and
 256 Figure 4-5. The values for both indices are comparable for poplar (PDN and PNM) grown at
 257 Guelph and Nappan sites. At these two sites, switchgrass MBSI values (Guelph – 1.68 ± 0.15 ,
 258 1.70 ± 0.15 ; Nappan – 2.08 ± 0.35 , 1.67 ± 0.21 , respectively for SGC and SGN) are relatively higher
 259 than NBSI values (Guelph – 1.09 ± 0.10 , 1.11 ± 0.07 ; Nappan – 1.52 ± 0.31 , 1.44 ± 0.19 , respectively
 260 for SGC and SGN). However, Kemptville site shows a different trend where MBSI is relatively
 261 higher than NBSI for poplar (PDN – 1.94 ± 0.40 , 1.30 ± 0.26 ; PNM – 0.82 ± 0.01 , 0.50 ± 0.002 ,
 262 respectively for MBSI and NBSI), but lower for switchgrass (SGC – 0.64 ± 0.10 , 0.72 ± 0.10 ; SGN
 263 – 0.75 ± 0.18 , 0.96 ± 0.24 , respectively for MBSI and NBSI). Analysis of variance results (Table 2)
 264 suggests that differences in MBSI and NBSI across sites are statistically non-significant however
 265 the respective *p* values (0.0669 and 0.0554) are close to threshold of significant difference ($\alpha =$
 266 0.05). At plant level, statistically significant differences in sustainability indices (MBSI and
 267 NBSI) between poplar clones (PDN – 1.23 ± 0.17 , 1.13 ± 0.11 , PNM – 0.68 ± 0.06 , 0.61 ± 0.05); and

between poplar (0.96 ± 0.11 , 0.87 ± 0.08) and switchgrass (1.42 ± 0.14 , 1.14 ± 0.10) suggest that switchgrass production is sustainable. When considered overall biomass production, both indices (MBSI and NBSI) suggest nutrient sustainability at Nappan site (1.41 ± 0.17 , 1.21 ± 0.13 , respectively) whereas, only MBSI (1.22 ± 0.14) suggests that Guelph site is sustainable. Overall, lower values of MBSI (0.93 ± 0.16) and NBSI (0.86 ± 0.12) at Kemptville show that biomass production is not sustainable at Kemptville (Table 3). When combined across all sites, all biomass plant production systems are sustainable except for PNM that received index values of 0.68 ± 0.06 and 0.61 ± 0.05 for MBSI and NBSI, respectively (Table 4).

DISCUSSIONS

There is growing interest to establish biomass crops to meet ever increasing demand of bioenergy, and to fulfil national and international obligations on reducing fossil fuel related carbon footprint. Studies suggest that nutrient requirements of purpose-grown biomass crops are relatively less and hence, these crops can be successfully grown on marginal lands (Schmer et al. 2005). Although, growing biomass crops on marginal lands is supported for many reasons such as it avoids conflict with food crop production and carbon emission from land use change; and land reclamation (Tilman et al. 2009; Dillen et al. 2013), the key focus is on biomass production for biofuels and bioproducts (Qin et al. 2015). In Canada, herbaceous biomass crops are generally harvested once a year whereas, woody crops are harvested over a short rotation of 3 - 4 years (Hangs et al. 2014b). Woody crops are considered better than herbaceous crops due to higher energy to mass ratio and lower overall ash contents, which are important considerations for industrial scale biomass production and easy processing (Mann 2012). Woody crops also have the advantage of shedding their leaves every year which add nutrients to the soil (Hangs et al. 2014a). Studies on biomass yield comparison of woody and herbaceous species on marginal

lands show that results are not consistent. For instance, Mann (2012) did not find any difference in biomass yield during first two growing seasons of poplar and switchgrass in southern Ontario. Later, similar results were reported for the fifth growing season for the same crops on the same site by Marsal et al. (2016). Amaducci et al. (2017) compared biomass yield of six biomass crops including poplar and switchgrass, and found that switchgrass biomass yield was significantly higher than poplar. Contrarily, our results show that stem biomass yields of poplar are significantly higher than switchgrass. The 2-year poplar stem biomass yield ranges between 7.0 – 15.5 t ha⁻¹, with significant differences between the two studied clones. This yield range is consistent with poplar biomass yield reported in many studies (Aylott et al. 2008; Dillen et al. 2013; Marsal et al. 2016). Similarly, switchgrass harvestable biomass yield (stem and leaves) ranged from 1.6 t ha⁻¹ (SGN) to 2.6 t ha⁻¹ (SGC), which can be compared with average yield of 3.5 t ha⁻¹ yr⁻¹ reported in other studies on marginal lands (Qin et al. 2015; Marsal et al. 2016). However, relatively lower SGC yields in this study, can be attributed mainly to the following establishment issues at the Kemptville site; crop failure due to early frost during establishment year (2014) and uncontrolled weeds during subsequent years.

Quantification of nutrients in leaf biomass and root biomass is important to assess biomass production system sustainability. We developed sustainability indices using macro-nutrients (MBSI) and N nutrient (NBSI) to assess biomass system sustainability. Lower values of MBSI (0.68±0.06) and NBSI (0.61±0.05) for high yielding biomass crop (PNM) suggest that the system is undergoing nutrient losses. This has implications to maintain high yield over successive rotations, unless the system is fertilized. In poplar, and many other woody crops, redistributions of nutrients, especially N, is fundamental element of nutrient economy. During the growing season, poplar leaves store about 50% of total plant N (Cooke and Weih 2005). To

maintain long term productivity, some of this N is translocated (N – resorption) to stem during leaf senescence. The N – resorption rates vary among poplar clones, and clones with higher N – resorption rates exhibit increased growth rates (Cooke and Weih 2005). The higher N – resorption also affects nutrient quality of leaf litter, and thereby less N cycling through leaf litter decomposition. Our data (not presented in this paper) shows that N – resorption rate in PNM is much higher than in PDN, which explains the higher yield in PNM as well as low sustainability indices values. This also explains the lower NBSI (0.61 ± 0.05) than MBSI (0.68 ± 0.06) values as the former is only N based index. These findings suggest a trade-off between high yield and long-term sustainability in purpose-grown woody biomass crops.

Higher index values for switchgrass (SGC – 1.47 ± 0.22 , 1.11 ± 0.15 and SGN – 1.37 ± 0.16 , 1.17 ± 0.12) for MBSI and NBSI, respectively, suggest that despite low stem biomass yield, switchgrass biomass production systems are sustainable. Nutrient input and output allocations in switchgrass are different from poplar. Unlike poplar leaf litter, switchgrass leaves are harvested with stem, and thereby any nutrients in leaves are nutrient lost at harvesting. Hence, the main nutrient input component is root biomass that is rich in N nutrient. The sustainability of switchgrass production is mainly attributed to higher N concentration in switchgrass root biomass that was significantly higher (SGC – $4.34 \pm 0.61 \text{ t ha}^{-1}$, SGN – $2.68 \pm 0.44 \text{ t ha}^{-1}$) than poplar (PDN – 1.38 ± 0.25 , PNM – 1.41 ± 0.21).

Conclusion

The overall goal of this study is to assess sustainability of purpose-grown biomass crops (poplar and switchgrass) on marginal lands in Canada. Since these crops are mainly grown for biomass production, the common interest is to select high yield woody (clones) and herbaceous (cultivars) crops. Our results suggest that, from a nutrient perspective, sustainable biomass production

systems can be established on marginal lands in Canada. However, during the establishment phase high-yielding biomass crops (PNM) may require external nutrient inputs, via fertilization, to compensate for nutrients that are lost at harvest. Having said the above, it should be mentioned that results presented in this study were derived from young stands established only for two years. As the stands mature, more nutrient inputs from aboveground and belowground plant components can be expected with corresponding higher biomass yields and nutrient removals. Therefore, further investigation is warranted on these stands at mature stage (5 or more years after establishment) in order to be more conclusive.

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460 Table 1: Baseline site characteristics of experimental plots. Data was collected in 2014 prior to
461 the establishment of experimental plots.

	Nappan	Kemptville	Guelph
Geo-climate			
Latitude	45° 45' 42.6" N	45° 00' 24.6" N	43° 32' 28" N
Longitude	64° 14' 31.6" W	75° 37' 26.9" W	80° 12' 32" W
Elevation (m)	15 m	96 m	325 m
Frost Free Days	152	130	144
Mean Annual Precipitation (mm)	1155 mm	868 mm	904 mm
Mean Summer Precipitation (mm)	273 mm	325 mm	338 mm
Soil Texture			
Sand (%)	60.33	60.00	49.67
Silt (%)	32.00	33.00	42.67
Clay (%)	7.67	7.00	7.67
Soil Nutrient Properties			
Soil N (g kg ⁻¹)	0.83	1.32	1.18
Total Soil C (g kg ⁻¹)	18.69	20.71	19.72
Inorganic Soil C (g kg ⁻¹)	0.71	0.73	3.62
Organic Soil C (g kg ⁻¹)	17.97	19.97	16.10
Soil EC (µS)	72.52	118.25	84.67
Soil pH	6.70	6.59	7.50
Organic Matter (g kg ⁻¹)	26.00	34.90	29.00
P (mg kg ⁻¹)	28.50	46.69	24.90
K (mg kg ⁻¹)	44.50	101.67	85.70
Ca (mg kg ⁻¹)	1167.40	1165.33	2134.80
Mg (mg kg ⁻¹)	76.00	219.08	218.30

N (nitrogen), C (carbon), P (phosphorus), K (potassium), Ca (calcium), Mg (magnesium)

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Table 2: Significance level (p-value) for the analysis of variance (ANOVA). Source of variations include site, plant and their interaction as main effects on different biomass variables (leaf, stem and root), and sustainability indices (MBSI and NBSI). The effects were tested on 2-year data of two poplar clones (DN34 and NM6) and two switchgrass cultivars (Cave-in-Rock and Nebraska) from three experimental sites namely Guelph (ON), Kemptville (ON), and Nappan (NS) in Canada.

Source of Variation	df	Leaf Biomass	Stem Biomass	Root Biomass	MBSI ¹	NBSI ²
Site	2	<0.0001	0.8289	0.0264	0.0669	0.0554
Block in Site	9	0.1339	0.1583	0.8999	0.8215	0.7966
Plant ³	3	<0.0001	<0.0001	<0.0001	0.0018	0.0010
Poplar vs Switchgrass	1	<0.0001	<0.0001	<0.0001	0.0102	0.0200
Poplar (PDN vs PNM)	1	0.0001	<0.0001	0.8446	0.0016	0.0005
Switchgrass (SGC vs SGN)	1	0.1354	0.1957	0.0003	0.8533	0.5766
Plant *Site	6	<0.0001	0.1251	<0.0001	0.0030	0.5668
CV	-	38.21	33.32	39.03	37.55	39.74
R-Square	-	0.8899	0.9340	0.8531	0.7798	0.6571

¹ Macro-nutrients Based Sustainability Index

² Nitrogen Based Sustainability Index

³ The partitioning of treatment (plant) sum of square was done using orthogonal contrasts approach.

Table 3: Comparison of 2-year means of leaf biomass, stem biomass, root biomass, and two sustainability indices (MBSI and NBSI) for Guelph (ON), Kemptville (ON), and Nappan (NS) in Canada. Data is for two poplar clones (DN34 and NM6) and two switchgrass cultivars (Cave-in-Rock and Nebraska).

Sites	Leaf Biomass (t ha ⁻¹)	Stem Biomass (t ha ⁻¹)	Root Biomass (t ha ⁻¹)	MBSI ¹	NBSI ²
Guelph	3.26±0.17 ^b	6.70±1.26 ^a	2.71±0.42 ^a	1.22±0.14 ^a	0.94±0.07 ^b
Kemptville	5.59±1.25 ^a	5.03±1.89 ^a	1.86±0.22 ^b	0.93±0.16 ^b	0.86±0.12 ^b
Nappan	2.53±0.32 ^b	6.47±1.43 ^a	2.81±0.63 ^a	1.41±0.17 ^a	1.21±0.13 ^a

Within column, values (means and standard errors) with the same letter are not statistically different as determined by Duncan’s new multiple range test (MRT) at $\alpha = 0.05$.

¹ Macro-nutrients Based Sustainability Index

² Nitrogen Based Sustainability Index

Table 4: 2-year means of leaf biomass, stem biomass, root biomass, and two sustainability indices (MBSI and NBSI) in two poplar clones (DN34 and NM6) and two switchgrass cultivars (Cave-in-Rock and Nebraska). Data is based on three experimental sites in Guelph (ON), Kemptville (ON), and Nappan (NS) in Canada.

Plant	Leaf Biomass (t ha ⁻¹)	Stem Biomass (t ha ⁻¹)	Root Biomass (t ha ⁻¹)	MBSI ¹	NBSI ²
PNM	6.73±1.26 ^a	15.27±15.27 ^a	1.41±0.21 ^c	0.68±0.06 ^b	0.61±0.05 ^b
PDN	4.06±0.80 ^b	7.02±0.54 ^b	1.38±0.25 ^c	1.23±0.17 ^a	1.13±0.11 ^a
SGC	2.65±0.27 ^c	2.57±0.28 ^c	4.34±0.61 ^a	1.47±0.22 ^a	1.11±0.15 ^a
SGN	1.74±0.30 ^c	1.45±0.22 ^c	2.68±0.44 ^b	1.37±0.16 ^a	1.17±0.12 ^a

Within column, values (means and standard errors) with the same letter are not statistically different as determined by Duncan's new multiple range test (MRT) at $\alpha = 0.05$.

¹ Macro-nutrients Based Sustainability Index

² Nitrogen Based Sustainability Index

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Table 5: Significance level (p-value) for partitioned analysis of the LS-means for site and plant interaction for four plants (poplar – DN34 and NM6, and switchgrass – Cave-in-Rock and Nebraska) sliced by three experimental sites (Guelph, Kemptville, and Nappan) in Canada.						
Site	df	Leaf Biomass	Stem Biomass	Root Biomass	MBSI ¹	NBSI ²
Guelph	3	0.5930	<0.0001	<0.0001	0.0003	0.0556
Kemptville	3	<0.0001	<0.0001	0.0563	0.0211	0.0667
Nappan	3	0.0180	<0.0001	<0.0001	0.0017	0.0109
¹ Macro-nutrients Based Sustainability Index						
² Nitrogen Based Sustainability Index						

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494 Table 6: Macro nutrient concentration (g kg^{-1}) in stem, leaf and root biomass of poplar (PDN and
 495 PNM) and switchgrass (SGC and SGN) across all three sites.

Site	Plant	Stem Biomass			Leaf Biomass			Root Biomass		
		N	P	K	N	P	K	N	P	K
Guelph	PDN	7.80	1.13	4.10	16.48	1.65	5.74	10.30	2.70	9.88
	PNM	6.78	0.88	3.78	12.38	1.74	3.48	5.88	2.13	8.33
	SGC	3.25	0.78	3.30	7.16	1.01	1.03	6.13	1.20	8.90
	SGN	4.10	0.95	2.18	7.04	1.04	0.81	5.83	0.83	6.90
Kemptville	PDN	9.70	1.45	6.15	14.00	2.21	16.74	5.70	1.53	9.48
	PNM	10.35	1.35	5.75	9.63	2.05	13.28	4.70	1.35	8.83
	SGC	4.53	1.35	7.43	9.68	2.26	4.83	6.20	0.78	4.28
	SGN	5.18	1.25	6.98	10.63	1.58	2.35	10.65	1.15	3.95
Nappan	PDN	6.38	1.10	3.40	15.68	2.24	6.98	8.05	1.93	6.83
	PNM	5.60	0.95	2.70	11.53	3.01	6.55	4.75	1.75	5.58
	SGC	3.05	0.83	2.55	11.40	3.10	4.69	5.35	1.18	8.20
	SGN	3.73	1.00	2.55	9.99	3.63	4.39	5.63	0.95	5.83

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Figure 1: Layout of double row design used in poplar plots. Blue dots represent poplar cutting plantings at 0.6 m distance apart. Red boxes show how a sampling quadrant (60 cm x 60 cm) was laid out around each selected poplar plant. Actual location of sampling quadrants varied in each plot and depended on selected plants that represent average crop condition. Distances shown are not to scale.

Figure 2: Box-plots of leaf biomass across sites (a) and plants (b); stem biomass across sites (c) and plants (d); and root biomass across sites (e) and plants (f). Abbreviations are G (Guelph), K (Kemptonville), N (Nappan), PDN (Poplar DN34), PNM (poplar NM6), SGC (Switchgrass Cave in rock) and SGN (Switchgrass Nebraska). The y-axis units are $t\ ha^{-1}$.

Figure 3: Root, leaf and stem biomass of different plants (poplar – PDN and PNM, and switchgrass – SGC and SGN) in each experimental site (Guelph, Kemptonville and Nappan). Values are based on 2-year data. Means in each site labeled with the same letter(s) are not significantly different as determined by Least Significant Difference (LSD) at $\alpha = 0.05$.

Figure 4: Box-plots of MBSI (Macro-nutrients Based Sustainability Index) across sites (a) and plants (b); and NBSI (Nitrogen Based Sustainability Index) across sites (c) and plants (d). Abbreviations are G (Guelph), K (Kemptonville), N (Nappan), PDN (Poplar DN34), PNM (poplar NM6), SGC (Switchgrass Cave in rock) and SGN (Switchgrass Nebraska). The y-axis units are $t\ ha^{-1}$.

Figure 5: Sustainability Indices comparing the performance of different plants (poplar – PDN and PNM, and switchgrass – SGC and SGN) in each experimental site (Guelph, Kemptonville and Nappan). MBSI and NBSI indicates are input/output ratio of macro-nutrients (NPK) and nitrogen respectively. Values are based on 2-year data. Means in each site labeled with the same letter(s) are not significantly different as determined by Least Significant Difference (LSD) at $\alpha = 0.05$.

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Figure 2: Box-plots of leaf biomass across sites (a) and plants (b); stem biomass across sites (c) and plants (d); and root biomass across sites (e) and plants (f). Abbreviations are G (Guelph), K (Kemptonville), N (Nappan), PDN (Poplar DN34), PNM (poplar NM6), SGC (Switchgrass Cave in rock) and SGN (Switchgrass Nebraska). The y-axis units are $t\ ha^{-1}$.

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543 Figure 5: Sustainability indices comparing the performance of different plants (poplar – PDN
 544 and PNM, and switchgrass – SGC and SGN) in each experimental site (Guelph, Kemptville and
 545 Nappan). MBSI and NBSI indicates are input/output ratio of macro-nutrients (N, P, and K) and
 546 nitrogen respectively. Values are based on 2-year data. Means in each site labeled with the same
 547 letter(s) are not significantly different as determined by Least Significant Difference (LSD) at α
 548 = 0.05.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5