



# Permanent site characteristics exert a larger influence than atmospheric conditions on leaf mass, foliar nutrients and ultimately aboveground biomass productivity of *Salix miyabeana* 'SX67'

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## ABSTRACT

*Salix* species are widely used for wood production, but the interactive effects of soil types and atmospheric conditions on *Salix* foliar nutrients and aboveground biomass productivity have not yet been elucidated. The objectives of this study were fourfold: (1) to assess the variation in foliar nutrients and leaf mass of *Salix miyabeana* 'SX67' grown as short rotation coppice (SRC) across three growing seasons and nine locations in Quebec with different permanent site characteristics and atmospheric conditions, (2) to test if atmospheric conditions and permanent site characteristics could explain the variation in foliar nutrients and leaf mass, (3) to develop models that consider foliar traits and nutrient interactions to produce more robust predictions of annual aboveground biomass yields, and (4) to compare nutritional requirements of 'SX67' to other *Salix* cultivars used for SRC. Leaf samples were collected over three growing seasons at all sites. For each site, atmospheric conditions were simulated and foliar nutrient levels were measured to perform centered log ratio (clr) transformations for each foliar nutrient. This approach considered foliar nutrient interactions and dealt with clr scores as linearly independent. The clr scores were more largely influenced by permanent site characteristics than by atmospheric conditions, despite large variations in degree-days. However, some foliar nutrients and leaf mass were linearly related to atmospheric variables within sites. Strong relationships between annual aboveground biomass yields and leaf mass were computed (e.g. adjusted  $R^2 = 0.62$ ), likely due to a proportional allocation between foliage and wood. Although significant linear relationships between clr scores (i.e. N, Ca and Mn) and annual aboveground biomass yields were detected, yields were more robustly explained non-linearly by thresholds (i.e. N, Ca and P) (e.g.  $R^2 = 0.85$ ), likely due to permanent characteristics specific to each of the sites and climatic limitations during the growing seasons studied. The thresholds detected by non-linear models suggested high N and P use efficiencies and a large Ca requirement of 'SX67'.

## 1. Introduction

Species of *Salix* exhibit high physiological and growth plasticity (He and Dong, 2003). They can be grown under a wide range of soil and climatic conditions (Tahvanainen and Rytkönen, 1999; Sannervik et al., 2006; Aylott et al., 2008; Ens et al., 2013), but yields of a species or genotype can be dramatically influenced by soil nutrients and thus, they can vary substantially among sites. Genotypes of *Salix* grown as short rotation coppice (SRC) are more nutrient-demanding than those growing in the wild (Weih, 2001) and are capable of building very high contents of N, P, K, Ca and Mg (Adegbi et al., 2001). Soil N

availability influences foliar N and aboveground yields (Ens et al., 2013; Toillon et al., 2013). Ericsson (1981a) also demonstrated strong causal relationships between nutrient availability (including N, P, K, Ca and Mg), foliar nutrients and aboveground biomass yields for three *Salix* cultivars grown hydroponically. Various studies have also showed the benefits of fertilization on foliar nutrients such as N, P or K and aboveground biomass yields of *Salix* (Marmioli et al., 2012; Quayle et al. 2015; Labrecque and Teodorescu, 2001; Labrecque and Teodorescu, 2003).

Monitoring of foliar nutrients over successive years following the fertilization of forests or plantations has often been done (Huettl et al.,

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1990; Wilmot et al., 1996), including *Salix* (Labrecque et al., 1998), but very few studies have established a relationship between year-to-year variations in atmospheric conditions and foliar nutrients (or other leaf traits). A better understanding of the year-to-year variation in foliar nutrients and other leaf traits of woody crops such as willow SRC could provide valuable information to support nutrient and water management in the context of climate change. Duquesnay et al. (2000) showed that foliar N, P, K, Ca and Mn of European beech (*Fagus sylvatica* L.) varied over several growing seasons within the same plots. The combined year-to-year and plot effects explained more than 70% of the variation in foliar nutrients. Kudo (2003) observed lower N concentrations in leaves of Miyabe willow (*Salix miyabeana* ‘Seemen’) during warm summers compared to cooler ones, suggesting that year-to-year atmospheric variations influenced *Salix* nutrition. In Mediterranean Italy, year-to-year atmospheric variations and soil conditions influenced leaf traits of *Fagus sylvatica* L., Turkey oak (*Quercus cerris* L.) and holm oak (*Q. ilex* L.), which in turn impacted aboveground productivity (Bussotti et al., 2000). In particular, leaf surface area and foliar N, P and S decreased and leaf mass per area ( $\text{g m}^{-2}$ ) increased under water stress. Leaf mass per area was the most robust predictor of site productivity under such conditions. In three plantations established on heterogeneous soils in northern France, leaf area and aboveground biomass of six hybrid *Salix* genotypes as well as soil N availability were highest where soil water availability was high (Toillon et al., 2013).

Studies on plant nutrition generally rely on nutrient concentrations, hence denying nutrient interactions (Wilkinson et al., 2000). Nutrient interactions are traditionally described as dual or stoichiometric ratios (Ingestad, 1987; Walworth and Sumner, 1987). Ratios are scale-dependent and generate spurious correlations (Chayes, 1960). Ratios should be averaged using geometric means (Fleming and Wallace, 1986), hence requiring log transformations to compute variance. Because nutrients in a leaf are compositional, they can be modelled using the more robust log ratio transformations of compositional data analysis (Aitchison, 1986; Parent and Dafir, 1992; Souza et al., 2016). To refine our understanding of nutrient cycling and optimize nutrient diagnoses for woody shrubs and trees in the context of global change, there is a need to investigate the relationships between foliar nutrients, atmospheric conditions and biomass production. A large network of *Salix miyabeana* ‘SX67’ plantations grown as SRC was used to address this question.

We assumed causal relationships between foliar nutrients and atmospheric conditions and in turn, we hypothesized that nutritional factors limiting aboveground biomass yield change across growing seasons. In this respect, we first assessed variations in foliar nutrients and leaf mass over three growing seasons across nine sites in Quebec with various soils and atmospheric conditions. Using different statistical models, we then tested if year-to-year variation in atmospheric conditions (within sites) as well as permanent site characteristics (e.g. parent material depth and soil texture, slope and aspect) could explain the variability in foliar nutrients and leaf mass. Using the centred log ratio transformation, we also developed models that consider nutrient interactions as a mean to come up with the most robust predictions of annual aboveground biomass yields. Finally, we compared the nutritional requirements of highly productive ‘SX67’ in Quebec to other *Salix* cultivars used for SRC elsewhere.

## 2. Methods

### 2.1. Sites

Nine sites of ‘SX67’ grown as SRC in Quebec (i.e. ABI, BEL, BOI, HTG, LAV, MTL, RXP, SJPJ and STR, Fig. 1) were used for this study.

Field design, previous land use and soil properties are described in detail in Fontana et al. (2016, 2017), however, we provide a brief description here. Using a mechanical type planter, ‘SX67’ cuttings (i.e. 20–25 cm in length) were inserted in the soil at a depth of 15–20 cm at a

spacing of 30 cm along a single row design. Rows were separated by 1.8 m, giving an approximate density of 18,500 stools  $\text{ha}^{-1}$ . Row lengths of ‘SX67’ were over 100 m for nearly all sites (i.e. ABI, BOI, LAV, MTL, RXP, SJPJ and STR). At the HTG site, ‘SX67’ was planted in  $10 \times 12$  m as part of a clonal trial with a randomized split-block design. Plowing and cross-disking were carried out before SRC establishment, except at BOI and SJPJ due to the stony nature of the land. Before plowing in the fall, roundup ProTM (41% glyphosate, the active ingredient (a.i.)) was applied once at a rate of  $2\text{--}4 \text{ L ha}^{-1}$  ( $0.85\text{--}1.7 \text{ a.i. ha}^{-1}$ ), depending on weed type and abundance. Coping was generally performed after the first growing season and harvesting was done between three to five years depending on site. All the SRC were established on mineral soils, except for RXP where soil organic C ( $C_{\text{org}}$ ) concentration is  $> 60\%$ . Soil particle size distribution varies considerably across sites, with sand content ranging from 19 to 74% and clay content ranging from 4 to 43%. Soil chemistry also varies substantially across sites. Soil pH ranges between 5.1 and 7.5, where BOI and HTG have a higher pH than the others. Except for RXP, which exhibits high soil total N and P concentrations ( $9.0 \text{ mg N kg}^{-1}$  and  $0.78 \text{ mg P kg}^{-1}$ ), all other sites have relatively low soil total N ( $1.7\text{--}3.4 \text{ mg N kg}^{-1}$ ) and P ( $0.06\text{--}0.17 \text{ mg P kg}^{-1}$ ) concentrations. The STR, MTL and LAV sites have the lowest  $C_{\text{org}}$  values ( $< 7.5\%$ ) and exchangeable Ca ( $< 5.1 \text{ cmol}_c \text{ kg}^{-1}$ ) and Mg ( $< 0.8 \text{ cmol}_c \text{ kg}^{-1}$ ) concentrations, likely due to their high sand content ( $> 50\%$ ).

### 2.2. Estimation of annual aboveground biomass yields

At each site, five plots ( $5 \times 5.4$  m, 45 plots in total) were randomly selected for crop establishment. Annual aboveground biomass yields ( $\text{Mg ha}^{-1}$ ) were estimated within all plots using aboveground biomass and basal area increment ( $\text{mm}^2$ ) data (Fontana et al., 2016). Because a few growing seasons are needed for the stools to fully establish and reach their maximum yield potential, productivity was estimated only for growing seasons that had reached their maximum yield potential. This procedure provided a robust comparison between sites, despite their different ages and number of rotations. First growing seasons of subsequent rotations were also excluded from the database because yields were systematically lower than maximum yield potential.

### 2.3. Foliage sampling, leaf mass and nutrient analysis

Foliage was sampled between the last week of August and the first week of September (i.e. before any night frost) of 2011, 2012 and 2013. The number of sampled sites varied between growing seasons because of the complex logistics of sampling nine sites across a large geographical range in a short period of time. The number of sampled plots and stools also varied between growing seasons and depended upon available resources for sampling and laboratory analysis. In 2011, four contiguous healthy stools were sampled in each of the five plots on seven of the sites, ABI, BOI, LAV, MTL, RXP, SJPJ and STR (140 samples from 35 plots). In 2012, one stool in each of the five plots was sampled on six of the sites, ABI, BOI, LAV, MTL, RXP and HTG (30 samples from 30 plots). In 2013, four contiguous healthy stools were sampled in three of the five plots on eight sites, ABI, BOI, BEL, HTG, LAV, MTL, RXP and SJPJ. In this last case only, we made a composite sample using the four samples collected in each plot (24 samples from 24 plots). For each stool that was sampled in 2011, 2012 and 2013, we collected a minimum of 10 mature and healthy leaves in the upper-third of the canopy (full sunlight) from the stem with the largest basal diameter.

Leaves were oven-dried at  $40^\circ\text{C}$  to a constant mass. For each sample, at least ten leaves were weighed and then finely ground using a ball mill (Vibratory Miro Mill Pulverisette 0, Fritsch GmbH, Idar-Oberstein, Germany). Average leaf mass was measured. Carbon and N levels were determined on ground samples using a Vario MicroCube (Elementar, Hanau, Germany) or an EA1108 CHNS-O Analyzer (Thermo Fisons, MA, USA). Ground samples were digested in

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