



Accelerating the selection process for Populus and Salix clones using short-term photosynthetic acclimation responses under greenhouse conditions

Takamitsu Mamashita, Guy R. Larocque, Annie DesRochers, Jean Beaulieu, Barb R. Thomas, Alex Mosseler, John Major & Derek Sidders

To cite this article: Takamitsu Mamashita, Guy R. Larocque, Annie DesRochers, Jean Beaulieu, Barb R. Thomas, Alex Mosseler, John Major & Derek Sidders (2017) Accelerating the selection process for Populus and Salix clones using short-term photosynthetic acclimation responses under greenhouse conditions, *Écoscience*, 24:1-2, 59-73, DOI: [10.1080/11956860.2017.1361508](https://doi.org/10.1080/11956860.2017.1361508)

To link to this article: <http://dx.doi.org/10.1080/11956860.2017.1361508>



Published online: 11 Aug 2017.



Submit your article to this journal [↗](#)



Article views: 33



View related articles [↗](#)



View Crossmark data [↗](#)

ARTICLE



Accelerating the selection process for *Populus* and *Salix* clones using short-term photosynthetic acclimation responses under greenhouse conditions

Takamitsu Mamashita ^a, Guy R. Larocque^a, Annie DesRochers^b, Jean Beaulieu^c, Barb R. Thomas^d, Alex Mosseler^e, John Major^e and Derek Sidders^f

^aNatural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, Canada; ^bInstitut de Recherche sur les Forêts, Université du Québec en Abitibi-Témiscamingue, Amos, Canada; ^cNatural Resources Canada, Canadian Forest Service, Canadian Wood Fibre Centre, Quebec, Canada; ^dDepartment Renewable Resources, University of Alberta, Edmonton, Canada; ^eNatural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, Canada; ^fNatural Resources Canada, Canadian Forest Service, Canadian Wood Fibre Centre, Edmonton, Canada

ABSTRACT

Leaf photosynthetic characteristics could be determinant factors to identify the most productive clones of hybrid poplars (*Populus* spp.) and willows (*Salix* spp.). Photosynthetic acclimation of hybrid poplars and willows was studied under greenhouse conditions. Seven *Populus* and five *Salix* clones were grown for 3 months at three spacings [20 × 20, 35 × 35, and 60 × 60 cm] and two nitrogen (N) levels (20 and 200 μg g⁻¹). There were no significant spacing effects on leafless aboveground biomass per tree (AGBT) and height. Clonal acclimation to higher density was associated to increases in leaf area index (LAI) by 347% and specific leaf area (SLA) by 13% despite decreased leaf N content per unit leaf area (N_{area}) by 31%. There were no changes in net CO₂ assimilation rate (A) and photosynthetic N-use efficiency in the ambient light condition (PNUE_{amb}) within different spacings. The N addition alleviated competition effects by maximizing leaf area (LA) and SLA. Compared with less productive clones, more productive clones had 28% greater SLA, greater LA and AGBT per unit of increase in N_{area} over all treatments. The increased development of LA and SLA under high planting density is a key indicator of more productive clones.

RÉSUMÉ

Les caractéristiques photosynthétiques des feuilles peuvent être déterminantes pour identifier les clones les plus productifs de peupliers (*Populus* spp.) et de saules (*Salix* spp.) hybrides. L'acclimation photosynthétique de clones de peupliers et de saules hybrides a été étudiée en serre. Sept clones de *Populus* et cinq clones de *Salix* ont cru pendant trois mois selon trois espacements [20×20, 35 × 35 et 60 × 60 cm] et deux niveaux d'azote (20 et 200 μg g⁻¹). Il n'y avait pas d'effet significatif de l'espacement sur la biomasse aérienne sans feuilles par arbre (AGBT) et sur la hauteur. L'acclimation des clones à des densités plus élevées était associée à une augmentation de 347% de l'indice de surface foliaire (LAI) et de 13% de la surface foliaire spécifique (SLA) malgré une diminution de 31% du contenu foliaire en azote par unité de surface foliaire (N_{area}). Il n'y a pas eu de changement du taux net d'assimilation de CO₂ (A) et de l'efficacité d'utilisation photosynthétique de l'azote sous conditions de lumière ambiante (PNUE_{amb}) et selon différents espacements. L'ajout d'azote a diminué les effets de la compétition en maximisant la surface foliaire (LA) et la SLA. Comparativement aux clones moins productifs, les clones plus productifs avaient des SLA 28% plus élevées, ainsi que des LA et AGBT plus élevées par unité d'augmentation de N_{area} dans tous les traitements. L'augmentation de LA et de SLA dans les plantations à forte densité est un indicateur clé de clones plus productifs.

ARTICLE HISTORY

Received 4 June 2017
Accepted 26 July 2017

KEYWORDS

Populus; *Salix*; spacing; nitrogen; photosynthesis; leaf area display

MOTS CLÉS

Populus; *Salix*; espacement; azote; photosynthèse; surface foliaire

Introduction

Short rotation forestry (SRF) plantations using hybrid poplars (*Populus* spp. including aspens) and willows (*Salix* spp.) have generated interest in Canada for manufacturing a variety of products including bioenergy. SRF

can produce appreciable amounts of wood fibre in less than 10 years in densely spaced stands (O'Neill et al. 2010). Generally, energy-crop productivity in *Populus* spp. can attain 10 dry Mg ha⁻¹ yr⁻¹, with values approaching 20 dry Mg ha⁻¹ yr⁻¹ for genotypes that are

well adapted to local site conditions (Zalesny et al. 2012; Truax et al. 2014). To achieve these production targets, a number of *Populus* and *Salix* clonal and progeny trials were established to evaluate growth rates, adaptability, and disease resistance across Canada over several decades (Dominy et al. 2010). However, there is a continuous need to develop and select better clones that could show higher growth performance and productivity, thereby producing economic returns on investment in SRF plantations (McKenney et al. 2011).

Since productivity is determined by both the genetic background and environmental factors such as initial tree spacing, site quality, weed management, fertilization, and local climate (Weih 2004; Mosseler et al. 2014a), it is necessary to better understand the variation in physiological and morphological traits of available plant material under different environmental conditions in order to select the best performers. In *Populus* and *Salix* improvement programmes, photosynthetic capacity is an important trait as it is highly correlated to plant growth and productivity (Dickmann et al. 2001). Crop trees in SRF are commonly planted in spacings ranging from 0.5 to 1.5 m between trees for willows and poplars (Weih 2004), and from 2.5 to 3.0 m between trees for hybrid aspens (Tullus et al. 2012). These high-density conditions may substantially reduce photosynthetically active radiation (PAR) received by leaves within crowns (Benomar et al. 2011). Thus, crop trees that can achieve greater photosynthetic capacity under low PAR have the potential to better acclimate to higher stand density and produce more biomass (Dickmann et al. 2001). The identification of physiological traits associated with more productive clones can be used to accelerate growth and may result in considerable savings in time and silvicultural expenses compared to conventional field selection processes that require several years to obtain results (Lamhamedi et al. 2007).

Physiological acclimation to high stand density varies within crowns, due to different PAR conditions and nutrients received by leaves (Benomar et al. 2011). In the upper canopy, increased crown competition may result in a greater proportion of leaf nitrogen (N) allocated to newly produced leaves to maintain greater photosynthetic capacity (Knops and Reinhart 2000; Benomar et al. 2011). In the lower canopy, reduced PAR and leaf N content associated with increased crown competition might induce an increase in specific leaf area (SLA; ratio of leaf area to leaf mass), which increase photosynthetic- and N-use efficiency (NUE) (Rosati et al. 1999, 2000). The increase in SLA is associated with larger leaf area per unit of leaf biomass that intercepts more PAR and decreases leaf thickness

for better PAR transmittance, allowing plants to capture low PAR effectively under shaded conditions (Vile et al. 2005). The increase in SLA would also contribute to making more effective use of decreased N for photosynthesis by increasing N allocation to photosynthetic machinery (Evans and Poorter 2001), leading to greater photosynthetic N-use efficiency (PNUE) on the leaves under low PAR (Benomar et al. 2011). As a result, greater SLA and PNUE may partially offset the negative effects of competition by increasing light-use efficiency under low PAR. However, as SLA is inversely associated with leaf longevity (Reich et al. 1992), an increase in plant competition might cause earlier loss of high SLA shaded leaves (Fang et al. 1999).

Nitrogen fertilization generally increases leaf N content as a greater proportion of N is distributed to leaves, particularly to Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), a critical enzyme for photosynthesis (Evans 1989). Thus, positive correlations between leaf N content and net CO₂ assimilation rate (*A*) have been reported (e.g., Reich et al. 1991; Sheriff and Nambiar 1991; Thompson and Wheeler 1992). The increased whole photosynthetic capacity should lead to greater plant biomass productivity (Cooke et al. 2005; Weih and Rönnerberg-Wästljung 2007). In addition, N fertilization should result in the expansion of leaf area (LA) and increase in SLA in densely planted fast-growing species plantations (Dickmann et al. 2001; Cooke et al. 2005; Weih and Rönnerberg-Wästljung 2007), leading to efficiently maximizing total photosynthetic leaf area per unit of allocated leaf mass under shaded conditions. However, the amount of SLA and the distribution of leaf N content per unit leaf area (N_{area}) vary among species and also depend on environmental factors such as irradiance, stand density, and temperature, which also cause variations in *A* (Evans 1989; Benomar et al. 2011).

These different studies suggest that basic relationships between PAR, N availability and leaf photosynthesis are understood only in general terms. However, there is still a lack of knowledge on the type of these relationships for leaves located in different sections of the crown and trees growing in different stand densities. A better knowledge of the type of the relationship is important to determine leaf photosynthetic characteristics leading to greater productivity under different planting densities and N availability.

The primary objective of this study was to examine the types of relationships among *A*, PAR, N availability, and leaf characteristics for hybrid poplar and willow clones under different planting densities. Our hypotheses were as follows: (I) despite greater crown closure under higher density, clones should maintain a

relatively high productive capacity because of photosynthetic acclimation to increased competition by increasing SLA and photosynthetic N-use efficiency in ambient PAR conditions (PNUE_{amb}); (II) The addition of N should alleviate competition effects under decreased spacing by maximizing *A* and leaf area development per unit leaf mass (SLA); (III) Compared with the large deltoid-shaped leaves of *Populus*, *Salix* leaves should show greater photosynthetic capacity under higher planting density as the small-narrow leaves of *Salix* could have better light interception in the lower canopy by reducing the degree of leaf aggregation.

Materials and methods

Study location and experimental design

The research took place in a greenhouse at the Laurentian Forestry Centre in Quebec City (46°46'N, 71°16'W). The experimental design was set up as an incomplete block design with six hybrid poplar, one hybrid aspen, and five willow clones (Table 1). These clones were selected from the Poplar and Willow Council of Canada database (Derbowka 2012) and have been commonly used in either research or commercial plantations in Canada for the purpose of biomass production under SRF conditions or site reclamation (Larocque 1999; Yu et al. 2001; Labrecque and Teodorescu 2005; Benomar et al. 2012; Mosseler et al. 2014a, 2014b).

As there was not enough space in the greenhouse to conduct all the experimental work simultaneously, the

experiment was repeated in time (within six different time periods) and was conducted over a 3-year period (Table 1). Within each time period (cycles of 13 weeks of growth), there were six clones randomly selected among 36 clones and each clone had two N levels (20 and 200 µg g⁻¹) and three spacings [20 (20 × 20), 35 (35 × 35), 60 (60 × 60) cm]. Thus, 36 planting boxes established for each time period were randomly positioned in the greenhouse. The planting box sizes were 1.0 × 1.0 × 0.4 m for the 20 cm spacing, 1.4 × 1.4 × 0.4 m for the 35 cm spacing, and 1.2 × 1.2 × 0.4 m for the 60 cm spacing. Each clone was replicated three times, once in each of three different time periods over the 3 years (Table 1). For the 20 and 35 cm spacings, 16 dormant, unrooted cuttings 10 cm in length were planted per planting box but only the plants arising from the four central cuttings were measured for statistical analyses. For the 60 cm spacing, five cuttings were planted but only one plant arising from one central cutting was measured for subsequent analyses. Unrooted dormant cuttings (hybrid poplars and willows) and rooted dormant cuttings (hybrid aspen clone 2782) were respectively stored at +2 to +4°C for storage (< 1 month), and at -2 to -4°C for storage (> 1 month) (Stanturf et al. 2001). As plants are required to be warmed to greenhouse temperature prior to planting, unrooted dormant cuttings were soaked for a day or two in freshwater at greenhouse temperature and rooted dormant cuttings were not soaked but placed at greenhouse temperature for 2–3 days (Stanturf et al. 2001). For hybrid aspen, 20 cm long rooted plants originating

Table 1. Parents and origins of the clones used in the experiment.

Block	Growing period	Tested clones	Clone name	Taxon	Growing periods	Origin
Hybrid poplar						
1	December 2009–February 2010		915319	<i>Populus maximowiczii</i> × <i>P. balsamifera</i>	1, 4, 5	Université du Québec en Abitibi-Témiscamingue, QC
2	May 2010–July 2010		747210	<i>P. balsamifera</i> × <i>P. trichocarpa</i>	1, 3, 6	Université du Québec en Abitibi-Témiscamingue, QC
3	December 2010–February 2011		1081	<i>P. deltoides</i> × <i>P. balsamifera</i>	1, 2, 4	Canadian Wood Fibre Centre, Natural Resources Canada
4	June 2010–August 2011		DN-74	<i>P. deltoides</i> × <i>P. nigra</i>	1, 5, 6	Canadian Wood Fibre Centre, Natural Resources Canada
5	January 2012–March 2012		NM6	<i>P. maximowiczii</i> × <i>P. nigra</i>	1, 2, 3	Canadian Wood Fibre Centre, Natural Resources Canada
6	June 2012–August 2012		Walker	<i>P. deltoides</i> × <i>P. × petrowskyana</i>	1, 4, 6	Canadian Wood Fibre Centre, Natural Resources Canada
Hybrid aspen						
			2782	<i>P. tremuloides</i> × <i>P. tremula</i>	2, 3, 5	Alberta-Pacific Forest Industries Inc.
Willow						
			HAN-A5	<i>Salix amygdaloides</i>	2, 3, 6	Jardin botanique de Montréal
			LEV-D5	<i>S. discolor</i>	3, 4, 6	Jardin botanique de Montréal
			SHE-E9	<i>S. eriocephala</i>	2, 5, 6	Jardin botanique de Montréal
			LAF-I4	<i>S. interior</i>	3, 4, 5	Jardin botanique de Montréal
			TCH-N4	<i>S. nigra</i>	2, 4, 5	Jardin botanique de Montréal

Growing periods 1: December 2009–February 2010; 2: May 2010–July 2010; 3: December 2010–February 2011; 4: June 2010–August 2011; 5: January 2012–March 2012; 6: June 2012–August 2012.

from in-vitro culture were used because aspens are difficult to root from dormant stem cuttings (Stanturf et al. 2001).

Fertilization

A 100 cm³ aliquot of tap water containing all macro- and micro-nutrients was provided twice a week using the irrigation system (O'Jet Olson Bleu 300, Harnois Inc., St-Thomas, QC, Canada). Therefore, the 20 and 35 cm spacings were supplied with 1600 cm³ of nutrients per planting box and the 60 cm spacing was supplied with 500 cm³ of nutrients per planting box. Two N levels (20 and 200 µg g⁻¹) were applied as ammonium nitrate (NH₄NO₃) twice a week. Phosphorus (1.5 g P) was provided as monobasic calcium orthophosphate [Ca(H₂PO₄)₂·H₂O] to each cutting. Calcium (1.55 g Ca) per cutting was supplied from above as calcium orthophosphate as well as CaCO₃ for pH control. Potassium (50 mg K), magnesium (11.9 mg Mg), and sulphur (15.7 mg S) of macro-nutrient solutions were supplied to each plant as K₂SO₄ and MgSO₄ · 7H₂O, respectively. Micronutrients were also provided per cutting, including 1.35 mg of iron (chelated Fe), 0.5 mg of manganese (Mn), 0.013 mg of molybdenum (Mo), 0.04 mg of copper (Cu), and 0.39 mg of boron (B). These nutrients were provided using the O'Jet Olson Bleu 300 irrigation system (Harnois Inc., St-Thomas, QC, Canada).

Greenhouse conditions

Greenhouse temperature was set at 24°C during the day and 18°C during the night. Daily photoperiod was set at 16 h (06h00–22h00) and natural light was supplemented with artificial light (400 W high pressure sodium bulbs, Litmore Distributors Ltd., Edmonton, AB, Canada) as needed. PAR above the canopy in the greenhouse varied within a range of 500–1122 µmol m⁻² s⁻¹ on sunny days and 50–500 µmol m⁻² s⁻¹ on cloudy days, depending on the degree of overcast. Soil pH in the boxes averaged 6 and ranged from 5.5–7. Soil mixture in the planting boxes consisted of peat, vermiculite, and sand (1:1:1 by volume), based on a commercial potting substrate PRO-MIX (Premier Horticulture Ltd, Dorval, QC, Canada).

Gas exchange measurements

Leaf gas exchange measurements were conducted randomly on one of the four central trees selected in the 20 and 35 cm spacings and one central tree in the 60 cm spacing at the end of 13-week growing periods.

The crown of each sampled tree was divided equally into three layers from the bottom to the top of the crown from which two leaves per crown section were randomly selected for leaf gas exchange measurements. Net CO₂ assimilation rate (*A*) and PAR under ambient light conditions were conducted, using a portable photosynthesis system LI-6400 (LI-COR Inc., Lincoln, NE, USA). Flow rate and CO₂ concentration in the leaf cuvette were maintained at 200 mL min⁻¹ and 400 µmol mol⁻¹, respectively. Measurements were made at 50–75% relative humidity and 0.8–1.8 kPa vapour pressure deficit. All measurements were taken between 11h00 and 13h00.

Sampling

After each 13-week growing period, the plants in the centre of each planting box were harvested and stems (including branches), roots, and leaves were separated for biomass measurements. LA was measured with a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NE, USA). Then, stems, roots, and leaves were oven-dried at 55°C until no change in mass was detected.

Leaf area index (LAI) was calculated as the ratio of leaf area (cm²) to a given spacing ground occupied by each plant (cm²). SLA was calculated as the ratio of leaf area (cm²) to leaf dry mass (g). Leaf N concentration (mg g⁻¹) was measured using a NA-2000 dry combustion N-analyser (Fissons Instruments, Milan, Italy), and N concentration per unit dry mass (mg g⁻¹) was then converted to N content per unit leaf area (N_{area}, g m⁻²). Photosynthetic N-use efficiency under ambient light condition (PNUE_{amb}) was calculated as the ratio of *A* to N_{area}.

Statistical analyses

All data were analysed using the Mixed Procedure in SAS (version 9.2, SAS Institute, Cary, NC, USA). The model contained fixed effects for genus or clone, N level, spacing, and their interactions in a factorial treatment structure. In this model, the dependent variables were height, leafless AGBT, LA, and LAI and measured at the end of the growing period.

$$Y_{\text{bgcns}} = \mu + \beta_{\text{gc}} + \beta_{\text{n}} + \beta_{\text{s}} + \beta_{\text{gc}^*\text{n}} + \beta_{\text{n}^*\text{s}} + \beta_{\text{gc}^*\text{n}^*\text{s}} + E_{\text{t}} + E_{\text{p}} + E_{\text{gc}^*\text{n}^*\text{s}^*\text{p}} + E_{\text{r}} \quad (1)$$

where Y_{bgcns} is the dependent variable, μ is the overall mean, β_{gc} is the fixed effect of genus or clone, β_{n} is the fixed effect of N, β_{s} is the fixed effect of spacing, $\beta_{\text{gc}^*\text{n}}$ is the genus or clone by N interaction, $\beta_{\text{n}^*\text{s}}$ is the N by

spacing interaction, β_{gc^*s} is the genus or clone by spacing interaction, $\beta_{gc^*n^*s}$ is the genus or clone by N by spacing interaction, E_t , E_p , and $E_{gc^*n^*s^*p}$, are the random effects for time period, planting box, and tree, respectively. Er is the residual error.

Analyses for SLA, N_{area} , A , PAR, and $PNUE_{amb}$ were examined using a mixed model. The model contained fixed effects for clone, N, spacing, crown section, and their interactions in a factorial treatment structure, with three replicates per combination. In this model, the dependent variables are SLA, N_{area} , A , and PAR and the model is as follows:

$$\begin{aligned}
 Y_{bgcncswp} = & \mu + \beta_{gc} + \beta_n + \beta_s + \beta_{cw} + \beta_{gc^*n} \\
 & + \beta_{n^*s} + \beta_{gc^*s} + \beta_{gc^*cw} + \beta_{n^*cw} \\
 & + \beta_{s^*cw} + \beta_{gc^*n^*s} \\
 & + \beta_{gc^*n^*cw} + \beta_{n^*s^*cw} + \beta_{gc^*s^*cw} + \beta_{gc^*n^*s^*cw} \\
 & + E_t + E_p + E_{gc^*n^*s^*p} + Er
 \end{aligned} \quad (2)$$

where $Y_{bgcncswp}$ is the dependent variable, β_{cw} is the fixed effect of canopy layer or crown section, β_{gc^*cw} is the genus or clone by crown section interaction, β_{n^*cw} is the N by crown section interaction, β_{s^*cw} is the spacing by crown section interaction, $\beta_{gc^*n^*cw}$ is the genus or clone by N by crown section interaction, $\beta_{n^*s^*cw}$ is the N by spacing by crown section interaction, $\beta_{gc^*s^*cw}$ is the genus or clone by spacing by crown section interaction, $\beta_{gc^*n^*s^*cw}$ is the genus or clone by N by spacing by crown section interaction.

Linear regression analyses were used to examine the relationships between A and N_{area} for *Populus* and *Salix* clones (GLM procedure in SAS, version 9.2). More productive and less productive clones were identified based on leafless AGBT and aboveground biomass per m^2 (AGBM) (Mamashita et al. 2015). In addition, linear regression analyses were also undertaken to verify relationships between AGBT or leaf area

and N_{area} for more and less productive clones. Slopes between more productive and less productive clones were compared using analysis of covariance (ANCOVA). Relationships between A and PAR and between $PNUE_{amb}$ and PAR were analysed using the best-fit light response equation as follows:

$$y = a(1 - e^{-bx}) \quad (3)$$

where y is A or $PNUE$, x is PAR, and a and b are fitted constants ($p < .05$) (NLIN procedure in SAS, version 9.2).

Results

Tree height and biomass

There were significant differences in height for individual clones in response to changes in N and spacing ($C \times N \times S$ interaction; Tables 2 and 3). Although there was no spacing effect under the same N level on height for all clones, the addition of N within some of the same spacing increased height for some clones such as 915,319, DN-74, NM06, Walker, HAN-A5, LEV-D5, and LAF-I4 (Table 3). Leafless AGBT increased by 53% from 10.8 ± 0.75 g at $20 \mu\text{g g}^{-1}$ N to 16.5 ± 0.8 g at $200 \mu\text{g g}^{-1}$ N levels (Table 2) and showed differences among clones (Figure 1 and Table 2).

Leaf area display

The decrease in spacing from 60 to 20 cm significantly reduced LA by 50% ($p < .001$), and the addition of N in each spacing increased LA by 30, 47, and 88% in the 20, 35, and 60 cm spacings, respectively ($N \times S$ interaction; Figure 2, Table 2). On the other hand, LAI increased from 60 to 20 cm spacing by 347% and by 42% with the addition of N (Figure 3(a,b), Table 2).

Trees growing under the high N level had 14% significantly SLA values that varied from

Table 2. Analysis of variance showing sources of variation, degrees of freedom (DF), F values, and associated probabilities (p) for height, leafless above-ground biomass per tree (AGBT), leaf area (LA), and leaf area index (LAI).

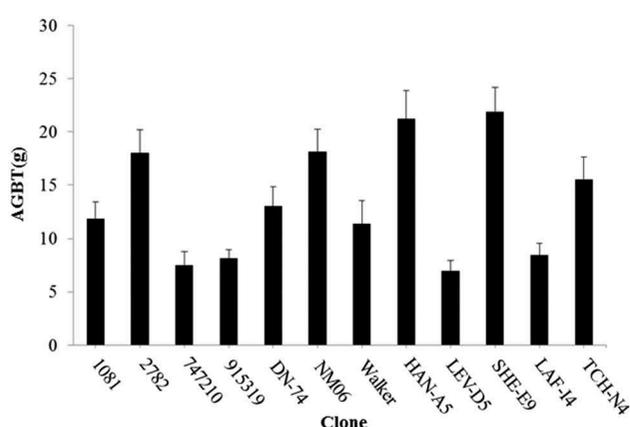
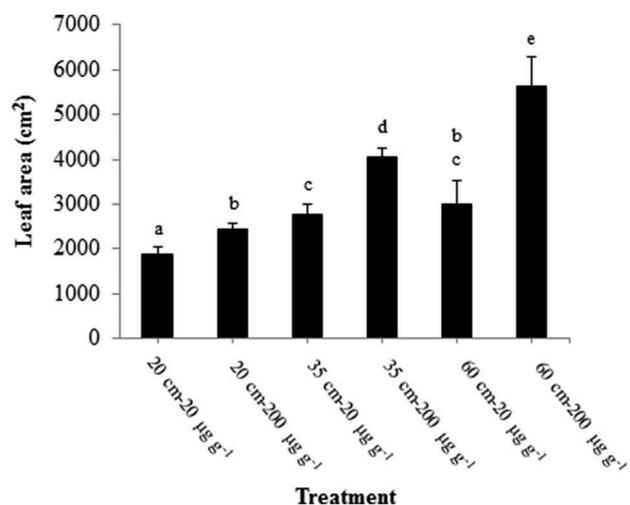
Source of variation	DF	Height		AGBT		LA		LAI	
		F	p	F	p	F	p	F	p
G	1	1.5	.250	2.4	.122	3.2	.073	7.8	.006
N	1	37.2	< .001	38.2	< .001	65.8	< .001	15.2	< .001
S	2	0.8	.471	2.7	.069	33.8	< .001	30.5	< .001
G × N	1	0.3	.628	0.7	.418	0.0	.877	0.1	.801
G × S	2	0.2	.823	0.6	.557	2.1	.128	0.9	.425
N × S	2	0.1	.878	1.5	.234	4.3	.010	0.4	.681
G × N × S	2	0.7	.532	0.1	.907	1.7	.186	0.3	.726
C	11	13.0	< .001	3.5	< .001	5.0	< .001	6.7	< .001
C × N	11	2.5	.006	1.0	.440	2.2	.016	0.4	.949
C × S	22	1.7	.020	0.4	.994	1.0	.426	1.0	.494
C × N × S	22	2.4	< .001	0.6	.943	1.4	.140	0.5	.980

In source of variation, G: genus (hybrid poplars vs willows), N: nitrogen, S: spacing, and C: clone. Significant p values are indicated in bold.

Table 3. Mean height (cm) for each *Populus* and *Salix* clone under the three spacings (20, 35, and 60 cm) and two N levels (20 and 200 $\mu\text{g g}^{-1}$) after 13 weeks of growth.

Clone	Spacing (cm)–Nitrogen ($\mu\text{g g}^{-1}$)					
	20–20	20–200	35–20	35–200	60–20	60–200
Height (cm)						
1081	88.4±11.9 ^a	87.2±9.1 ^a	88.8±12.9 ^a	99.2±12.0 ^a	76.0±25.5 ^a	96.3±24.1 ^a
2782	123.0±12.2 ^a	140.9±13.6 ^a	117.6±8.9 ^a	152.0±9.5 ^a	152.3±27.0 ^a	159.0±27.5 ^a
747210	66.0±7.1 ^a	100.8±7.4 ^a	105.8±14.4 ^a	109.4±10.7 ^a	77.7±9.3 ^a	94.0±9.5 ^a
915319	85.5±7.5 ^{ab}	100.6±5.3 ^b	73.1±9.5 ^{ab}	114.4±4.9 ^b	62.3±8.9 ^a	107.7±17.1 ^b
DN-74	77.4±13.1 ^a	129.7±8.8 ^{bc}	98.7±16.1 ^a	156.9±3.5 ^c	102.7±28.4 ^{ab}	162.0±35.5 ^c
NM06	106.3±9.8 ^a	138.4±11.6 ^{ab}	101.0±14.4 ^a	159.6±8.8 ^b	129.3±36.4 ^{ab}	146.3±5.4 ^b
Walker	74.0±7.0 ^a	114.5±5.1 ^{bc}	66.6±14.6 ^a	157.4±13.2 ^c	100.0±5.0 ^{ab}	146.0±26.0 ^{bc}
HAN-A5	93.5±12.6 ^a	125.1±7.6 ^{ab}	124.1±6.4 ^{ab}	126.0±9.8 ^b	88.7±35.1 ^a	162.0±10.6 ^b
LEV-D5	49.2±5.3 ^a	87.3±15.5 ^{bc}	61.6±9.3 ^{ab}	94.2±12.5 ^c	67.8±9.9 ^{ab}	114.0±25.2 ^c
SHE-E9	109.0±8.9 ^a	116.3±14.0 ^a	125.7±8.5 ^a	137.1±12.6 ^a	145.4±17.5 ^a	145.8±5.9 ^a
LAF-I4	73.7±5.1 ^{ab}	110.8±11.1 ^b	88.4±9.2 ^b	117.9±16.8 ^b	43.8±0.9 ^a	100.6±10.6 ^b
TCH-N4	85.9±12.8 ^a	111.6±12.9 ^{ab}	89.6±10.2 ^{ab}	111.9±5.4 ^b	108.0±29.3 ^{ab}	125.2±4.1 ^b

1. Within a row, means followed by the same letter do not significantly differ at $\alpha = 0.05$ and 2. Percentage of clones is indicated at Table 1.

**Figure 1.** Mean leafless aboveground biomass per tree (AGBT) for each clone after 13 weeks of growth.**Figure 2.** Mean tree leaf area (LA) for all clones combined under three spacings (20, 35, and 60 cm) and two N levels (20 and 200 $\mu\text{g g}^{-1}$) after 13 weeks of growth. Means followed by the same letter on the bar do not significantly differ at $\alpha = 0.05$.

238.8 ± 2.9 $\text{cm}^2 \text{g}^{-1}$ at 20 $\mu\text{g g}^{-1}$ N to 273.1 ± 3.6 $\text{cm}^2 \text{g}^{-1}$ at 200 $\mu\text{g g}^{-1}$ N (Table 4). SLA significantly varied with crown section and spacing (Tables 4 and 5). SLA increased in the 20 cm spacing from the top to bottom crown sections, was the lowest in the middle crown section in the 35 cm spacing, and showed no significant difference in the crowns of the 60 cm spacing. These results explain the significant $S \times \text{CWS}$ interaction (Tables 4 and 5). *Populus* clones had greater SLA compared with *Salix* clones (Tables 4 and 6). Despite the $S \times \text{C}$ interaction, the decrease in spacing from 60 to 20 cm increased SLA similarly between more productive clones 2782, NM06, HAN-A5, SHE-E9, TCH-N4 (14%) and less productive clones 747,210, 915,319, LEV-D5 and LAF-I4 (13%) (Table 7).

Leaf N content per unit leaf area (N_{area}) decreased by 28% from the top to bottom crown section under the low N level and decreased by 34% under the high N level ($N \times \text{CWS}$ interaction, Figure 4(a), Table 4). N_{area} also decreased from the top to the bottom crown section by 37 and 30% in the 20 and 35 cm spacings, respectively, and showed no significant changes from the middle to bottom crown sections in the 60 cm spacing ($S \times \text{CWS}$ interaction, Tables 4 and 5). At the genus level, *Populus* showed 26 and 11% lower N_{area} in the 20 and 35 cm spacings, respectively, compared with *Salix*, while the two genera had similar values at the 60 cm spacing ($G \times S$ interaction, Figure 4(b), Table 4).

Crown light environment and photosynthesis

In the 20 and 35 cm spacings, there were significant decreases in PAR by 71% and 50%, respectively, from the top to the bottom crown sections, while there was no significant change in PAR between the middle and

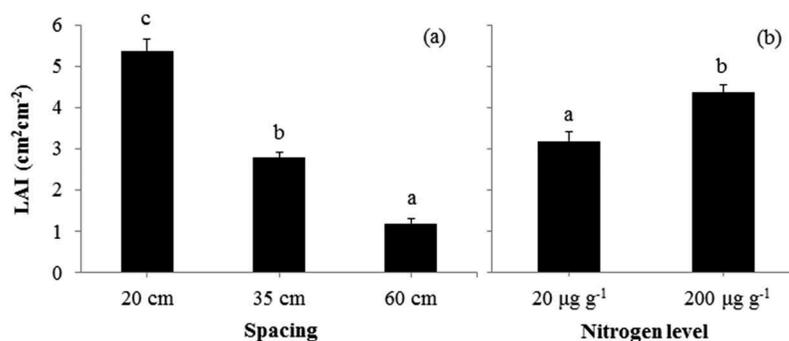


Figure 3. Mean leaf area index (LAI) for all clones combined under three spacings (20, 35, and 60 cm) (a) and two N levels (20 and 200 $\mu\text{g g}^{-1}$) (b) after 13 weeks of growth. Means followed by the same letter on the bar do not significantly differ at $\alpha = 0.05$.

Table 4. Analysis of variance showing sources of variation, degrees of freedom (DF), F values, and associated probabilities (p) for specific leaf area (SLA), N content per unit leaf area (N_{area}), photosynthetically active radiation (PAR), net CO_2 assimilation rate (A), and photosynthetic N-use efficiency under ambient light conditions (PNUE_{amb}) at the genus and clonal levels.

Source of variation	DF	SLA		N_{area}		PAR		A		PNUE_{amb}	
		F	p	F	p	F	p	F	p	F	p
G	1	15.1	<.001	61.5	<.001	0.1	.970	5.1	.030	1.8	.780
N	1	49.5	<.001	53.5	<.001	1.9	.172	0.8	.369	18.8	<.001
S	2	14.2	<.001	33.3	<.001	6.3	.003	1.7	.197	0.1	.906
CWS	2	8.4	<.001	117.0	<.001	126.9	<.001	36.5	<.001	3.8	.020
G × N	1	0.4	.555	2.3	.136	0.6	.440	4.6	.040	0.4	.557
G × S	2	2.5	.090	5.8	.005	1.6	.211	0.3	.722	0.1	.898
G × CWS	2	0.3	.722	2.6	.080	0.1	.950	0.5	.627	0.4	.670
N × S	2	0.2	.795	0.5	.604	1.3	.284	1.1	.330	0.4	.661
N × CWS	2	0.0	.991	4.0	.020	1.7	.189	2.1	.124	0.2	.830
S × CWS	4	6.0	<.001	6.0	<.001	10.3	<.001	2.3	.061	1.7	.143
G × N × S	2	0.2	.792	0.2	.827	0.1	.878	0.6	.531	1.7	.195
G × N × CWS	2	0.4	.676	1.3	.275	0.1	.980	0.1	.962	0.1	.942
G × S × CWS	4	1.1	.338	1.0	.397	0.6	.641	0.6	.653	0.4	.821
N × S × CWS	4	0.5	.758	1.3	2.662	0.9	.497	0.6	.645	0.4	.892
G × N × S × CWS	4	0.3	.854	0.6	.683	0.2	.930	0.5	.770	1.0	.396
C	11	23.6	<.001	25.1	<.001	0.9	.574	3.7	<.001	11.0	<.001
C × N	11	1.4	.168	1.5	.235	0.3	.985	1.7	.095	1.1	.346
C × S	22	1.7	.030	2.0	.006	1.3	.232	1.3	.229	2.4	.018
C × CWS	22	1.6	.045	2.3	.036	1.3	.200	0.9	.645	0.8	.641
C × N × S	22	0.9	.681	0.5	.984	0.5	.953	0.9	.575	1.4	.217
C × N × CWS	22	0.9	.635	0.3	.999	0.4	.990	0.4	.996	0.5	.835
C × S × CWS	44	0.8	.780	0.4	.999	1.0	.550	0.7	.946	0.8	.726
C × N × S × CWS	44	0.9	.741	0.4	.999	0.6	.960	0.6	.952	0.5	.967

In source of variation, G: genus (hybrid poplars vs willows), N: nitrogen, S: spacing, CWS: crown section, and C: clone. Significant p values are indicated in bold.

bottom crown sections in the 60 cm spacing (S × CWS interaction, Tables 4 and 5).

Net CO_2 assimilation rate (A) decreased by 36% from the top to the bottom crown sections (Figure 5(a), Table 4). At the genus level, A for *Salix* was significantly greater than *Populus* (Tables 4 and 6). The increase in N level significantly decreased A by 22% for *Salix* but showed no change for *Populus* clones (G × N interaction, Figure 5(b), Table 4). All the clones showed no significant differences in A from the 60 to 20 cm spacings despite the significant decrease in PAR (Tables 4 and 5). The largest significant difference in mean A between *Populus* and *Salix* clones occurred between clones 747,210 ($2.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) and LAF-I4 ($8.6 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Tables 4 and 6).

Photosynthetic capacity

Mean photosynthetic N-use efficiency under ambient light conditions (PNUE_{amb}) for all clones significantly decreased by 15% from the top to the bottom crown section (Figure 6 (a)). The increase in N level significantly decreased PNUE_{amb} by 34% (Figure 6(b)). PNUE_{amb} also varied depending on clone and spacing (C × S interaction, Tables 4 and 8). However, for all clones, there were no significant differences in PNUE_{amb} between the 20 and 60 cm spacings (Table 8). Differences between the 35 and the 20 spacing and between the 35 and the 60 cm spacing were obtained only for the hybrid aspen clone 2782 and HAN-A5.

Table 5. Mean specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), N content per unit leaf area (N_{area} , g m^{-2}), and photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$) for all clones at each crown section under three spacings (20, 35, and 60 cm) after 13 weeks of growth.

Variable	Crown section	Spacing		
		20 cm	35 cm	60 cm
SLA	Top	255.0±5.9 ^b	259.5±7.2 ^b	248.6±10.4 ^a
	Middle	257.2±6.0 ^b	242.2±4.5 ^a	223.2±8.3 ^a
	Bottom	280.3±7.2 ^c	258.7±6.1 ^b	230.7±10.6 ^a
N_{area}	Top	1.1±0.03 ^c	1.3±0.03 ^d	1.3±0.06 ^d
	Middle	0.9±0.02 ^b	1.1±0.02 ^c	1.2±0.04 ^c
	Bottom	0.7±0.02 ^a	0.9±0.02 ^b	1.1±0.04 ^c
PAR	Top	354.6±34.6 ^d	329.8±32.8 ^d	302.3±28.4 ^d
	Middle	200.9±25.9 ^c	207.9±28.2 ^c	205.7±22.0 ^{bc}
	Bottom	102.9±21.1 ^a	148.2±21.0 ^b	179.0±21.4 ^b

For each variable, means followed by the same letter do not significantly differ at $\alpha = 0.05$.

Table 6. Mean specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), N content per unit leaf area (N_{area} , g m^{-2}), net CO₂ assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), and photosynthetic N-use efficiency under ambient light condition (PNUE_{amb} , $\mu\text{mol CO}_2 \text{s}^{-1} \text{g}^{-1} \text{N}$) for each clone for all spacings (20, 35, and 60 cm) and N levels (20 and 200 $\mu\text{g g}^{-1}$) combined after 13 weeks of growth.

Clone	Variable			
	SLA	N_{area}	A	PNUE_{amb}
<i>Populus</i>				
1081	318.9±11.8	0.8±0.04	5.5±0.6	7.5±0.9
2782	275.5±5.6	0.9±0.03	5.1±0.5	6.5±0.6
747210	218.3±5.0	1.0±0.04	2.3±0.4	2.5±0.5
915319	240.4±7.5	1.0±0.04	4.7±0.4	5.4±0.6
DN-74	329.0±8.9	1.0±0.03	3.2±0.4	3.5±0.5
NM06	263.0±7.2	1.0±0.03	3.6±0.6	5.0±1.0
Walker	303.6±8.3	0.9±0.03	3.6±0.5	4.4±0.6
Mean	278.4±3.3	0.9±0.01	4.0±0.2	4.8±0.3
<i>Salix</i>				
HAN-A5	266.4±5.4	1.1±0.03	4.6±0.6	4.1±0.6
LEV-D5	195.3±9.4	1.2±0.03	5.8±0.6	5.0±0.5
SHE-E9	238.3±5.8	1.0±0.03	3.8±0.3	4.4±0.4
LAF-I4	169.8±3.0	1.3±0.04	8.6±0.7	6.4±0.6
TCH-N4	275.0±5.6	1.0±0.03	4.9±0.4	5.9±0.5
Mean	246.7±3.1	1.1±0.02	5.0±0.3	5.0±0.2

Parentage of clones is indicated at Table 1.

For any PAR in the ambient light conditions, A for both *Populus* and *Salix* clones were higher in the middle crown section compared with the bottom and top crown sections (Figure 7(a,b)). Under any given PAR, PNUE_{amb} in the bottom crown section for *Populus* and *Salix* clones was relatively higher than the top and middle crown sections (Figure 7(c,d)). *Populus* and *Salix* clones showed different acclimations to the incoming PAR; PNUE_{amb} for *Salix* reached a plateau at 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR for all crown sections (Figure 7(d)) while PNUE_{amb} for *Populus* did not level off in the measured ambient light conditions (Figure 7(c)). *Populus* and *Salix* clones also showed different A - N_{area} relationships, as A was positively related to N_{area} for *Salix* ($p < .05$) (Figure 8), but not *Populus* ($p < .05$) (not shown).

More productive vs less productive clones

Compared with the less productive clones 747,210, 915,319, LEV-D5 and LAF-I4, the more productive clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 had 153%, 263%, 136%, and 28% greater AGBT under the low N level, leafless AGBM, and SLA, respectively (Table 9). However, A for more productive clones was 20% lower than less productive clones (Table 9). Leaf area and AGBT for the more and less productive clones were positively associated with N_{area} ($p < .05$) (Figure 9(a,b)). Compared with less productive clones, more productive clones had greater AGBT and leaf area per unit increase in N_{area} ($p < .05$) (Figure 9(a,b)).

Discussion

Characteristics of more productive clones

It is desirable to identify clones that can grow well under intensive plant competition and low nitrogen (N) level so that these clones can maximize biomass production per unit area with reduced fertilization and associated silviculture costs. In this study, more productive clones had 28% greater SLA under all treatments relative to less productive clones (Table 9). This indicated that more productive clones had a greater LA development capacity relative to leaf mass under high plant density when leaf N content per unit leaf area (N_{area}) was significantly reduced (Table 5). As a consequence, more productive clones had greater LA per unit increase in N_{area} and, subsequently, leafless AGBT per unit increase in N_{area} , compared with the less productive clones (Figure 9(a,b)). More productive clones thus had a greater N-use efficiency (NUE) for photosynthetic area gain and for biomass production, which resulted in 263% greater AGBT for more productive clones under the low N level compared with less productive clones (Table 9). However, we conclude that greater productivity of clones was not due to the photosynthetic capacity of individual leaves under ambient light conditions. First, despite a relationship between net CO₂ assimilation rate (A) and N_{area} for *Salix* spp., there were no relationships between A and AGBT and between photosynthetic N-use efficiency in the ambient light condition (PNUE_{amb}) and AGBT between any other clones (Data not shown). Second, there were no significant differences in PNUE_{amb} between more productive and less productive clones (Table 9). Lastly, A for more productive clones was 20% lower than less productive clones (Table 9).

Table 7. Mean specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$) and leaf N content per unit leaf area (N_{area} , g m^{-2}) for each *Populus* and *Salix* clone under the three spacings (20, 35, and 60 cm) and three crown sections (bottom, middle, and top) after 13 weeks of growth.

Clone	Spacing			Crown section		
	20 cm	35 cm	60 cm	Bottom	Middle	Top
SLA						
1081	367.7±22.4 ^b	270.7±9.7 ^a	304.9±18.0 ^a	352.4±24.0 ^b	309.2±19.4 ^{ab}	290.1±14.4 ^a
2782	292.7±11.0 ^c	269.1±5.2 ^b	234.4±12.1 ^a	289.1±12.9 ^b	251.9±7.7 ^a	286.5±7.6 ^b
747210	237.5±8.0 ^b	201.3±6.5 ^a	200.1±8.5 ^a	226.9±10.1 ^a	216.1±8.8 ^a	212.1±6.5 ^a
915319	250.9±6.4 ^b	238.2±15.8 ^b	206.6±11.6 ^a	245.1±18.6 ^a	239.4±8.2 ^a	236.3±8.9 ^a
DN-74	319.9±13.3 ^b	357.1±11.2 ^c	272±31.2 ^a	348.5±16.7 ^a	336±14.5 ^a	306.4±14.8 ^a
NM06	281.3±13.2 ^b	249.5±6.9 ^a	236.7±20.2 ^a	268.3±10.8 ^a	249.7±10.5 ^a	271.5±15.6 ^a
Walker	295.9±14.8 ^a	307.4±8.8 ^a	319.2±29.2 ^a	300.4±11.7 ^b	280.4±10.5 ^a	329.9±18.5 ^b
HAN-A5	257.8±8.5 ^b	286.3±7.6 ^b	234±11.4 ^a	281.3±10.2 ^b	260.9±8.1 ^a	256.1±9.4 ^a
LEV-D5	196±5.4 ^a	195.5±20.6 ^a	192±10.3 ^a	198.8±6.7 ^{ab}	173.2±4.4 ^a	213.5±26.9 ^b
SHE-E9	251.1±9.1 ^b	232.5±8.3 ^b	212±15.5 ^a	234.7±10.4 ^{ab}	229.4±8.8 ^a	250.6±10.7 ^b
LAF-I4	168.7±5.6 ^a	174.6±3.3 ^a	154.9±6.0 ^a	175.1±5.9 ^a	170.3±4.8 ^a	164.1±4.8 ^a
TCH-N4	268.6±8.2 ^a	282.4±8.5 ^a	269.4±16.3 ^a	282.5±10.1 ^a	263.5±9.3 ^a	278.6±9.5 ^a
1081	0.7±0.04 ^a	1.0±0.07 ^b	0.9±0.10 ^a	0.6±0.04 ^a	0.9±0.06 ^b	1.1±0.08 ^c
2782	0.8±0.04 ^a	0.9±0.04 ^{ab}	1.1±0.12 ^b	0.7±0.04 ^a	0.9±0.04 ^b	1.0±0.05 ^c
747210	0.7±0.04 ^a	1.1±0.07 ^b	1.2±0.08 ^b	0.7±0.04 ^a	0.9±0.06 ^b	1.2±0.08 ^c
915319	0.8±0.04 ^a	1.1±0.06 ^b	1.2±0.10 ^b	0.8±0.04 ^a	0.9±0.04 ^b	1.3±0.08 ^c
DN-74	0.8±0.04 ^a	1.1±0.06 ^b	1.2±0.09 ^b	0.8±0.05 ^a	0.9±0.04 ^b	1.2±0.06 ^c
NM06	0.9±0.05 ^a	1.0±0.04 ^a	1.1±0.09 ^a	0.8±0.03 ^a	1.0±0.04 ^b	1.2±0.07 ^c
Walker	0.8±0.04 ^a	1.0±0.05 ^a	1.0±0.08 ^a	0.8±0.04 ^a	1.0±0.05 ^b	1.0±0.06 ^c
HAN-A5	1.1±0.04 ^a	1.2±0.03 ^b	1.2±0.09 ^a	1.0±0.04 ^a	1.1±0.04 ^b	1.3±0.05 ^c
LEV-D5	1.1±0.03 ^a	1.3±0.06 ^{ab}	1.2±0.06 ^{ab}	1.1±0.04 ^a	1.2±0.04 ^b	1.3±0.08 ^b
SHE-E9	0.9±0.04 ^a	1.0±0.05 ^{ab}	1.2±0.07 ^b	0.9±0.05 ^a	0.9±0.04 ^a	1.1±0.06 ^b
LAF-I4	1.3±0.06 ^a	1.3±0.05 ^a	1.6±0.09 ^a	1.0±0.09 ^a	1.3±0.04 ^b	1.7±0.07 ^c
TCH-N4	0.9±0.04 ^a	1.0±0.05 ^{ab}	1.2±0.09 ^b	0.8±0.04 ^a	1.0±0.05 ^b	1.2±0.06 ^c

1. Within a row, means followed by the same letter do not significantly differ at $\alpha = 0.05$ and 2. Parentage of clones is indicated at Table 1.

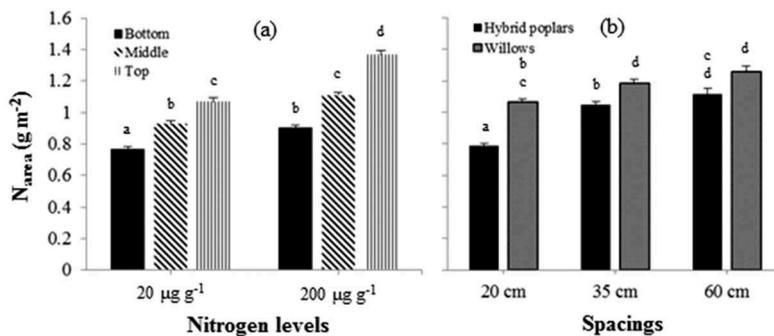


Figure 4. Mean nitrogen concentration (N_{area}) for each N levels (20 and $200 \mu\text{g g}^{-1}$) for crown section (bottom, middle, and top) (a) and three spacings (20, 35, and 60 cm) for *Populus* and *Salix* (b) after 13 weeks of growth. In each figure, means followed by the same letter do not significantly differ at $\alpha = 0.05$.

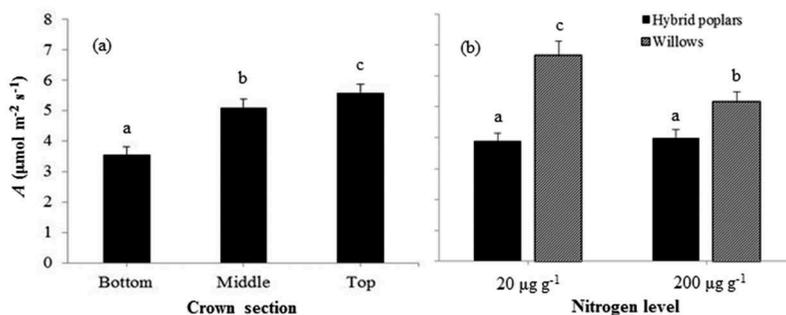


Figure 5. Mean net CO_2 assimilation rate (A) under three crown sections (bottom, middle, and top) for all clones (a) and under two N levels (20 and $200 \mu\text{g g}^{-1}$) comparing *Populus* and *Salix* (b) after 13 weeks of growth. Means followed by the same letter do not significantly differ at $\alpha = 0.05$.

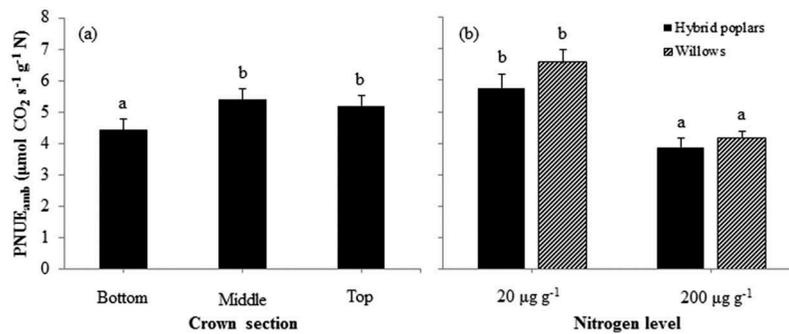


Figure 6. Mean photosynthetic N-use efficiency under ambient light conditions (PNUE_{amb}) for all clones under three crown sections (bottom, middle, and top) (a) and for *Populus* and *Salix* under two N levels (20 and 200 µg g⁻¹) (b) after 13 weeks of growth. Means followed by the same letter do not significantly differ at $\alpha = 0.05$.

Table 8. Mean photosynthetic N-use efficiency under ambient light condition (PNUE_{amb}, µmol CO₂ s⁻¹ g⁻¹ N) for each *Populus* and *Salix* clone and spacing (20, 35, and 60 cm) after 13 weeks of growth.

Clone	Spacing		
	20 cm	35 cm	60 cm
1081	6.0±1.5 ^a	8.5±1.4 ^a	8.2±2.1 ^a
2782	6.7±1.1 ^b	4.1±0.7 ^a	8.8±1.0 ^b
747210	3.8±1.4 ^a	1.6±0.7 ^a	2.1±0.5 ^a
915319	5.5±1.2 ^a	6.1±1.4 ^a	4.6±0.8 ^a
DN-74	3.7±0.8 ^a	3.7±1.1 ^a	3.1±0.7 ^a
NM06	6.5±2.6 ^a	3.9±1.1 ^a	4.8±1.4 ^a
Walker	4.5±1.2 ^a	4.3±1.3 ^a	4.5±0.9 ^a
HAN-A5	5.5±1.3 ^b	2.4±0.5 ^a	4.4±0.7 ^b
LEV-D5	4.6±0.9 ^a	6.2±1.0 ^a	4.3±0.5 ^a
SHE-E9	5.3±0.7 ^a	3.8±0.6 ^a	4.2±0.6 ^a
LAF-I4	6.3±1.2 ^a	6.3±0.7 ^a	6.6±1.1 ^a
TCH-N4	6.0±0.9 ^a	5.6±0.7 ^a	6.0±1.1 ^a

1. Within a row, means followed by the same letter do not significantly differ at $\alpha = 0.05$ and 2. Parentage of clones is indicated at Table 1.

Spacing and nitrogen effects

Each clone had different degrees of height response to N addition under different spacings (Table 3); however, there was no spacing effect on height for clones overall ($P = 0.471$) (Table 2). In addition, there were no significant clone \times nitrogen \times spacing interactions for AGBT, LA, SLA, N_{area}, A, and PNUE_{amb}, suggesting similar productive and photosynthetic acclimation to different degrees of plant density and N availability among clones (Tables 2 and 4). Specifically, the plants appeared to acclimate well to intensive competition with more limited resource availability because there was no spacing effect on AGBT for clone (Table 2). The greater growth capacity in close spacings for all clones was primarily explained by greater LA expansion capacity and photosynthetic acclimation under competitive conditions as follows: (1) LA per unit of given spacing ground (LAI) increased from 60 to 20 cm spacing by 347% with an increase in SLA by 13% (Figure 3, Table 5) and (2) shade leaves utilized N

more efficiently for greater A compared with sun leaves for the both *Populus* and *Salix* (Figure 7(c,d)).

There were higher PAR, thicker leaves (lower SLA) and higher N_{area} from the upper canopy or in the largest spacing (Table 5), probably due in part to greater N allocation needed in the extra layers of palisade or longer palisade cells (Evans and Poorter 2001). The development of palisade cells increases the number of chloroplasts and the quantity of photosynthetic enzymes (Evans and Poorter 2001) and thereby enhances photosynthetic capacity. In this study, A significantly increased from the bottom to the top crown section with greater N_{area} and increasing PAR (Figure 5(a), Table 5). However, in spite of the pronounced decrease in PAR in the bottom crown section of the 20 cm spacing, shade leaves utilized N more efficiently for greater A under given PAR compared with sun leaves for both *Populus* and *Salix* (Figure 7(c, d)). This photosynthetic acclimation under low PAR that resulted from thin leaves with higher SLA and lower N_{area} likely allowed trees to increase LAI by 347% from the 60 to 20 cm spacings with no major loss of shade leaves in this study (Figure 3, Table 2). Higher SLA and lower N_{area} may have induced a greater proportion of N allocated to photosynthetically derived compounds such as Rubisco (Hikosaka and Terashima 1995), thereby increasing photosynthetic capacity (Poorter and Evans 1998; Evans and Poorter 2001). It is widely recognized that shade or high-SLA leaves have greater photosynthetic N-use efficiency at either ambient light or saturating light conditions (Poorter and Evans 1998; Evans and Poorter 2001). These results show that the shade leaves partially offset the negative impact of competition on A by maximizing PAR and NUE for A. Consequently, there was no significant difference in A under the decrease in spacing.

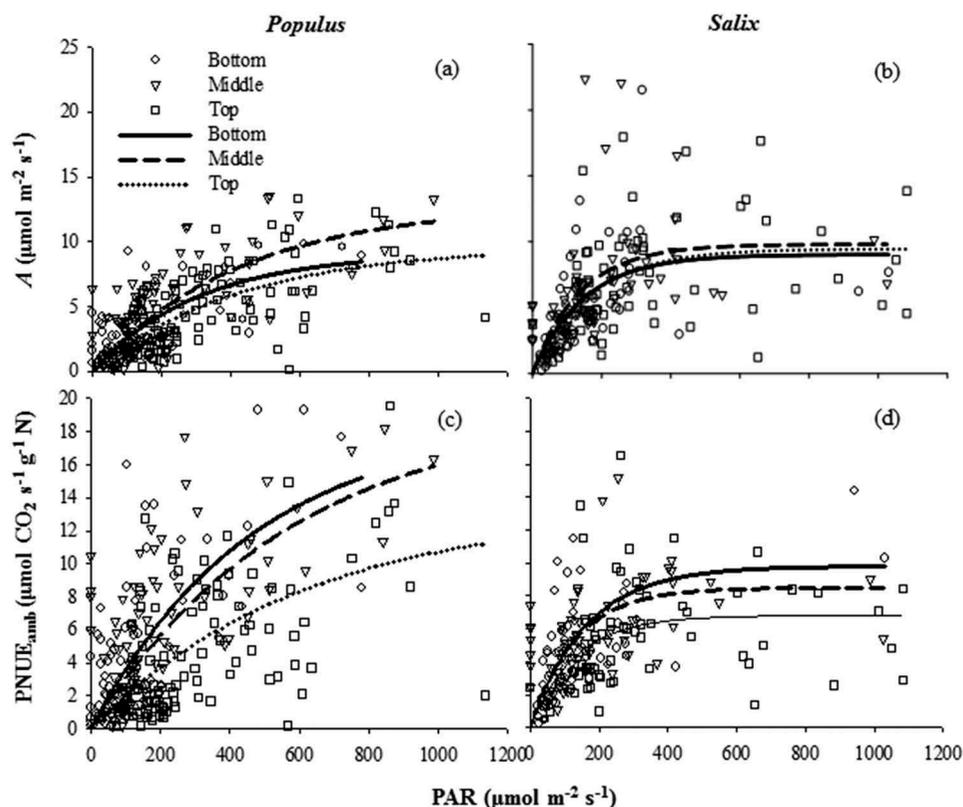


Figure 7. Net CO₂ assimilation rate (*A*)–photosynthetically active radiation (PAR) response curve for the three crown sections (bottom, middle, and top) for (a) *Populus* and (b) *Salix* and photosynthetic N-use efficiency under ambient light conditions (PNUE_{amb})–PAR response curve under three crown sections for (c) *Populus* and (d) *Salix* after 13 weeks of growth. $p < .001$ and $R^2 > 0.2$ for all regressions.

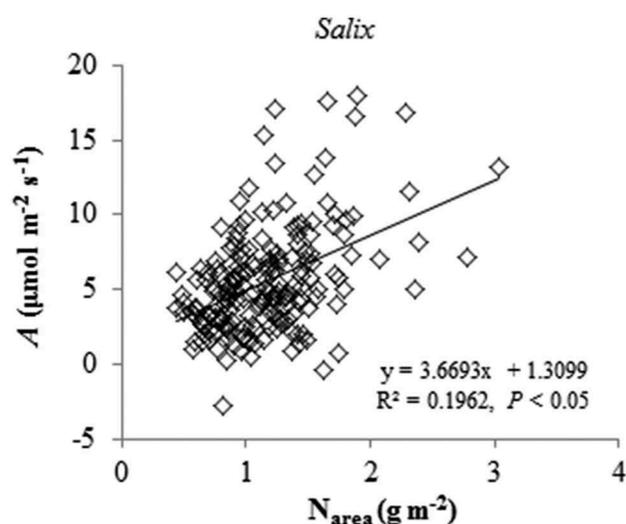


Figure 8. Relationships between net CO₂ assimilation rate (*A*) and nitrogen concentration (N_{area}) for *Salix* after 13 weeks of growth ($p < .05$). No significant relationships for hybrid poplars.

The addition of N significantly increased N_{area} in each crown section (Figure 4(a)), which was consistent with other studies as a great portion of N may have been distributed to leaves, particularly Rubisco, a

Table 9. Mean leafless aboveground biomass per tree (AGBT), AGBT under low N (Low N AGBT), leafless aboveground biomass per m² (AGBM), specific leaf area (SLA), net CO₂ assimilation rate (*A*), and photosynthetic N-use efficiency in the ambient light condition (PNUE_{amb}) of more productive clones (2782, NM06, HAN-A5, SHE-E9, and TCH-N4) compared with less productive clones (747,210, 915,319, LEV-D5, and LAF-I4) as a group after 13 weeks of growth.

Variable	Superior clones	Inferior clones
AGBT (g)	19.7 ± 1.1 ^b	7.8 ± 0.5 ^a
Low N AGBT (g)	17.4 ± 1.5 ^b	4.8 ± 0.5 ^a
AGBM (g m ⁻²)	259.0 ± 18.2 ^b	110.0 ± 9.0 ^a
SLA (cm ² g ⁻¹)	263.6 ± 2.7 ^b	205.3 ± 3.6 ^a
<i>A</i> (μmol m ⁻² s ⁻¹)	4.4 ± 0.2 ^a	5.5 ± 0.3 ^b
PNUE _{amb} (μmol CO ₂ s ⁻¹ g ⁻¹ N)	5.2 ± 0.3 ^a	4.9 ± 0.3 ^a

Within a row, means followed by the same letter do not significantly differ at $\alpha = 0.05$.

critical leaf protein for photosynthesis (Rosati et al. 2000; Clearwater and Meinzer 2001; Dickmann et al. 2001). As the increase in N_{area} was significantly related to LA expansion in this study (Figure 9(a)), increased LA under high N level caused 9% lower PAR compared with low N level, which resulted in 14% greater SLA. This result was similar to previous findings to the effect that the change in SLA was related to both canopy light

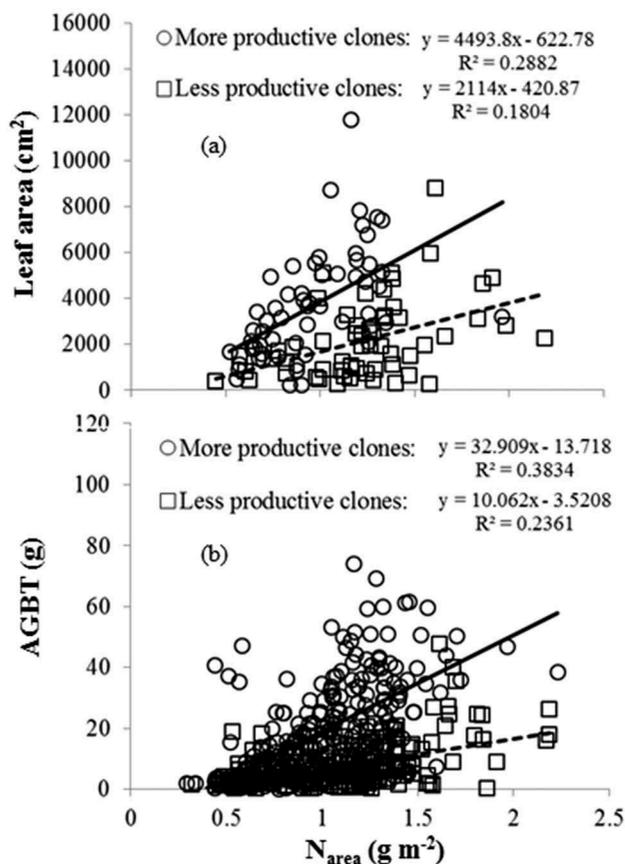


Figure 9. Relationship between (a) leaf area and (b) leafless aboveground biomass per tree (AGBT) vs leaf N concentration (N_{area}) for more productive clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 and less productive clones 747,210, 915,319, LEV-D5, and LAF-I4 after 13 weeks of growth ($p < .05$).

gradients and nitrogen fertilization (Knops and Reinhart 2000; Wang et al. 2012). Therefore, SLA could be a useful indicator to select more productive clones under high plant density, as SLA represents the competitive status of clones. In this study, although SLA was 20% greater for less productive clones from 20 to 200 $\mu\text{g g}^{-1}$ relative to 8% for more productive clones, more productive clones had 22% greater SLA under high N level relative to less productive clones (Data not shown). Interestingly, despite the decrease in PAR from the 60 to 20 cm spacings, the addition of N did not produce greater SLA in the 20 cm spacing compared with the 60 cm spacing (non-significant $N \times S$; Table 1). This was because the decrease in spacing also decreased N_{area} (Tables 4 and 5), which likely limited further development of LA.

The decrease in A with the addition of N for *Salix* likely resulted from an increased degree of self-shading (Figure 5(b)). In fact, A patterns paralleled the light environment where *Salix* clones reduced PAR by 10% and *Populus* by only 2% following increased N level

(Data not shown). This result did not support our hypothesis that the addition of N would increase A under intensive competition. The increased amount of N_{area} under the high N level may have overinvested into chlorophyll production that was not efficiently used under the available PAR (Boadman 1977). Therefore, the benefit of a higher amount of Rubisco in photosynthesis under low irradiance may be partially lessened by a lower Rubisco activity state in high-N leaves (Cheng and Fuchigami 2000; Ray et al. 2003).

Characteristics of *Populus* and *Salix* clones

Populus and *Salix* clones also showed different degrees of shade tolerance under tree competition. *Salix* exhibited greater A approximately up to 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR in the bottom crown section while *Populus* did not reach the light saturation point under ambient light conditions (Figure 7(a,b)). The narrower leaves of *Salix* may reduce light interception in the lower canopy by reducing the degree of leaf aggregation compared with the deltoid-shaped leaves for *Populus* (Percy and Yang 1998; Takenaka et al. 2001; Casella and Sinoquet 2007). In this study, *Salix* had greater ratios of leaf length to leaf width and relatively greater PAR (132 $\mu\text{mol m}^{-2} \text{s}^{-1}$) compared with *Populus* (110 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in the bottom crown sections (Data not shown). In fact, narrow leaves can increase light harvesting during the establishment year (Niinemets 1998; Casella and Sinoquet 2007), indicating perhaps a beneficial impact on photosynthetic capacity under intensive competition. Under ambient light conditions, PNUE_{amb} was greater for *Salix* at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR compared with *Populus* (Figure 7(c,d)). However, *Populus* had greater PNUE_{amb} after 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (Figure 7(c,d)), indicating greater efficiency of *Populus* under higher PAR.

Salix clones also had a greater amount of leaf N within the canopy compared with *Populus* clones (Figure 4(b)). Greater N_{area} for *Salix* may be due to greater nitrogen uptake capacity in relation to root morphology (Al Afas et al. 2008) or distribution of root biomass (Johnson and Biondini 2001). Curiously, although N is an important nutrient for photosynthesis, the significant relationship between A and N_{area} observed for *Salix* was not found for *Populus* clones (Figure 8). This was probably because the relationship of A to leaf N is highly variable depending on species (Peterson et al. 1999). *Salix* may have allocated a greater proportion of N to photosynthetic functions compared to *Populus* or the activation state of Rubisco differs among species (Poorter and Evans

1998). The activity of Rubisco for *Salix* may have responded more efficiently to leaf N changes.

Salix clones LEV-D5 and LAF-I4 exhibited the highest mean N_{area} and relatively greater A compared with the other clones (Table 6). Greater A of these clones could have been the result of compensatory photosynthesis after aphid feeding, as aphids caused a loss of foliage in this study, which enhanced light penetration within the crown (Way and Cammell 1970; Collins et al. 2001). Also, a large loss of leaves for these clones may have caused an increase in N_{area} in the remaining leaves as a result of compensatory photosynthesis (Collins et al. 2001).

Differences in leaf structure between greenhouse and field conditions

Compared with a field study using the same *Populus* clones 747,210 and 915,319 (Benomar et al. 2011), this greenhouse study showed greater values of SLA, 200–251 $\text{cm}^2 \text{g}^{-1}$, compared with 69–142 $\text{cm}^2 \text{g}^{-1}$ in the field. We also had lower N_{area} 0.7–1.3 g m^{-2} values compared with 1.1–3.4 g m^{-2} in the field (Benomar et al. 2011). Although the field study used wider spacings of $1 \times 1\text{--}5 \times 5 \text{ m}$, the measurements were taken 5 years after plantation establishment when trees were under fairly competitive conditions (Benomar et al. 2011). Since greenhouse light conditions may represent 60–80% of field light conditions (Sharpe et al. 1999; Keyhaninejad et al. 2012), the higher SLA and lower N_{area} may be primarily due to much lower light levels in the greenhouse. In this study, the highest PAR measured was 1082 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Data not shown). However, in the open field, PAR often reaches up to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in North America (Fredericksen et al. 1996; Cadenasso et al. 1997).

Applicability to long-term field performance?

Despite the short-term period (13-growing weeks) in this study, all clones experienced intensive competition between trees: the 20 and 35 cm spacings mostly showed 90–100% canopy closure and 60 cm spacing was 60–90% at the end of growing period according to visual estimates (Data not shown). The high degree of intensive competition between trees is also common in the SRF studies at the end of a growing period (Fang et al. 1999; Proe et al. 2002; Benomar et al. 2011). Therefore, physiological and morphological characteristics of more productive clones assessed under intensive competition in this study would be applicable to understand long-term characteristics of more productive clones; however, field study remains necessary to

investigate the clonal adaptability to local site and climate. In fact, the Ministère des Ressources naturelles et de la Faune du Québec has been achieving early selection of more productive clones by using desirable morpho-physiological traits, instead of applying conventional breeding programmes that would require more silviculture costs and time in the field (Lamhamedi et al. 2007).

Conclusion

Results for both biomass productivity and photosynthetic acclimation to changes in nitrogen (N) and spacing density among *Populus* and *Salix* clones were similar. Despite decreased N content per unit leaf area (N_{area}) by 31% from 60 to 20 cm spacing, *Populus* and *Salix* clones had increases in LAI by up to 347% and SLA by up to 13% and no changes in net CO_2 assimilation rate (A) and photosynthetic N-use efficiency under ambient light condition (PNUE_{amb}). Shade leaves also utilized N more efficiently with greater A compared with sun leaves under a given level of PAR. This greater LA-expansion and photosynthetic capacity under low irradiance in a higher density planting led to no spacing effect on leafless AGBT for all clones. The addition of N alleviated competition effects under decreased spacing by maximizing LA and SLA. More productive clones had 28% greater SLA, significantly greater LA per unit of N_{area} , and AGBT per unit of N_{area} under all treatments. However, compared with less productive clones, more productive clones showed no significant difference in PNUE_{amb} and even had 20% lower A with the decrease in spacing, indicating that greater productivity of clones was likely owing to greater LA production capacity and not to the photosynthetic capacity of individual leaves under ambient light conditions. *Salix* clones had greater PNUE_{amb} under low PAR ($< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$), and A and N_{area} than *Populus* clones, suggesting a greater photosynthetic capacity of *Salix* under higher plant density when canopy closure was accelerated.

Acknowledgements

The authors would like to express their appreciation for contributions of research support received from the following organizations: Institut de Recherche sur les Forêts, Université du Québec en Abitibi-Témiscamingue, Jardin botanique de Montréal, Alberta-Pacific Forest Industries Inc., Canadian Wood Fibre Centre, Natural Resources Canada, and Canadian Forest Service, Laurentian Forestry Centre.

Disclosure statement

Mr. Takamitsu Mamashita received research funding from Natural Resources Canada, which is investigating forestry products related to the research described in this paper. The author served as a research assistant to Natural Resources Canada and received compensation for these services. The terms of this arrangement have been reviewed and approved by Natural Resources Canada in accordance with its conflicts of interest policies.

Funding

This study was funded by the Program of Energy Research and Development (PERD) of Natural Resources Canada under Grant [PERD number I11.009].

ORCID

Takamitsu Mamashita  <http://orcid.org/0000-0002-0100-7672>

References

- Al Afas N, Marron N, Zavalloni C, Ceulemans R. 2008. Growth and production of a short-rotation coppice culture of poplar—IV: fine root characteristics of five poplar clones. *Biomass Bioenerg.* 32:494–502.
- Benomar L, DesRochers A, Larocque GR. 2011. Changes in specific leaf area and photosynthetic nitrogen-use efficiency associated with physiological acclimation of two hybrid poplar clones to intraclonal competition. *Can J For Res.* 41:1465–1476.
- Benomar L, DesRochers A, Larocque GR. 2012. The effects of spacing on growth, morphology and biomass production and allocation in two hybrid poplar clones growing in the boreal region of Canada. *Trees.* 26:939–949.
- Boadman NK. 1977. Comparative photosynthesis of sun and shade plants. *Annu Rev Plant Physiol.* 28:355–377.
- Cadenasso ML, Traynor MM, Pickett STA. 1997. Functional location of forest edges: gradients of multiple physical factors. *Can J For Res.* 27:774–782.
- Casella E, Sinoquet H. 2007. Botanical determinants of foliage clumping and light interception in two-year-old coppice poplar canopies: assessment from 3-D plant mock-ups. *Ann For Sci.* 64:395–404.
- Cheng L, Fuchigami LH. 2000. Rubisco activation state decreasing with increasing nitrogen content in apple tree. *J Exp Bot.* 51:1687–1694.
- Clearwater MJ, Meinzer FC. 2001. Relationships between hydraulic architecture and leaf photosynthetic capacity in nitrogen-fertilized *Eucalyptus grandis* trees. *Tree Physiol.* 21:683–690.
- Collins CM, Rosado RG, Leather SR. 2001. The impact of the aphids *Tuberolachnus salignus* and *Pterocomma salicis* on willow trees. *Ann Appl Biol.* 138:133–140.
- Cooke JEK, Martin TA, Davis JM. 2005. Short-term physiological and developmental responses to nitrogen availability in hybrid poplar. *New Phytol.* 167:41–52.
- Derbowka DR. 2012. Poplar and willow cultivation and utilization in Canada: 2008–2011 Canadian country progress. Dehradun (India): Poplar Council of Canada.
- Dickmann DI, Isebrands JG, Blake TJ, Kosola K, Kort J. 2001. Physiological ecology of poplars. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. *Poplar culture in North America*. Ottawa: NRC Research Press; p. 77–118.
- Dominy SWJ, Gilsenan R, McKenney DW, Allen DJ, Hatton T, Koven A, Cary J, Yemshanov D, Sidders D. 2010. A retrospective and lessons learned from Natural Resources Canada's Forest 2020 afforestation initiative. *For Chron.* 86:339–347.
- Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia.* 78:9–19.
- Evans JR, Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ.* 24:755–767.
- Fang S, Xu X, Lu S, Tang L. 1999. Growth dynamics and biomass production in short-rotation poplar plantations: 6-year results for three clones at four spacings. *Biomass Bioenerg.* 17:415–425.
- Fredericksen TS, Kolb TE, Skelly JM, Steiner KC, Joyce BJ, Savage JE. 1996. Light environment alters ozone uptake per net photosynthetic rate in black cherry trees. *Tree Physiol.* 16:485–490.
- Hikosaka K, Terashima I. 1995. A model of the acclimation of photosynthesis in the leaves of C₃ plants to sun and shade with respect to nitrogen use. *Plant Cell Environ.* 18:605–618.
- Johnson HA, Biondini ME. 2001. Root morphological plasticity and nitrogen uptake of 59 plant species from the Great Plains grasslands, U.S.A. *Basic Appl Ecol.* 2:127–143.
- Keyhaninejad N, Richins RD, O'Connell MA. 2012. Carotenoid content in field-grown versus greenhouse-grown peppers: different responses in leaf and fruit. *HortScience.* 47:852–855.
- Knops JMH, Reinhart K. 2000. Specific leaf area along a nitrogen fertilization gradient. *Am Midl Nat.* 144:265–272.
- Labrecque M, Teodorescu TI. 2005. Field performance and biomass production of 12 willow and poplar clones in short-rotation coppice in southern Quebec (Canada). *Biomass Bioenerg.* 29:1–9.
- Lamhamedi MS, Renaud M, Desjardins P, Veilleux L. 2007. Early selection and clonal variation of hybrid poplar clones in a Québec forest nursery. Québec City (Canada): Ministère des Ressources naturelles et de la Faune.
- Larocque GR. 1999. Performance and morphological response of the hybrid poplar DN-74 (*Populus deltoides* × *nigra*) under different spacings on a 4-year rotation. *Ann For Sci.* 56:275–287.
- Mamashita T, Larocque GR, DesRochers A, Beaulieu J, Thomas BR, Mosseler A, Major J, Sidders D. 2015. Short-term growth and morphological responses to nitrogen availability and plant density in hybrid poplars and willows. *Biomass Bioenerg.* 81:88–97.
- McKenney DW, Yemshanov D, Fraleigh S, Allen D, Preto F. 2011. An economic assessment of the use of short-rotation coppice woody biomass to heat greenhouses in southern Canada. *Biomass Bioenerg.* 35:374–384.

- Mosseler A, Major JE, Labrecque M. 2014b. Growth and survival of seven native willow species on highly disturbed coal mine sites in eastern Canada. *Can J For Res.* 44:340–349.
- Mosseler A, Major JE, Labrecque M, Larocque GR. 2014a. Allometric relationships in coppice biomass production for two North American willows (*Salix* spp.) across three different sites. *For Ecol Manag.* 320:190–196.
- Niinemets U. 1998. Adjustment of foliage structure and function to a canopy light gradient in two co-existing deciduous trees. Variability in leaf inclination angles in relation to petiole morphology. *Trees.* 12:446–451.
- O'Neill M, Shock C, Lombard K, Heyduck R, Feibert E, Smeal D, Arnold R. 2010. Hybrid poplar (*Populus* ssp.) selections for arid and semi-arid intermountain regions of the western United States. *Agrofor Syst.* 79:409–418.
- Pearcy RW, Yang W. 1998. The functional morphology of light capture and carbon gain in the Redwood forest understorey plant *Adenocaulon bicolor* Hook. *Funct Ecol.* 12:543–552.
- Peterson AG, Ball JT, Luo Y, Field CB, Reich PB, Curtis PS, Griffin KL, Gunderson CA, Norby RJ, Tissue DT, et al. 1999. The photosynthesis — leaf nitrogen relationship at ambient and elevated atmospheric carbon dioxide: a meta-analysis. *Glob Change Biol.* 5:331–346.
- Poorter H, Evans JR. 1998. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia.* 116:26–37.
- Proe MF, Griffiths JH, Craig J. 2002. Effects of spacing, species and coppicing on leaf area, light interception and photosynthesis in short rotation forestry. *Biomass Bioenerg.* 23:315–326.
- Ray D, Scheshshayee ME, Mukhopadhyay K, Bindumadhava H, Prasad TG, Kumar MU. 2003. High nitrogen use efficiency in rice genotypes is associated with higher net photosynthetic rate at lower Rubisco content. *Biol Planta.* 46:251–256.
- Reich PB, Walters MB, Ellsworth DS. 1991. Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area, and photosynthesis in maple and oak trees. *Plant Cell Environ.* 14:251–259.
- Reich PB, Walters MB, Ellsworth DS. 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecol Monogr.* 62:365–392.
- Rosati A, Day KR, Dejong TM. 2000. Distribution of leaf mass per unit area and leaf nitrogen concentration determine partitioning of leaf nitrogen within tree canopies. *Tree Physiol.* 20:271–276.
- Rosati A, Esparza G, Dejong TM, Pearcy RW. 1999. Influence of canopy light environment and nitrogen availability on leaf photosynthetic characteristics and photosynthetic nitrogen-use efficiency of field-grown nectarine trees. *Tree Physiol.* 19:173–180.
- Sharpe WE, Swistock BR, Mecum KA, Demchik MC. 1999. Greenhouse and field growth of northern red oak seedlings inside different types of treeshelters. *J Arboric.* 25:249–257.
- Sheriff DW, Nambiar EKS. 1991. Nitrogen nutrition, growth and gas exchange in *Eucalyptus globulus* Labill. seedlings. *Aust J Plant Physiol.* 18:37–52.
- Stanturf JA, van Oosten C, Netzer DA, Coleman MD. 2001. Ecology and silviculture of poplar plantations. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. *Poplar culture in North America*. Ottawa: NRC Research Press; p. 153–203.
- Takenaka A, Takahashi K, Kohyama T. 2001. Effects of leaf blade narrowness and petiole length on the light capture efficiency of shoots. *Ecol Res.* 9:109–114.
- Thompson WA, Wheeler AM. 1992. Photosynthesis by mature needles of field-grown *Pinus radiata*. *For Ecol Manag.* 52:225–242.
- Truax B, Gagnon D, Fortier J, Lambert F. 2014. Biomass and volume yield in mature hybrid poplar plantations on temperate abandoned farmland. *Forests.* 5:3107–3130.
- Tullus A, Rytter L, Tullus T, Weih M, Tullus H. 2012. Short-rotation forestry with hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) in Northern Europe. *Scand J For Res.* 27:10–29.
- Vile D, Garnier E, Shipley B, Laurent G, Navas ML, Poumet C, Lavorel S, Diaz S, Hodgson JG, Lloret F, et al. 2005. Specific leaf area and dry matter content estimate thickness in laminar leaves. *Ann Bot.* 96:1129–1136.
- Wang D, Maughan MW, Sun J, Feng X, Miguez F, Lee D, Dietze MC. 2012. Impact of nitrogen allocation on growth and photosynthesis of *Miscanthus* (*Miscanthus* × *giganteus*). *Glob Change Biol Bioenerg.* 4:688–697.
- Way MJ, Cammell M. 1970. Aggregation behaviour in relation to food utilization by aphids. In: Watson A, editor. *Animal populations in relation to their food resources*. Aberdeen: British Ecological Society; p. 229–247.
- Weih M. 2004. Intensive short rotation forestry in boreal climates: present and future perspectives. *Can J For Res.* 34:1369–1378.
- Weih M, Rönnerberg-Wästljung AC. 2007. Shoot biomass growth is related to the vertical leaf nitrogen gradient in *Salix* canopies. *Tree Physiol.* 27:1551–1559.
- Yu Q, Tigerstedt PMA, Haapanen M. 2001. Growth and phenology of hybrid aspen clones (*Populus tremula* L. × *Populus tremuloides* Michx.). *Silva Fenn.* 35:15–25.
- Zalesny RS, Donner DM, Coyle DR, Headlee WL. 2012. An approach for siting poplar energy production systems to increase productivity and associated ecosystem services. *For Ecol Manag.* 284:45–58.