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Planting Density and Site Effects on Stem Dimensions, Stand Productivity, Biomass Partitioning, Carbon Stocks and Soil Nutrient Supply in Hybrid Poplar Plantations

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Abstract: In this study, planting density and site effects on hybrid poplar productivity and stem dimensions were evaluated on the mid-term and longer term (8 and 14 years) in southern Québec (Canada). We also evaluated the effects of planting density and site on biomass accumulation and carbon stocks in different plantation compartments, on biomass partitioning at the stand-level, on soil carbon stocks and on soil nutrient supply rate after 14 years. The experimental design consisted of three replicate poplar stands located along a site fertility gradient. Each stand contained six planting densities (ranging from 494 to 1975 trees/ha) and a single genotype (*Populus canadensis* × *P. maximowiczii* hybrid). Planting density had a large effect on stem dimensions, but a minor effect on stand volume, aboveground woody biomass production, and aboveground biomass carbon stocks. Site selection and tree survival were more important factors affecting these variables. At all sites, and independent of planting density, mean annual volume increments were also higher after 14 vs. 8 years. On fertile sites, strong correlations between area per tree at planting and biomass partitioning, carbon allocation belowground, soil nutrient supply rate and soil carbon stocks were observed. Aboveground, higher competition for light with increasing planting density resulted in an increase in the stem to branch ratio. Belowground, higher competition for soil resources with increasing planting density reduced soil macronutrient availability (except for potassium), which likely stimulated carbon allocation belowground and carbon accumulation in the soil. Over the longer-term, higher density plantations of poplars could provide greater benefits in terms of carbon storage belowground (soil and roots).

Keywords: spacing trial; stocking level; carbon sequestration; allocation; ecosystem services; abandoned farmland; *Populus canadensis* × *Populus maximowiczii*; southern Québec (Eastern Canada); short-rotation woody crops; intraspecific competition

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1. Introduction

In temperate ecosystems, fast-growing poplars and their hybrids (*Populus* × spp.) are among the most widely used species for afforestation and agroforestry [1–3]. Poplars are planted for the production of bioenergy, pulp and solid wood products, but also for the provision of other ecosystem services (atmospheric carbon dioxide sequestration, refuge for forest biodiversity, nurse crop for valuable hardwoods, fodder for livestock, soil stabilization, wind protection, soil phytoremediation, flood control) [1,2,4–6]. Thus, poplar afforestation has the potential to address both local and global environmental problems, while providing an opportunity to intensify biomass or wood production.

To reduce competition with food crops, abandoned farmland are increasingly used for poplar afforestation [7,8]. However, marginal agricultural land are often characterized by sub-optimal growth conditions [9], and appropriate management decisions and silvicultural practices are needed to maximize stand productivity. These include intensive site preparation, vegetation management, irrigation, fertilization and the selection of genotypes (clones) adapted to local environmental conditions [10]. Manipulation of tree spacing or planting density (i.e., the number of trees planted per hectare) can increase fast-growing plantation productivity by ensuring rapid and complete site occupancy [11,12]. Maximum site productivity, which generally occurs when mean annual biomass increment peaks in the rotation, is a function of the stocking level [12]. Hence, the selection of planting density has important economic consequences because it influences not only the rotation length, but also plantation establishment costs, weed control strategies, and the dimensions of stems at harvest [12]. Also, ecosystem services such as carbon (C) sequestration in tree biomass is directly linked to stand biomass productivity and may thus be affected by planting density.

Studies involving fast-growing species (*Populus*, *Eucalyptus*, *Pinus*) have shown strong positive effects of wider tree spacing on individual stem diameter, volume and quality [13–17]. For example, DeBell et al. [16] observed that diameter at breast height (DBH) of two hybrid poplar clones ranged 3.2–4.5 cm in the 0.5 × 0.5 m spacing, 6.0–7.7 cm in the 1 × 1 m spacing and 10.8–13.5 m in the 2 × 2 m spacing after 7 years. However, conflicting evidence of linkage between planting density and biomass productivity have been reported in both short-rotation coppices and more widely spaced poplar plantations. In India, 9 year-old *P. deltoides* plantations showed major increase in total biomass production (from 72 to 250 t/ha), with increasing planting density (from 278 to 2500 trees/ha) [18]. Similar observations were reported in the Eastern Canadian boreal zone after 6 growing seasons (3.2 t/ha at 400 trees/ha, 4.9 t/ha at 1111 trees/ha and 29.6 t/ha at 10,000 trees/ha) [19]. In China, C stocks in the total biomass of 10 year-old hybrid poplars increased significantly (from 58 to 72 t C/ha) with increasing planting density (from 500 to 1111 trees/ha) [20]. In England, biomass yields were generally higher in the 10,000 stems/ha plantations compared to 2500 stems/ha plantations, but the significance of the planting density effect varied between sites, clones and cutting cycles [21]. Spacing effect on poplar biomass yield was also found to vary with plantation design (inter-planted design vs. short-rotation coppice alone) [22].

On the other hand, several studies suggest that planting density has only minor and/or temporary effects on the yield of planted poplars. Although biomass production was found to increase exponentially with planting density after the first growing season, planting density (ranging 6666–33,333 cuttings/ha) was no longer a significant factor affecting productivity after 3 years across 12 sites in Spain [23]. In the Pacific Northwest region of the United States, little effect of planting density (2500, 10,000 and 40,000 cuttings/ha) was reported on the biomass productivity after 7 years, despite a trend towards higher productivity in narrower spacings earlier in the rotation [16]. Longer-term studies from southern British Columbia (Canada) and northern Wisconsin (United States) concluded that spacing has only a negligible effect on the productivity of planted poplars [15,24]. In the aforementioned studies, factors such as clone selection, site quality, irrigation, diseases, and weed control had greater effect on yield than stocking level.

Spacing trials also offer an opportunity to evaluate how varying levels of intraspecific competition affect biomass partitioning, C allocation to plantation compartments, soil nutrient status and soil C stocks. In the aboveground compartment, high competition for light in pioneer species tends to enhance the self-pruning process, and trees with proportionally smaller crowns are generally observed in highly stocked stands [25]. Poplars also tend to develop large and deep crowns under high light availability, while small and shallow crowns are typical of crowded stands [19,24,26]. Thus, the ratio of biomass allocated to stems vs. branches is expected to decrease with increasing spacing in planted poplars [19,24,26]. Over the years, root competition (i.e., the reduction in the availability of a soil resource to roots that is caused by other roots [27]) between neighboring trees may also be affected by planting density. More intense root competition in narrow spacings may accelerate soil nutrient depletion, especially in afforested plantations of fast-growing-trees with high nutrient requirements [28,29]. In response to increase competition for soil resources, poplars may enhance C allocation belowground to stimulate root production, root exudation and symbiosis with mycorrhizae [30–33]. Such a change in C allocation may enrich the soil C pool as C inputs derived from roots have been shown to be the major driver of soil carbon sequestration on marginal land afforested with poplars [34]. Over the long-term, increasing planting density in poplar plantations is expected to cause macronutrient depletion in the soil, a decrease in the shoot to root ratio at the stand-level, and an increase in soil C stocks [28–31,33,34]. However, past afforestation studies with fast-growing *Pinus* and *Eucalyptus* species have found little evidence of a planting density effect on soil C [14,35], despite the fact that higher planting density tends to increase total belowground C allocation on the short-term [31].

Additionally, by affecting the time to canopy closure, planting density also has an impact on plantation microclimate. In planted poplars, rapid canopy closure in narrow spacings can lead to a faster decrease of soil and air temperatures during the growing season, which can reduce nitrification and nitrogen mineralization rates [36]. Similarly, narrow spacings may provide favorable conditions for organic C accumulation, in both litter and mineral soil, as the decomposition of unprotected soil organic matter and plant litter is positively affected by temperature [37,38].

This study took place along a site fertility gradient in southern Québec (Eastern Canada). Three replicate stands of hybrid poplars containing six different planting density treatments (ranging 494–1975 trees/ha) and a single genotype (a *P. canadensis* × *P. maximowiczii* hybrid) were sampled. We evaluated to which extent planting density and site affected hybrid poplar productivity and stem dimensions on the mid-term and the longer term (8 vs. 14 years). We also evaluated, after 14 years, the effects of planting density and site on biomass accumulation and C stocks in different plantation compartments, on biomass partitioning at the stand-level, on soil C stocks and on soil nutrient supply rate.

First, we hypothesized that individual stem dimensions (diameter, volume) would be strongly and positively linked to spacing on the mid-term and the longer term. Second, we hypothesized that highest stand productivity would be observed in higher tree density plots in the mid-term only, as planting density effects on productivity tend to decrease as rotation length increases [12]. We also hypothesized that higher stem to branch ratio, but lower shoot to root ratio, would be observed at higher planting densities because intraspecific competition for light and soil resources is more intense. Finally, on the longer term, we expected greater soil C stocks at higher planting densities, but little effect of planting density on total biomass C stocks.

2. Materials and Methods

2.1. Study Sites and Experimental Design

This study was conducted in the Estrie region of southern Québec (Eastern Canada). The three plantation sites (Brompton, Mégantic, and Ogden) were located within a radius of 80 km along a regional elevation and soil fertility gradient. Site elevation above sea level, mean annual temperature (MAT), and mean annual precipitation (MAP) data, obtained from 30 year climatic averages (1981–2010) [39], are provided in Table 1. A continental subhumid moderate climate characterizes the Brompton and Ogden sites, whereas a continental subpolar-subhumid climate characterizes the Mégantic site [40]. Thick glacial till deposits of at least 2 m of depth and gentle slopes (<5%) also characterized the plantation sites [40].

Table 1. Site and soil characteristics of the studied hybrid poplar plantations. Soil variables were measured during the 14th growing season (2013).

				Soil Nutrient Supply Rate (µg/10 cm^2/42 days) [2]										
Site	**Elev.** [1] **(m)**	**MAT** [1] **(°C)**	**MAP** [1] **(mm)**	**NO_3**	**NH_4**	**P**	**K**	**Ca**	**Mg**	**S**	**pH**	**Clay (%)**	**Silt (%)**	**Sand (%)**
Brompton	165	5.6	1146	111.8	4.11	5.16	25	1810	246	54.2	5.27	16.2	58.5	25.3
Mégantic	470	4.2	1048	74.1	2.34	6.73	46	1531	287	27.6	5.00	13.1	41.4	45.5
Ogden	265	5.3	1264	2.8	3.83	2.74	138	808	198	37.0	5.17	14.6	43.2	42.2
SE [1]	-	-	-	41.6	0.52	0.64	21	156	33	8.3	0.05	1.6	1.3	1.2
p-value	-	-	-	<0.01	0.08	<0.01	<0.001	<0.01	0.21	0.12	<0.01	0.41	<0.001	<0.001

[1] Abbreviation used in Table: Elev. (Elevation above sea-level), MAT (Mean annual temperature), MAP (Mean annual precipitation), SE (Standard error of the mean). [2] A log transformation and a reciprocal transformation were respectively used in the ANOVA for soil NO_3 and K supply rate.

At each site, the hybrid poplar plantation was established on an abandoned field dominated by herbaceous vegetation. Site preparation included ploughing in the fall of 1999 and disking the following spring. In the spring of 2000, bare-root planting stock (±2 m-long) were planted manually at 30–40 cm depth. Planting stock (1–0) was provided by the Berthierville nursery of the Ministère des Forêts, de la Faune et des Parcs (MFFP) of Québec. For vegetation management, a glyphosate-based herbicide was applied over the entire plantation area in June 2000, and between plantation rows only in June 2001.

The experimental design contained six planting density treatments (494, 741, 988, 1111, 1481 and 1975 trees/ha) per site and three sites for a total of 18 plots. Twenty trees were planted in each plot, for a total of 360 trees. Details of spacing between trees, area per tree and plot size are provided in Table 2 for each planting density. All plots were separated by two buffer rows, with each buffer row having the same planting density as its adjacent plot. The DN×M-915508 clone was used in this study, which is a female hybrid between *Populus canadensis* (DN) and *P. maximowiczii* (M). It was developed in Québec and is recommended for commercial production in the study area [41] because of its high productivity across a wide range of environmental conditions [9,42].

Table 2. Initial planting density, spacing between trees, area occupied per tree and plot area.

Planting Density (trees/ha)	Spacing (m)	Area per Tree (m^2)	Plot Area (m^2/20 trees)
494	4.5 × 4.5	20.25	405
741	4.5 × 3	13.5	270
988	4.5 × 2.25	10.125	203
1111	3 × 3	9	180
1481	3 × 2.25	6.75	135
1975	2.25 × 2.25	5.0625	101

2.2. Soil Nutrient Supply

Soil macronutrient supply rates were determined using Plant Root Simulator (PRS^{TM}-Probes) technology from Western Ag Innovations Inc., Saskatoon, SK, Canada. The PRS-probes consist of an ion exchange membrane encapsulated in a thin plastic probe, which is inserted into the ground with little disturbance of soil structure. Nutrient supplies observed with this method are strongly correlated with nutrients concentrations or stocks obtained with conventional soil extraction methods over a wide range of soil types [43], and in poplar plantations of the study area [42].

On 6 August 2013 (14th growing season), four pairs of probes (an anion and a cation probe in each pair) were buried in the A horizon of each plot for a 42-day period. Probes were always buried in a plot area where there was full stocking. Each pair of probes was placed at the center point between four trees in the plot, in the competition zone between neighboring trees (Figure A1). After probes were removed from the soil, they were washed with distilled water, and returned to Western Ag Labs for analysis (NO_3, NH_4, P, K, Ca, Mg, S). PRS-probe samples where eluted in a 0.5 M HCl solution and determination of NO_3 and NH_4 concentrations in the eluate was made colorimetrically using an automated flow injection analysis system [44]. The concentration of other nutrients where determined using inductively-coupled plasma spectrometry [44]. Composite samples were made in each plot by combining the four pairs of probes.

2.3. Mineral Soil Characteristics and Carbon Stocks

In July 2013 (14th growing season), soil samples (0–20 depth) were obtained from a composite sample consisting of four soil cores (diameter of the corer = 5.2 cm) per plot. Prior to coring the mineral soil (A horizon), the O horizon (litter layer) was carefully removed. The soil cores were always extracted from a plot area where there was full stocking (see Figure A1 for the sampling design). Composite soil samples were air dried and sieved (mesh size 2 mm) to remove coarse fragments (stones, roots) and macroinvertebrates. Following sieving, air-dry mass of each soil sample was recorded and a subsample was taken to determine an oven-dry mass (105 °C) to air-dry mass ratio, and also to calculate dry mass of soil samples. Soil bulk density was calculated by dividing the dry mass of the fine earth fraction by the volume of soil cores [45].

Soil C concentration of each sample was determined by high-temperature combustion (960 °C) of the samples, followed by thermo-conductometric detection, on a Vario Macro analyzer (Elementar Analysensysteme, Hanau, Germany). These analyses were done by the Centre d'étude de la forêt (CEF) lab at the University of Sherbrooke (Québec, Canada). Soil C stocks were calculated by multiplying C concentration by the bulk density of soil. In each plot, a subsample from the soil cores was also used to determine soil texture and pH. The analyses were done by the Agridirect Inc. soil analysis lab in Longueuil (Québec, Canada). For particle size analyses, the Bouyoucos [46] method was used. The determination of soil pH was made using a 2:1 ratio of water to soil. Soil pH and texture are provided in Table 1 for each site. At each site, soil textural class was the same across the six spacing treatments (silty loam for the Brompton site, and loam for Mégantic and Ogden sites).

2.4. Fine Root, Herbaceous Vegetation, Leaf Litter and Fine Woody Debris Biomass

Fine root biomass samples (root diameter <2 mm) were collected following sieving of the soil cores (see previous subsection). At Brompton and Mégantic sites, where understory vegetation was almost absent, fine root samples consisted of poplar roots only. At the Ogden site, there was a well-developed herbaceous cover in the understory; poplar and herbaceous fine root biomass were not separated in samples. At all sites, fine root biomass samples included both live and dead roots. Fine root samples were washed and dried at 65 °C to determine dry weight and C concentrations.

Herbaceous vegetation biomass was determined by collecting five samples (50 × 50 cm/sample) in each plot during August 2013 (14th growing season). In each plot, the samples were collected at random locations, where there was full stocking. The five samples from each plot were combined and dried at 65 °C to determine dry weight. An herbaceous vegetation subsample from each plot was used for C concentration determination. Woody vegetation in plantation understory was not sampled as it was only a minor biomass component at each site.

Fine woody debris biomass (large end diameter ranging 1–10 cm) [47] was determined (October 2013, 14th growing season) by collecting a single sample in each plot on an area equivalent to the area occupied by a single tree (see Table 2). In each plot the sample was collected in a randomly selected rectangular or square area delineated by four neighboring trees. Each sample was dried at 65 °C to determine dry weight and a subsample from each plot was used for C concentration determination.

Leaf litter and very fine woody debris (large end diameter <1 cm) biomass was determined by collecting three samples (50 × 50 cm/sample) in each plot in late October 2013 (14th growing season), just after leaf fall. In each plot, the samples were collected directly on the ground at random locations, where there was full stocking. The three samples from each plot were combined and dried at 65 °C to determine dry weight. A litter subsample from each plot was used for C concentration determination.

2.5. Hybrid Poplar Biomass and Stem Volume

In each plot, stem volume per tree and stand volume were estimated after 8 and 14 years, while aboveground woody biomass and coarse root biomass were estimated after 14 years only. Those estimates were obtained using allometric relationships between diameter at breast height (DBH) as the predictor variable of stem volume (outside bark) or compartment biomass (stem with bark, branches and coarse roots) at the tree-level. DBH data (mean of two diameter measurements taken perpendicularly at 1.3 m above ground-level) were collected in late October 2007 and 2013. The DBH of all trees in the experimental design was measured.

For stem volume estimations after 8 years, we used a clone-specific allometric relationship that had been previously developed for clone DN×M-915508 in 8 year-old hybrid poplar plantations of the study region [7]. For estimations of stem volume, stem biomass, branch biomass and coarse root biomass after 14 years, we used site-specific allometric relationships that were developed in the studied experimental design with 14 year-old trees [48]. Site-specific relationships were chosen over generic models because allometry of clone DN×M-915508 was found to be plastic across the studied plantation environments, especially for coarse root biomass, branch biomass and stem volume [48]. During destructive harvest procedures, subsamples from stem, branch and coarse root biomass were collected for C concentration determination in 14-year-old poplars. Given that destructive harvest procedures were only done in the 494, 1111 and 1975 trees/ha density treatments at each site, only three samples of stem, branch and coarse root biomass were collected at each site. For each compartment, each of these subsamples consisted in a composite sample collected from two average-sized trees within the plot (see Fortier et al. [48] for a complete description of stem subsamples collection). At each site, and for a given compartment (stem, branch or coarse root biomass), we used C concentration data obtained in the 494, 1111 and 1975 trees/ha treatment as an estimate of C concentration of 741, 988 and 1481 trees/ha treatments respectively.

2.6. Carbon Concentrations and Stocks in Biomass and in the O Horizon

Biomass and litter subsamples were ground in a mill (Pulverisette 15, Fritsch) to a particle size of <0.5 mm to insure adequate sample homogeneity. For biomass and litter C concentration determination, ground, dried aliquots of samples (approximately 100 mg) were encapsulated in tin prior to analysis. Total C was determined by high-temperature combustion (960 °C), followed by thermo-conductometric detection, on a Vario Macro analyzer (Elementar Analysensysteme, Hanau, Germany). These analyses were done by the CEF lab at the University of Sherbrooke. In each plot, C stocks in the different compartments were obtained by multiplying C concentrations determined in subsamples by the total mass of the compartment. Carbon stocks from litter and fine woody debris were summed to obtain a single C stock value for the O horizon soil layer. All C stocks and biomass data were scaled-up to one-hectare area for comparison purposes with other studies.

2.7. Statistical Analysis

Because each planting density treatment is not replicated within sites, site was used as a blocking factor [49], as in other spacing trials [50]. Site and Planting density effects on studied variables were analyzed using ANOVA in a fixed factorial design [49]. Degrees of freedom were the following: Total, 17; Site, 2; Planting density, 5; Error, 10. All of the ANOVAs were run with the complete set of data (3 Sites × 6 Planting densities = 18 experimental plots).

Following ANOVAs, normality of residual was verified using the Shapiro-Wilk W-test. Variables showing non-normal residual distribution were transformed and ANOVA was repeated on transformed variables. Being proportions, survival data were logit transformed [51], while a log transformation and a reciprocal transformation were respectively used for soil NO_3 and K supply rates. Site and Planting density effects were declared statistically significant for three levels of significance ($p < 0.05$, $p < 0.01$ and $p < 0.001$). *A priori* contrasts were further used to test specific hypotheses between particular sets of means [49,52,53]. Two contrasts were tested on key variables: (1) 494 trees/ha vs. 1975 trees/ha and (2) 741 trees/ha vs. 1481 trees/ha, which corresponds to a four-fold and a two-fold variation in planting density, respectively.

Because the Site (i.e., Block) effect was strong and significant for most of the studied variables, we also tested site-specific correlations between planting density and key variables related to stem dimensions, stand and soil characteristics. The area per tree (m^2/tree) at planting was preferred to planting density in pairwise correlation analysis as it yields stronger correlations, based on the Pearson correlation coefficient (r). Based on results of the pairwise correlations, we then developed regression models (linear and non-linear) between area per tree, as predictor variable, and selected response variables. All statistical analyses were done using JMP 11 from SAS Institute (Cary, NC, USA).

3. Results

3.1. Site and Planting Density Effects on Individual Stem Dimensions

The ANOVA revealed strong and significant Site and Planting density effects ($p < 0.01$ or $p < 0.001$) on all variables related to individual stem DBH and volume (Table 3). Larger trees were produced at the fertile sites of Brompton and Mégantic, while lower planting density treatments produced trees with greater DBH and stem volume. After 8 years, mean tree DBH and stem volume respectively ranged 10.01–15.54 cm and 41.7–132.7 dm^3 across the planting density treatments, while after 14 years DBH and stem volume respectively ranged 14.53–25.18 cm and 128.4–434.9 dm^3 across planting density treatments. Planting density treatments also significantly affected the gain in DBH or in stem volume between the end of the 8th and 14th growing seasons, with greater gains being observed at lower planting density (Table 3). Contrasts analysis further suggests that quadrupling or doubling planting density (from 494 to 1975 or from 741 to 1481 trees/ha) resulted in a significant increase of stem dimensions after 8 and 14 years (Table 4).

Table 3. Site and Planting density effects on survival rate, DBH, stem volume, stand volume and volume yield after 8 and 14 years in hybrid poplar plantations.

		DBH (cm)			**Stem Volume (dm^3/tree)**			**Stand Volume (m^3/ha)**			**Volume Yield (m^3/ha/year)**	
Site/Plant. Density	**Surv.** [1] **(%)**	**8 years**	**14 years**	**Δ 8–14 years**	**8 years**	**14 years**	**Δ 8–14 years**	**8 years**	**14 years**	**Δ 8–14 years**	**8 years**	**14 years**
Brompton	100.0	15.43	21.03	+5.60	119.7	307.3	+187.5	115.9	292.7	+176.8	14.48	20.91
Mégantic	86.7	13.24	20.48	+7.24	86.5	285.9	+199.4	69.2	230.3	+161.1	8.65	16.45
Ogden	89.2	9.06	15.01	+5.95	30.8	124.4	+93.6	28.6	108.8	+80.3	3.57	7.77
SE	4.0	0.59	0.69	0.30	10.7	27.3	17.7	6.5	14.2	9.2	0.82	1.02
p-value	<0.05	<0.001	<0.001	<0.01	<0.001	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
494 trees/ha	86.7	15.54	25.18	+9.64	132.7	434.9	+302.2	58.5	189.4	+130.9	7.32	13.53
741 trees/ha	95.0	14.97	22.12	+7.16	113.2	313.1	+199.9	80.9	222.2	+141.4	10.11	15.87
988 trees/ha	93.3	13.07	19.26	+6.20	81.4	238.2	+156.8	78.6	227.5	+148.8	9.83	16.25
1111 trees/ha	83.3	10.01	15.68	+5.67	50.7	160.9	+110.2	51.0	150.4	+99.4	6.37	10.74
1481 trees/ha	93.3	11.58	16.26	+4.68	54.6	159.7	+105.1	76.0	220.6	+144.7	9.50	15.76
1975 trees/ha	100.0	10.29	14.53	+4.24	41.7	128.4	+86.7	82.3	253.6	+171.3	10.29	18.11
SE	5.7	0.83	0.98	0.43	15.2	38.6	25.0	9.2	20.1	13.1	1.15	1.44
p-value	0.34	<0.01	<0.001	<0.001	<0.01	<0.01	<0.01	0.16	0.06	0.05	0.16	0.06

[1] Survival rate (Surv.) was the same after 8 and 14 years.

Table 4. Contrast probabilities (*p*-values) between pairs of planting density treatments for different plantation characteristics in hybrid poplar plantations.

Plantation Characteristics	**Response Variable**	**494 vs. 1975 trees/ha**	**741 vs. 1481 trees/ha**
Stem dimensions	DBH 8 years (cm)	<0.01	<0.05
	DBH 14 years (cm)	<0.001	<0.001
	Stem volume 8 years (dm^3)	<0.01	<0.05
	Stem volume 14 years (dm^3)	<0.001	<0.05
Stand volume	Stand volume 8 years (m^3/ha)	0.10	0.72
	Stand volume 14 years (m^3/ha)	<0.05	0.92
Stand biomass (14 years)	Aboveground woody (t/ha)	0.13	0.75
	Belowground (t/ha)	<0.01	0.50
Biomass partitioning (14 years)	Stem to branch ratio	<0.01	<0.05
	Shoot to root ratio	<0.001	<0.05
Biomass C stocks (14 years)	Aboveground (t C/ha)	0.15	0.68
	Belowground (t C/ha)	<0.01	0.53
	Total (t C/ha)	0.09	0.81
Mineral soil C (14 years)	C Concentration (mg C/g soil)	<0.05	<0.05
	C Stocks (t C/ha)	<0.05	<0.05

3.2. Site and Planting Density Effects on Stand Volume, Biomass and Biomass Carbon Stocks

Stand-level variables (stand volume, compartment biomass and biomass C stocks) indicated significant site effects ($p < 0.01$ or $p < 0.001$) on most variables, with the exception of coarse root biomass, total root biomass, and belowground biomass C stocks (Figure 1a, Tables 3 and 5). Overall, C stocks in the belowground and aboveground biomass respectively represented 14% and 86% of the total biomass C stocks at Brompton and Mégantic, while at Ogden those C stocks respectively represented 26% and 74% of the total biomass C stocks (Figure 1a). After 14 years, a near two-fold difference was observed in total (aboveground + belowground) biomass C stocks between sites (60.5 t C/ha at Brompton vs. 32.9 t C/ha at Ogden).

Results also show that at each site, more volume was cumulated between the end of the 8th and of the 14th growing seasons (a 6 year period), than during the first 8 years of the rotation (Table 3). Thus, higher mean annual volume increment was observed after 14 years than after 8 years for a given site (Table 3). After 14 growing seasons the volume yield observed at the higher fertility sites (Brompton and Mégantic) was more than twice the yield at Ogden (20.91 and 16.45 m^3/ha/year vs. 7.77 m^3/ha/year).

For the Planting density effect, the ANOVA showed significant ($p < 0.05$) or non-significant effects on stand-level volume, biomass and biomass C stocks in the different plantation compartments (Figure 1b, Tables 4 and 5). The Planting density effect was also nearly significant ($p = 0.06$) for the total C stocks stored in plantation biomass after 14 years (Figure 1b). The lowest stand volume, aboveground woody biomass and belowground biomass were observed in the 1111 trees/ha treatment, followed by the 494 trees/ha treatment. Biomass C stocks followed the same pattern (Figure 1b). However, in both of these treatments, survival was relatively low (83.3% at 1111 trees/ha and 86.7% at 494 trees/ha) compared to the other treatments, where survival ranged 93.3–100% (Table 3). The survival rate between the 741 and 1481 trees/ha treatments was comparable (93.3% vs. 95%, respectively), and the contrast analysis between this pair of treatments was far from statistical significance for stand volume, aboveground woody biomass, belowground biomass and for biomass C stocks (Table 4). Table A2 also shows the p-value of the Planting density effect when the ANOVA is run without the 1111 trees/ha treatment, where the survival rate was the lowest. In Table A2, all stand-level variables were far from significance, with the exception of belowground biomass and C stocks ($p = 0.05$). Moreover, for the C stocks in total and aboveground biomass, the contrast analysis revealed no significant differences between the 494 and 1975 trees/ha treatments, while a significant difference ($p < 0.01$) was observed for belowground C stocks (Table 4).

Table 5. Site and Planting density effects on main biomass components at the stand level, on the shoot to root biomass ratio and on the stem to branch biomass ratio in 14 year-old hybrid poplar plantations.

Site/Density	Aboveground Biomass					Belowground Biomass			Total Biomass (t/ha)	Shoot: Root [2]	Stem: Branch
	Stem (t/ha)	Branch (t/ha)	Woody [1] (t/ha)	Herb.[1] (t/ha)	Total (t/ha)	Coarse Roots (t/ha)	Fine Roots (t/ha)	Total (t/ha)			
Brompton	94.2	21.3	115.4	0.02	115.5	16.1	2.03	18.1	133.6	7.24	4.73
Mégantic	74.2	23.4	97.7	0.01	97.7	14.3	1.31	15.6	113.2	6.84	3.28
Ogden	41.6	11.1	52.7	0.76	53.5	14.7	3.99	18.7	72.2	3.59	3.78
SE	4.6	2.0	6.3	0.07	6.3	1.1	0.30	1.2	7.4	0.11	0.25
p-value	<0.001	<0.01	<0.001	<0.001	<0.001	0.51	<0.001	0.21	<0.001	<0.001	<0.01
494 trees/ha	60.6	22.6	83.2	0.36	83.5	12.1	2.21	14.3	97.8	6.58	2.85
741 trees/ha	73.2	22.7	96.0	0.25	96.2	15.7	2.07	17.8	114.0	6.20	3.29
988 trees/ha	74.8	20.5	95.3	0.16	95.5	15.4	2.45	17.8	113.3	5.96	3.65
1111 trees/ha	50.1	11.1	61.2	0.37	61.6	10.5	2.24	12.7	74.3	5.61	4.35
1481 trees/ha	74.9	16.8	91.8	0.28	92.0	17.0	2.54	19.5	111.6	5.58	4.50
1975 trees/ha	86.4	17.9	104.3	0.16	104.4	19.4	3.16	22.5	127.0	5.43	4.94
SE	6.5	2.9	8.9	0.09	8.9	1.6	0.43	1.7	10.4	0.16	0.35
p-value	<0.05	0.12	0.08	0.46	0.08	<0.05	0.56	<0.05	0.06	<0.01	<0.05

[1] Woody = stem + branch biomass. Herb. = Herbaceous plant biomass. [2] The shoot to root ratio excludes fine root biomass.

On the other hand, stand-level biomass partitioning between shoot and roots, but also between stem and branches was significantly affected by both plantation site and planting density (Table 5). Much higher shoot to root ratio was observed on the more fertile sites (Brompton and Mégantic), while a slight decrease in the shoot to root ratio was observed with increasing planting density (Table 5). Conversely, the stem to branch ratio was found to increase with increasing planting density, from 2.85 at 494 trees/ha, up to 4.94 at 1975 trees/ha. Both contrasts tested were also significant for the shoot to root ratio and the stem to branch ratio (Table 4).

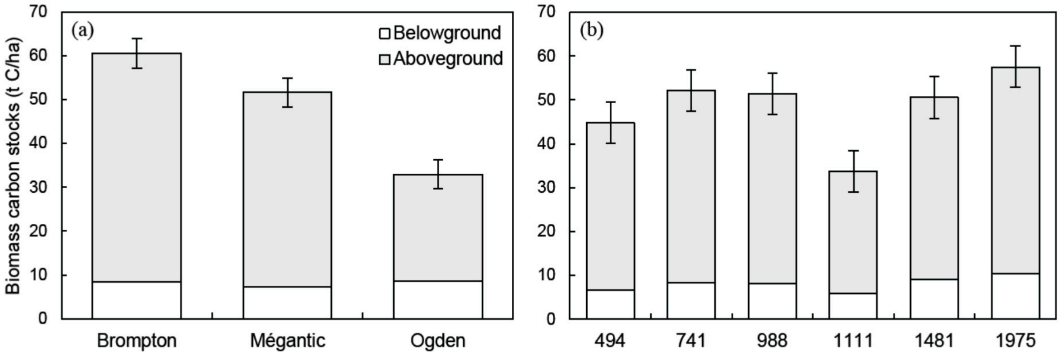

Figure 1. Site effect (**a**) and Planting density effect (**b**) on total biomass C stocks and its distribution between the belowground and aboveground compartments in 14 year-old hybrid poplar plantations. Vertical bars represent standard error of the mean for total C stocks in biomass. *p*-value for the Site effect are the followings: total biomass C ($p < 0.001$), belowground biomass C ($p = 0.21$) and aboveground biomass C ($p < 0.001$). *p*-value for the Planting density effect are the followings: total biomass C ($p = 0.06$), belowground biomass C ($p < 0.05$) and aboveground biomass C ($p = 0.08$).

3.3. Site and Planting Density Effects on Soil Characteristics

The ANOVA showed significant Site effects ($p < 0.01$) for soil NO_3, P, K and Ca supply rates and a marginally significant Planting density effect for soil Mg ($p = 0.05$) supply rate measured during the 14th growing season (Tables 1 and A1). The Ogden site showed the lowest NO_3, P and Ca supply rates, while Mg supply rate was especially high for the 494 and 741 trees/ha treatments (Tables 1 and A1).

For soil C concentration in the fine earth fraction (0–20 cm layer), the Site and Planting density effects were both significant ($p < 0.05$) (Table 6). While the Site effect was highly significant ($p < 0.001$) for soil C stocks (fine earth fraction, O horizon and total soil C), the Planting density effect was nearly significant for C stocks in the fine earth fraction ($p = 0.10$) and for total soil C stocks (mineral soil + O horizon) ($p = 0.07$). There was a trend towards higher soil C concentration, mineral soil C stocks and total soil C stocks with increasing planting density (Table 6). Contrasts analysis showed that C concentrations and stocks in the fine earth fraction (0–20 cm layer) where lower ($p < 0.05$) in the 494 trees/ha vs. the 1975 trees/ha treatment, and in the 741 trees/ha vs. the 1481 trees/ha treatments (Table 4).

3.4. Area per Tree at Planting as a Predictor of Tree-Level, Stand-Level and Soil Characteristics

Because the Site effect was strong on most response variables in this study, we also examined site-specific relationships between area per tree at planting and variables related to individual stem dimensions, stand characteristics and soil characteristics. Several site-specific correlations were strong and significant, mostly for stem dimensions, biomass partitioning and soil characteristics (Table 7).

Table 6. Site and Planting density effects on soil stoniness, bulk density, carbon stocks and concentration in 14 year-old hybrid poplar plantations (0–20 cm layer).

		Fine Earth Fraction (Mineral Soil)				
Site/Planting Density	**Stoniness (% mass basis)**	**Bulk Density (g/cm^3)**	**C Concentration (mg C/g soil)**	**C Stocks (t C/ha)**	**O Horizon C Stocks (t C/ha)**	**Total Soil C Stocks (t C/ha)**
Brompton	0.6	1.02	35.1	71.1	2.16	73.2
Mégantic	11.4	0.97	34.2	65.3	2.89	68.1
Ogden	18.8	0.82	27.3	44.7	1.56	46.2
SE	1.6	0.04	1.7	2.4	0.17	2.2
p-value	<0.001	<0.05	<0.05	<0.001	<0.001	<0.001
494 trees/ha	12.9	1.02	25.7	52.0	1.80	53.8
741 trees/ha	7.0	1.01	27.5	56.1	2.28	58.4
988 trees/ha	11.2	0.91	34.0	61.8	2.47	64.3
1111 trees/ha	10.0	0.92	33.8	62.1	2.01	64.2
1481 trees/ha	8.6	0.92	36.3	66.7	2.22	68.9
1975 trees/ha	12.0	0.86	36.0	63.2	2.44	65.6
SE	2.3	0.06	2.4	3.3	0.24	3.2
p-value	0.48	0.34	<0.05	0.10	0.39	0.07

Table 7. Site-specific pairwise correlations (r) between area per tree at planting (m^2/tree) and selected variables related to individual stem dimensions, stand-level characteristics and soil characteristics. Correlations with $p < 0.10$ are indicated in bold.

		Brompton		**Mégantic**		**Ogden**	
Characteristics	**Variables**	**r**	***p*-value**	**r**	***p*-value**	**r**	***p*-value**
Stem dimensions	DBH 8 years (cm)	**0.94**	**<0.01**	**0.88**	**<0.05**	0.51	0.30
	Volume 8 years (dm^3/tree)	**0.96**	**<0.01**	**0.94**	**<0.01**	0.58	0.22
	DBH 14 years (cm)	**0.96**	**<0.01**	**0.97**	**<0.01**	**0.81**	**0.05**
	Volume 14 years (dm^3/tree)	**0.98**	**<0.001**	**0.98**	**<0.001**	**0.82**	**<0.05**
	Δ DBH 8–14 years (cm)	**0.94**	**<0.01**	**0.97**	**<0.01**	**0.96**	**<0.01**
	Δ Volume 8–14 years (dm^3/tree)	**0.96**	**<0.01**	**0.98**	**<0.001**	**0.88**	**<0.05**
Stand volume	Volume 8 years (m^3/ha)	**−0.81**	**0.05**	0.13	0.81	−0.54	0.27
	Volume 14 years (m^3/ha)	−0.53	0.28	−0.04	0.93	−0.50	0.32
	Δ Volume 8–14years (m^3/ha)	−0.28	0.60	−0.15	0.78	−0.47	0.35
Stand biomass (14 years)	Abovegr. woody biomass (t/ha)	−0.23	0.66	0.05	0.92	−0.46	0.36
	Coarse root biomass (t/ha)	**−0.80**	**0.06**	−0.16	0.75	−0.61	0.20
	Fine root biomass (t/ha)	**−0.94**	**<0.01**	0.13	0.81	−0.27	0.61
	Herbaceous biomass (t/ha)	−0.46	0.36	−0.12	0.82	0.53	0.28
Stand-level partitioning (14 years)	Stem to branch biomass ratio	**−0.92**	**<0.05**	**−0.98**	**<0.001**	**−0.75**	**0.09**
	Shoot to root biomass ratio	**0.97**	**<0.01**	**0.99**	**<0.001**	**0.78**	**0.07**
Stand biomass C stocks (14 years)	Aboveground (t C/ha)	−0.20	0.70	0.08	0.87	−0.44	0.38
	Belowground (t C/ha)	**−0.87**	**<0.05**	−0.13	0.81	−0.57	0.24
	Total biomass (t C/ha)	−0.35	0.50	0.06	0.91	−0.48	0.34
Soil characteristics (14 years)	Bulk density (g/cm^3)	**0.94**	**<0.01**	**0.82**	**<0.05**	−0.10	0.85
	C conc. mineral soil (mg C/g)	**−0.89**	**<0.05**	**−0.86**	**<0.05**	−0.45	0.37
	Mineral soil C stocks (t C/ha)	**−0.84**	**<0.05**	**−0.79**	**0.06**	−0.44	0.39
	O horizon C stocks (t C/ha)	−0.05	0.92	−0.55	0.25	−0.69	0.13
	Total soil C stocks (t C/ha)	**−0.86**	**<0.05**	**−0.83**	**<0.05**	−0.46	0.35
	NO$_3$ supply (µg/10 cm^2/42 days)	**0.87**	**<0.05**	**0.77**	**0.07**	**−0.77**	**0.07**
	NH$_4$ supply (µg/10 cm^2/42 days)	−0.32	0.54	−0.56	0.25	0.16	0.76
	P supply (µg/10 cm^2/42 days)	**0.80**	**0.06**	0.54	0.26	0.00	1.00
	K supply (µg/10 cm^2/42 days)	**−0.91**	**<0.05**	−0.47	0.35	−0.40	0.43
	Ca supply (µg/10 cm^2/42 days)	**0.87**	**<0.05**	0.41	0.42	**0.93**	**<0.01**
	Mg supply (µg/10 cm^2/42 days)	**0.89**	**<0.05**	0.42	0.40	0.41	0.42
	S supply (µg/10 cm^2/42 days)	**0.87**	**<0.05**	0.12	0.82	**0.82**	**<0.05**

For individual stem dimensions, strong and significant positive correlations ($p \leq 0.05$) were observed at Brompton and Mégantic between area per tree and DBH or stem volume (8 years, 14 years and Δ 8–14 years) (Table 7). At the Ogden site, these correlations were only significant ($p \leq 0.05$) for stem DBH and volume after 14 years. Area per tree and stand productivity were only strongly correlated after 8 years at the Brompton site (r = −0.81 and $p = 0.05$ for stand volume). Significant site-specific relationships ($p \leq 0.05$) between area per tree and tree DBH after 14 years, stem volume per tree after 14 years, and stand volume after 8 years are presented in Figure 2.

For biomass partitioning at the stand-level, negative correlations were observed between area per tree and the stem to branch ratio at Brompton (r = −0.92, $p < 0.05$), Mégantic (r = −0.98, $p < 0.001$) and Ogden (r = −0.75, $p = 0.09$), while positive correlations were observed between area per tree and the shoot to root ratio at Brompton (r = 0.97, $p < 0.01$), Mégantic (r = 0.99, $p < 0.001$) and Ogden (r = 0.78, $p = 0.07$) (Table 7). At the Brompton site, significant or near significant negative correlations between area per tree at planting and fine root biomass (r = −0.94, $p < 0.01$), coarse root biomass (r = −0.80, $p = 0.06$), and belowground C stocks (r = −0.87, $p < 0.05$) were also observed (Table 7). Site-specific relationships between area per tree and the branch to stem ratio, the shoot to root ratio, fine root biomass or coarse root biomass at the stand-level are presented in Figure 3.

For soil nutrients, positive correlations between area per tree and nutrient supply rate were found at Brompton (NO_3, P, Ca, Mg and S, significant at $p = 0.06$ or less), Mégantic (NO_3, $p = 0.07$), and Ogden (Ca and S, significant at $p < 0.05$) (Table 7). However, area per tree and soil K were negatively correlated at Brompton ($p < 0.05$), while area per tree and soil NO_3 were negatively correlated at Ogden ($p = 0.07$). Site-specific relationships between area per tree and soil nutrient supply rates measured during the 14th growing season are presented in Figure 4. For soil C, negative correlations were observed between area per tree and mineral soil C concentration, mineral soil C stocks (0–20 cm layer) and total soil C stocks (mineral soil + O horizon) at Brompton ($p < 0.05$) and Mégantic ($p = 0.06$ or less). At those two sites, mineral soil bulk density (0–20 cm layer) was positively correlated ($p < 0.05$) with area per tree (Table 7). Site-specific relationships between area per tree at planting and soil C or bulk density measured during the 14th growing season are presented in Figure 5.

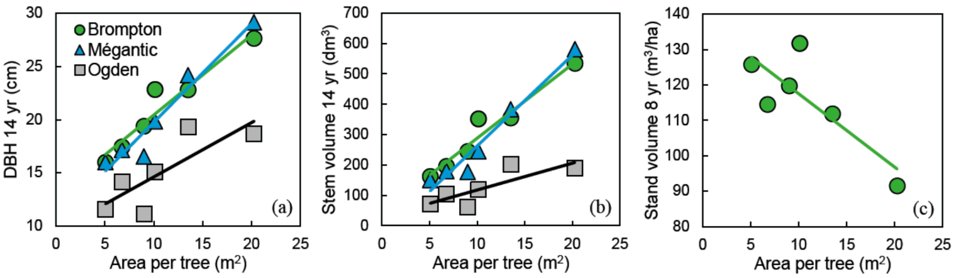

Figure 2. Site-specific linear relationships between area per tree at planting and (**a**) mean tree DBH after 14 years, (**b**) mean stem volume per tree after 14 years and (**c**) stand volume after 8 years in hybrid poplar plantations. Only relationships with $p \leq 0.05$ are presented. Fit and significance level of the relationships are the following for panel (**a**): Brompton site ($R^2 = 0.93$, $p < 0.01$), Mégantic site ($R^2 = 0.95$, $p < 0.01$), Ogden site ($R^2 = 0.65$, $p = 0.05$); for panel (**b**): Brompton site ($R^2 = 0.95$, $p < 0.001$), Mégantic site ($R^2 = 0.96$, $p < 0.001$), Ogden site ($R^2 = 0.67$, $p < 0.05$); for panel (**c**): Brompton site ($R^2 = 0.66$, $p = 0.05$).

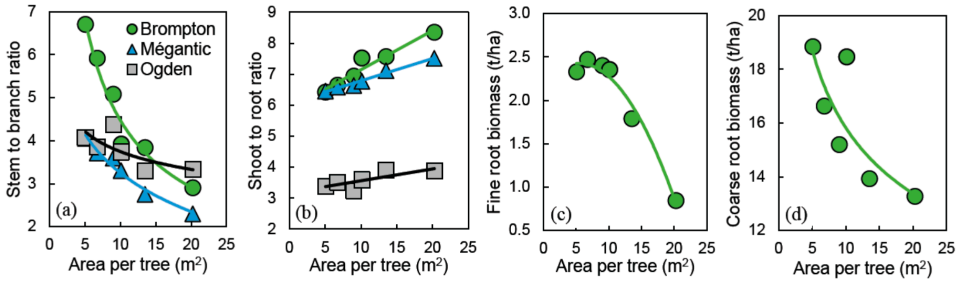

Figure 3. Site-specific relationships between the area per tree at planting and (**a**) the stem to branch biomass ratio at the stand-level, (**b**) the shoot to root biomass ratio at the stand-level, (**c**) fine root biomass and (**d**) coarse root biomass in 14 year-old hybrid plantations. Only relationships with $p < 0.1$ are presented. Model type, fit and significance level of the relationships are the following for panel (**a**): Brompton site (power model, $R^2 = 0.95$, $p < 0.001$), Mégantic site (logarithmic model, $R^2 = 0.97$, $p < 0.001$), Ogden site (power model, $R^2 = 0.56$, $p = 0.09$); for panel (**b**): Brompton site (linear model, $R^2 = 0.94$, $p < 0.01$), Mégantic site (linear model, $R^2 = 0.98$, $p < 0.001$), Ogden site (linear model, $R^2 = 0.61$, $p = 0.07$); for panel (**c**): Brompton site (polynomial model, $R^2 = 0.97$, $p < 0.01$). The shoot to root ratio exclude fine root biomass; for panel (**d**): Brompton site (power model, $R^2 = 0.68$, $p < 0.05$).

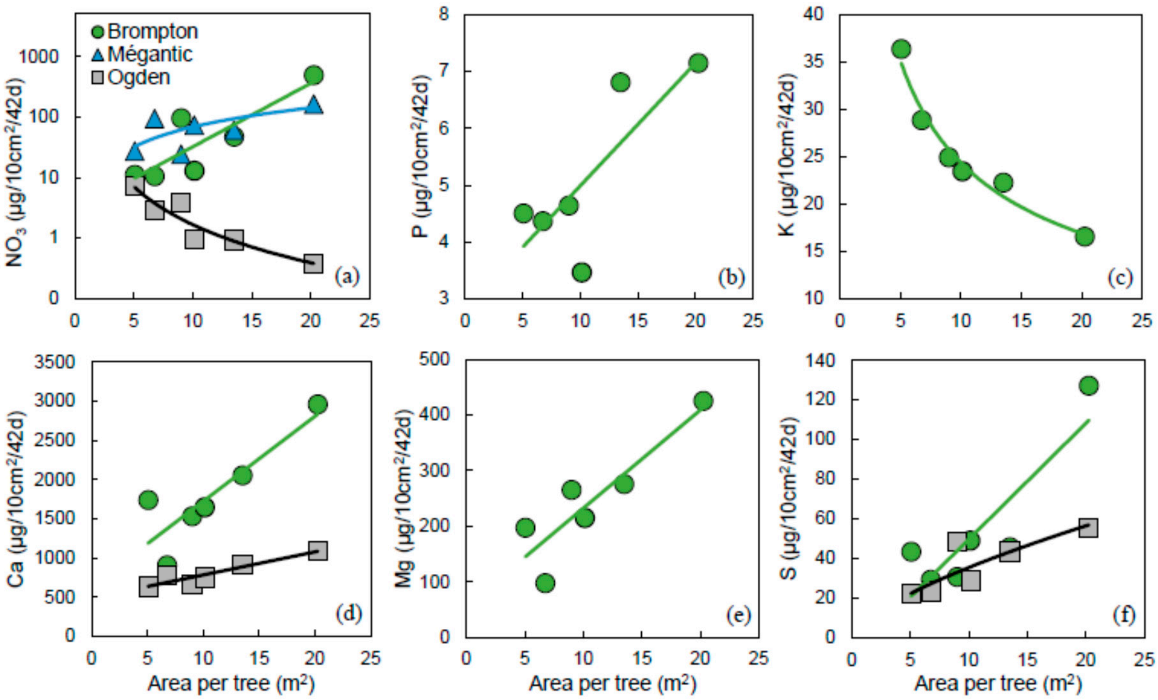

Figure 4. Site-specific relationships between the area per tree at planting and the supply rate of soil (**a**) nitrate, (**b**) phosphorus, (**c**) potassium, (**d**) calcium, (**e**) magnesium and (**f**) sulfur during the 14th growing season in hybrid poplar plantations. Only relationships with $p < 0.1$ are presented. Model type, fit and significance level of the relationships are the following for panel (**a**): Brompton site (exponential model, $R^2 = 0.74$, $p < 0.05$), Mégantic site (linear model, $R^2 = 0.60$, $p = 0.07$), Ogden site (linear model, $R^2 = 0.88$, $p < 0.01$); for panel (**b**): Brompton site (linear model: $R^2 = 0.64$, $p = 0.06$); for panel (**c**): Brompton site (power model, $R^2 = 0.97$, $p < 0.001$); for panel (**d**): Brompton site (linear model, $R^2 = 0.76$, $p < 0.05$), Ogden site (linear model, $R^2 = 0.87$, $p < 0.01$); for panel (**e**): Brompton site (linear model, $R^2 = 0.79$, $p < 0.05$); for panel (**f**): Brompton site (linear model, $R^2 = 0.76$, $p < 0.05$), Ogden site (linear model, $R^2 = 0.71$, $p < 0.05$). Nutrient supply rates were measured in hybrid poplar plantations during 42 days (6 August–17 September 2013).

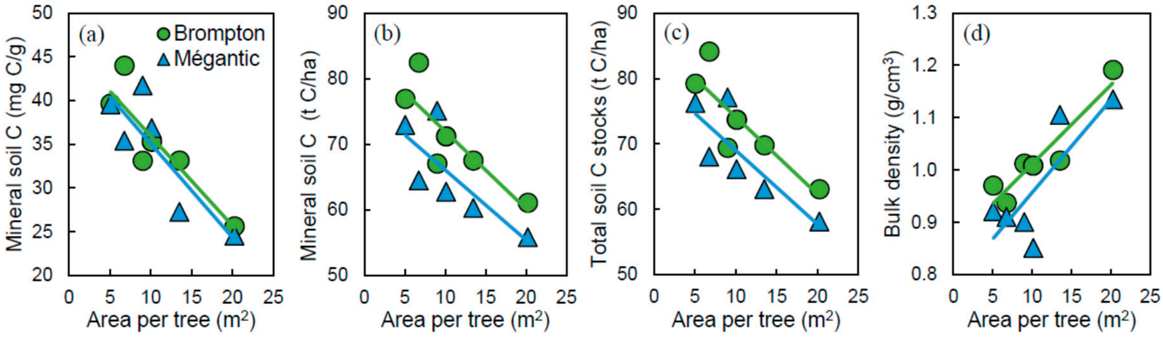

Figure 5. Site-specific linear relationships between the area per tree at planting and (**a**) mineral soil C concentration (0–20 cm layer), (**b**) mineral soil C stocks (0–20 cm layer), (**c**) total soil C stocks (mineral soil + O horizon) and (**d**) mineral soil bulk density (0–20 cm layer). Only relationships with $p < 0.1$ are presented. Fit and significance level of the relationships are the following for panel (**a**): Brompton site ($R^2 = 0.80$, $p < 0.05$), Mégantic site ($R^2 = 0.74$, $p < 0.05$); for panel (**b**): Brompton site ($R^2 = 0.71$, $p < 0.05$), Mégantic site ($R^2 = 0.62$, $p = 0.06$); for panel (**c**): Brompton site ($R^2 = 0.74$, $p < 0.05$), Mégantic site ($R^2 = 0.69$, $p < 0.05$): for panel (**d**): Brompton site ($R^2 = 0.88$, $p < 0.01$), Mégantic site ($R^2 = 0.67$, $p < 0.05$).

4. Discussion

4.1. Planting Density Effects on Stem Dimensions, Stand Productivity and Total Biomass Carbon Stocks

In fast-growing plantations, the selection of the planting density is an important management decision as it can maximize biomass or wood production, and optimize wood log size at harvest, which is important for specific production objectives [12]. As expected, there was a strong effect of planting density on individual stem DBH or volume after 8 and 14 years (Table 3, Figure 2a,b). However, we found little evidence of an important effect of planting density on stand volume after 8 and 14 years, or on woody biomass production after 14 years. Site selection was clearly a more important factor affecting stem volume and woody biomass production at the stand-level (Table 3). Across sites, low stand volume or woody biomass production was mainly observed in planting density treatments were the survival rate was the lowest (Table 3). Yet, when the 1111 trees/ha treatment (lowest survival rate) was excluded from the ANOVA, there was no significant Planting density effect on stand volume or woody biomass production (Table A2). At the Mégantic and Ogden sites, where mortality occurred (Table 3), stand volume and aboveground woody biomass were more strongly correlated with survival than with spacing (Tables 7 and A3). This indicates that if tree mortality occurs early in the rotation, substantial productivity loss will be observed on the long-term.

The strongest evidence of a linkage between planting density and productivity was a significant negative relationship between area per tree and stand volume after 8 years, only observed at Brompton ($R^2 = 0.66$, $p = 0.05$) (Figure 2c). Thus, our second hypothesis suggesting greater mid-term (8 years) stand volume at higher planting density only holds for the most productive site, mainly because of the low productivity of the wider spacing treatment (4.5 × 4.5 m or 484 trees/ha) (Figure 2c). Our results corroborate previous studies showing that productivity differences between planting density treatments in *Populus* plantations are generally minor and tend to decrease as rotation length increases [12,15,16,23,24]. In addition, given that the largest biomass C stock is located in aboveground biomass, planting density only had a marginal effect on the total biomass C storage capacity of 14 year-old poplar plantations (Figure 1b, Tables 7 and A2).

The absence of an aboveground productivity/spacing relationship may be related to the clone selected for this study. Clone DN×M-915508 is known for its high plasticity in biomass allocation to the crown and its high allocation to branch biomass compared to other hybrid types [9,42,48]. Such a biomass allocation strategy allows this clone to rapidly occupy available canopy space, thereby optimizing its light harvesting capacity under both narrow and wide spacings. Clone DN×M-915508 is also known to have a relatively high productivity across a wide range of environmental conditions, even on sites presenting nutrient limitations [7]. This generalist genotype was found to be highly proficient at resorbing growth limiting nutrients (N and P) from senesced leaves, especially when site fertility declined [54]. Such a nutrient cycling strategy may allow clone DN×M-915508 to cumulate aboveground biomass at similar rate in both narrower and wider spacings, despite that the supply of growth limiting nutrients (N and P) in soil tends to decline with increasingly narrow spacing on the more fertile sites (Brompton and Mégantic) (Figure 4a,b).

The small plot size (20 trees/plot at planting) and the presence of only two buffer rows between the planting density treatments were limitations in this study. Such an experimental design was not optimal in completely eliminating the effect of neighboring plots in terms of competition for light. Also, the yield results may not be fully representative of large-scale commercial plantations where mortality rates are rarely 0%, as observed at the Brompton site.

4.2. Planting Density Effects on Biomass Partitioning, Belowground Carbon Allocation, Soil Nutrient Supply and Soil Carbon

This study shows that planting density has important effects on biomass partitioning, on belowground C allocation, on soil nutrient supply rate and on soil C sequestration over the long-term in poplar plantations. As hypothesized, we observed a decrease in the stem to branch biomass ratio with increasing tree spacing (Table 5, Figure 3a), which suggest that poplars responded to high competition for light in narrow spacings by proportionally reducing allocation to branch vs. stem biomass. The negative relationship between spacing and the stem to branch ratio was particularly steep at the productive site of Brompton (Figure 3a), where canopy closure and self-pruning occurred the earliest in the rotation (B. Truax, field observations).

Also, there was evidence of higher root competition for nutrients with decreasing spacing as shown by the positive relationships between spacing and soil NO_3 (Brompton and Mégantic sites), P, Mg (Brompton site), Ca and Mg (Brompton and Ogden sites) (Figure 4). Resource depletion in the narrower spacings likely stimulated biomass allocation belowground (Table 5). As expected, a decrease in the shoot to root ratio was observed at all sites with decreasing spacing (Figure 3b). Significant relationships between spacing and fine or coarse root biomass were also observed at the productive site of Brompton (Figure 3c,d), where signs of nutrient depletion were mainly observed (Figure 4). Thus, at Brompton, higher amounts of soil nutrients may have been taken up by poplars in narrow spacings to enhance biomass production belowground and increase foraging capacity. Furthermore, smaller diameter trees, which were produced in narrower spacings at all sites (Table 3), tend to have greater macronutrient concentrations per unit of woody biomass [55]. This could also have contributed to soil nutrient depletion in narrower spacings, despite the lack of spacing/aboveground productivity relationships after 14 years. Recent studies have suggested that faster canopy closure in narrower spacings can lower nitrification and nitrogen mineralization rates after 5 years in widely spaced (400 trees/ha or less) poplar plantations [36]. However, such a hypothesis is not supported by our results related to soil NO_3 supply at Brompton and Mégantic, as closed canopy characterized all spacing treatments at these sites after 14 years (B. Truax and J. Fortier, field observations).

On the other hand, there was a strong negative relationship between tree spacing and soil K at Brompton (Figure 4c), which suggests little depletion of this soil nutrient with increasing root competition. Weaker negative correlations between spacing and soil K were also observed at Mégantic and Ogden (Table 7). In mature poplar plantations, approximately 90% of total net stand deposition of K originates from canopy leaching, and compared to other base cations, little K is lost by percolation in the soil [56]. Since canopy closure was reached earlier in narrow spacings (B. Truax, field observations), this may have enhanced canopy-derived K input to the soil over the years. A low magnitude negative relationship between soil NO_3 supply and spacing was also observed at the low fertility site of Ogden (Figure 4a), where herbaceous biomass was by far the highest across sites (Table 5). Such a relationship potentially reflects a higher NO_3 uptake by herbaceous vegetation underneath poplars in more widely spaced treatments, since spacing and herbaceous biomass were weakly correlated at Ogden ($r = 0.53$, $p = 0.28$). Ruderal herbaceous plants that are typically found in the understory of poplar plantations growing on old fields can be strong competitors for soil NO_3 [57,58].

As expected, decreasing spacing between poplars not only led to greater allocation to belowground biomass (Figure 3b–d), it also led to higher soil C concentration and C stocks after 14 years (Tables 4 and 7). Yet, significant relationships between spacing and soil C were only found on the more productive sites (Brompton and Mégantic) (Figure 5a–c). Like many other tree species, poplars growing in less favorable or more competitive soil environments tend to increase root production, and root exudation to support mycorrhizal networks and microorganisms in the rhizosphere, in order to improve the access to soil resources [33,59]. Such a positive feedback of high intraspecific competition on soil C was likely observed in this study. Also, faster canopy closure in narrower spacings may have led to a faster decrease of soil and air temperatures, potentially providing more favorable conditions for soil C accumulation [37].

While mineral soil C concentration decreased with spacing at Brompton and Mégantic (Figure 5a), soil bulk density tended to increase with spacing at those sites (Figure 5d). As a result, soil C concentration and bulk density were negatively correlated at Brompton ($r = -0.93$, $p < 0.01$) and Mégantic ($r = -0.91$, $p < 0.05$). Such a trend is consistent with the notion that soil organic matter, which was strongly correlated to soil C concentration across sites ($r = 0.92$, $p < 0.001$, $n = 18$), has an attractive effect on soil invertebrates that can create pores in the soil [60]. At Brompton, greater fine root biomass in narrower spacings (Figure 3c) could have also contributed to decrease soil bulk density.

4.3. Management Implications

When a particular log size is required for solid wood product applications or for pulpwood, planting density should be carefully selected (Table 3, Figure 2a,b). Particular care should also be given to obtaining a very high survival rate, as higher yields were always achieved in planting density treatments with the highest survival rates (Tables 3 and A3). However, compared to planting density, site selection was of overriding importance for achieving high volume or biomass yields at the stand level. Thus, poplar afforestation on low fertility abandoned farmland sites cannot provide interesting yields (above 10 m^3/ha/year) [9], even if a generalist genotype is planted at a high density (1975 trees/ha) over a relatively long rotation (14 years). On low fertility sites, such as Ogden, hybrid poplars tend to allocate a larger proportion of assimilate C belowground, at the expense of aboveground biomass production [30]. As a result, similar coarse root biomass was observed between sites, while aboveground woody biomass varied by more than two-fold (Table 5).

For the production of bioenergy with single-stem trees, hybrid poplar plantations with planting densities ranging 1000–2000 stems/ha and 5–8 year rotations are recommended in Europe and in North America [2,61]. Yet, this study shows that for planting densities ranging 484–1975 trees/ha, using longer rotations would allow much greater productivity. At all sites and independently of the planting density, more stand volume was cumulated between the end of 8th and 14th growing season (a 6 year period) than during the first 8 years (Table 3). Rotations longer than 10 years were also recommended in Germany for high density (4000–8000 trees/ha) hybrid aspen (*P. tremula* × *P. tremuloides*) plantations on agricultural land [62]. Besides, if nitrogen fertilizers are used, the management of poplar plantations on longer rotations may reduce environmental impacts in terms of NO_3 leaching and soil nitrous oxide emissions [63]. However, longer rotations are not always optimal from an economic perspective [12]. For the specific case of short-rotation coppice bioenergy plantations with high planting densities ($\geq$2500 cuttings/ha), high biomass yields (above 10 t/ha/year) were obtained with hybrid poplars managed on 4-year cutting cycles in southern Québec (Canada) and other temperate regions [21,64]. Coppicing is also known to increase poplar yield in the subsequent rotations [65]. Thus, optimal rotation length may vary greatly between hybrid poplar plantation types (e.g., single-stem vs. coppiced systems) [66].

The use of long rotations also increases the risks of tree exposure to diseases and climatic disturbances [13], such has wind and ice storms, which are common across northeastern America. Substantial stem breakages were observed in the 1975 and 494 trees/ha plots at Brompton during the 15th growing season (B. Truax and J. Fortier, field observations). This suggests that for the studied poplar clone (DN×M-915508), both the smaller diameter trees with narrow crowns and the larger diameter trees with wide crowns may have reached their physical limit to accumulate biomass under the climatic conditions of southern Québec.

The results from this study have important implications for ecosystem services provision in poplar plantations. While planting density had a minor effect on aboveground biomass C stocks, significant increases in belowground biomass C stocks and in mineral soil C stocks were observed with decreasing tree spacing (Table 7, Figures 3 and 5). Soil porosity also tended to increase with decreasing spacing (Figure 5d). Thus, on the longer term, more densely planted poplars may be more effective to sequester atmospheric CO_2 belowground and improve general soil health on marginal agricultural land. Such environmental benefits could be the trade-off compensating for the higher establishing costs of higher density plantations, especially if large planting stocks are used.

More generally, our results have potential implications for the design of multi-functional poplar buffers, which are increasingly used in agricultural watersheds for bioenergy production, streambank stabilization, phytoremediation and on-farm C storage [1,67,68]. Higher soil porosity, reduced soil NO_3 and P, enhanced soil organic C and higher belowground biomass in more densely planted poplars could provide more effective buffering capacity against main agricultural pollutants (sediments, N, P and organic pesticides) reaching streams [69]. Further studies are therefore needed to evaluate the effects of planting density on the provision of various ecosystem services in different types of *Populus* plantation systems.

5. Conclusions

Overall, this long-term study showed that planting density had major effects on stem dimensions, biomass partitioning and soil C, but minor effects on productivity and aboveground biomass C stocks. Site selection was a much more determinant factor in obtaining high yields for the studied hybrid poplar clone. On the higher fertility sites, surface area per tree was a significant negative predictor of fine root biomass, coarse root biomass and soil C, but a significant positive predictor for the supply of several macronutrients. Therefore, in higher density plantations of poplars, the high root competition for soil resources may lead to soil nutrient depletion, which may stimulate C allocation belowground and enhance soil C sequestration potential over the long-term.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix

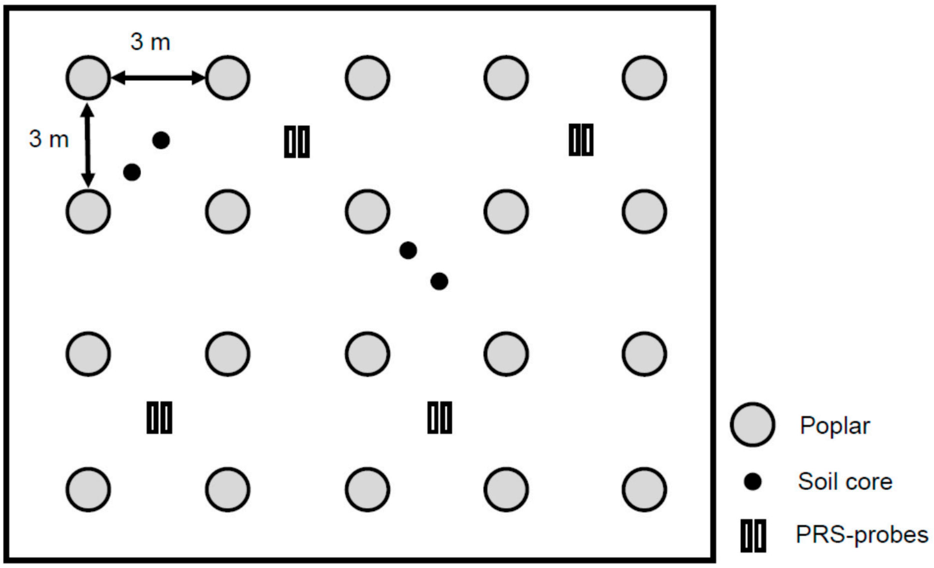

Figure A1. Schematic representation of the sampling design used for the soil core extractions and for the evaluation of soil nutrient supply rate with the PRS-probes. The experimental plot shown represents the 1111 trees/ha (3 × 3 m spacing) treatment.

Table A1. Planting density effect on soil nutrient supply rate, pH, clay, silt and sand content in 14 year-old hybrid poplar plantations.

	Soil Nutrient Supply Rate (μg/10 cm^2/42 days)										
Planting Density	**NO_3**	**NH_4**	**P**	**K**	**Ca**	**Mg**	**S**	**pH**	**Clay**	**Silt**	**Sand**
494 stems/ha	219.0	3.31	6.02	46	1880	309	70.4	5.20	15.9	45.8	38.3
741 stems/ha	36.5	2.29	4.85	48	1642	387	40.4	5.27	15.9	49.8	34.3
988 stems/ha	29.2	3.38	4.90	55	1324	200	34.5	5.20	14.3	50.1	35.6
1111 stems/ha	41.3	4.91	5.22	63	1123	194	33.3	4.97	13.5	44.4	42.0
1481 stems/ha	36.0	3.25	4.20	149	1136	188	27.6	5.20	13.9	46.7	39.4
1975 stems/ha	15.2	3.42	4.06	56	1194	186	31.7	5.03	14.2	49.4	36.4
SE	58.9	0.73	0.91	29	221	47	11.7	0.06	2.3	1.9	1.7
p-value	0.91	0.33	0.69	0.08	0.17	0.05	0.21	<0.05	0.95	0.24	0.14

Table A2. Probability value (*p*-value) of the Planting density effect on stand-level characteristics following the removal of the 1111 trees/ha treatment in the ANOVA.

Stand-Level Characteristics	*p*-value Planting Density Effect
Volume 8 years (m^3/ha)	0.15
Volume 14 years (m^3/ha)	0.21
Δ Volume 8–14 years (m^3/ha)	0.33
Aboveground woody biomass 14 year (t/ha)	0.49
Belowground biomass 14 years (t/ha)	0.05
Total biomass 14 years (t/ha)	0.37
Aboveground C stocks 14 years (t C/ha)	0.53
Belowground C stocks 14 years (t C/ha)	0.05
Total biomass C stocks 14 years (t C/ha)	0.40

Table A3. Site-specific pairwise correlations (r) between survival and selected stand-level characteristics in hybrid poplar plantations.

	Mégantic		Ogden	
Stand-Level Characteristics	**r**	***p*-value**	**r**	***p*-value**
Volume 8 years (m^3/ha)	0.92	<0.01	0.74	0.09
Volume 14 years (m^3/ha)	0.93	<0.01	0.69	0.13
Δ volume 8–14 years (m^3/ha)	0.86	<0.05	0.65	0.16
Aboveground woody biomass 14 years (t/ha)	0.91	<0.05	0.66	0.15
Aboveground biomass C stocks 14 years (t C/ha)	0.91	<0.05	0.66	0.15
Belowground biomass C stocks 14 years (t C/ha)	0.91	<0.05	0.74	0.09
Total biomass C stocks 14 years (t C/ha)	0.91	<0.05	0.69	0.13

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