



Quantitative genetic analysis of biomass and wood chemistry of *Populus* under different nitrogen levels

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Summary

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- The genetic control of carbon allocation and partitioning in woody perennial plants is poorly understood despite its importance for carbon sequestration, biofuels and other wood-based industries. It is also unclear how environmental cues, such as nitrogen availability, impact the genes that regulate growth, biomass allocation and wood composition in trees.

- We phenotyped 396 clonally replicated genotypes of an interspecific pseudo-backcross pedigree of *Populus* for wood composition and biomass traits in above- and below-ground organs. The loci that regulate growth, carbon allocation and partitioning under two nitrogen conditions were identified, defining the contribution of environmental cues to their genetic control.

- Sixty-three quantitative trait loci were identified for the 20 traits analyzed. The majority of quantitative trait loci are specific to one of the two nitrogen treatments, demonstrating significant nitrogen-dependent genetic control. A highly significant genetic correlation was observed between plant growth and lignin/cellulose composition, and quantitative trait loci co-localization identified the genomic position of potential pleiotropic regulators.

- Pleiotropic loci linking higher growth rates to wood with less lignin are excellent targets to engineer tree germplasm improved for pulp, paper and cellulosic ethanol production. The causative genes are being identified with a genetical genomics approach.

Abbreviations: eQTL, expression quantitative trait locus; LG, linkage group; LOD, logarithm of the odds ratio; py-MBMS, pyrolyzer coupled to a molecular beam mass spectrometer; QTL, quantitative trait locus; S/G ratio, ratio of S- to G-lignin; SSR, simple sequence repeat.

Introduction

As a growing body of evidence supports the negative effects of accumulation of CO₂ and other greenhouse gases in the atmosphere (Lenoir *et al.*, 2008; Rosenzweig *et al.*, 2008), society is increasingly turning to forests and forest management for the mitigation of atmospheric CO₂ (Canadell & Raupach, 2008). Forests store *c.* 45% of terrestrial carbon (Bonan, 2008) and cellulosic ethanol production from wood has

great potential to diminish the need for fossil fuels, limiting atmospheric CO₂ accumulation (Sticklen, 2008). Therefore, increasing the productivity of plantation forests could have a significant impact on society by simultaneously enhancing carbon sequestration and meeting greater demands for renewable wood and bioenergy products.

The chemical composition of wood, cellulose (45–50%), hemicellulose (25%) and lignin (25–35%) (Plomion *et al.*, 2001), is important for its conversion into products and for

carbon sequestration. Because lignin is richest in carbon and the most recalcitrant component of wood, higher proportions can translate into more carbon stored for longer periods of time. However, higher lignin contents may be undesirable for the production of pulp/paper and cellulosic ethanol. For these applications, lignin needs to be extracted from wood with relatively harsh chemical treatments and high energy inputs (Li *et al.*, 2003; Chen & Dixon, 2007; Sticklen, 2008). One strategy for increasing carbon sequestration and improving pulp, paper and cellulosic biofuel productivity is to raise the lignin content in nonharvested roots while reducing lignin in the woody stem. Therefore, the development of tree germplasm that is optimal for carbon sequestration and biomass conversion requires an understanding of the genetic regulation of growth, carbon allocation among plant organs and carbon partitioning into lignin, cellulose and hemicellulose within organs.

In woody plants, studies have identified a consistent, significant correlation between biomass growth and wood composition that is genetically regulated (Hu *et al.*, 1999; Wu *et al.*, 1999; Kirst *et al.*, 2004; Yu *et al.*, 2006). Pleiotropic genetic loci coordinating wood composition and stem growth are important targets for the enhancement of wood products. *Populus* is an excellent model species to identify these genetic elements, given the availability of several segregating pedigrees, easy clonal propagation, well-established transformation protocols and the genome sequence of a *P. trichocarpa* genotype (Tuskan *et al.*, 2006). Previous studies have identified quantitative trait loci (QTLs) for biomass accumulation and allocation in segregating *Populus* families (Bradshaw & Stettler, 1995; Wu, 1998; Wu *et al.*, 1998; Wullschleger *et al.*, 2005; Rae *et al.*, 2008). Above- and below-ground growth QTLs have also been mapped in poplar grown under ambient and elevated CO₂ (Rae *et al.*, 2007). However, few studies have attempted to map QTLs for wood chemical composition in poplar (Zhang *et al.*, 2006) or, more importantly, have dissected the coordinate regulation of stem growth and wood chemistry.

In addition to genetics, environmental cues at the level of nutrient availability have a tremendous impact on tree growth, biomass allocation and wood composition. Nitrogen is generally the most limiting nutrient for tree growth and carbon sequestration (Oren *et al.*, 2001; Finzi *et al.*, 2007). Poplar trees exhibit extensive phenotypic plasticity in response to nitrogen. Nitrogen fertilization of young (< 1 yr old) poplar trees increases shoot biomass and wood cellulose content, while decreasing lignin content and the ratio of S- and G-lignin (S/G ratio) (Cooke *et al.*, 2005; Pitre *et al.*, 2007a,b). Nitrogen supply has also been observed to increase the photosynthetic rate in mature leaves (Cooke *et al.*, 2005) and to alter xylem fiber anatomy by thickening and shortening (Pitre *et al.*, 2007a). Changes in mRNA abundance have also been observed in poplar in response to nitrogen treatments (Cooke *et al.*, 2003). Although these studies have contributed

to a better understanding of the effects of nitrogen on tree anatomy and physiology, little is known about how genotypes interact with nitrogen and how these interactions are regulated in forest species.

The objective of this study was to define the genetic loci controlling the phenotypic variation in biomass accumulation, carbon allocation and partitioning in an interspecific *Populus* pedigree grown under two different nitrogen conditions. To identify these regions, a QTL mapping experiment with 396 clonally replicated genotypes was completed. Extensive phenotypic plasticity in response to nitrogen availability was observed. The experimental design allowed the analysis of genotype by nitrogen interaction for the first time in a tree QTL mapping population, resulting in the identification of 51 loci that control traits only under one nitrogen condition. Biomass accumulation, cellulose and lignin levels in wood were strongly correlated, genetically and phenotypically. More importantly, some QTLs for biomass growth and wood composition mapped to the same genomic region, delineating potential pleiotropic regulators that coordinate these traits.

Materials and Methods

Poplar pseudo-backcross pedigree phenotyping

An interspecific pseudo-backcross pedigree (family 52-124) composed of 396 genotypes was created by crossing the hybrid female clone 52-225 [*P. trichocarpa* (clone 93-968) × *P. deltoides* (clone ILL-101)] with the male *P. deltoides* clone D124. Six cuttings of each progeny genotype were planted in pots 41 cm deep (TPOT2, Stuewe & Sons, Inc., Corvallis, OR, USA) with Fafard 4MIX soil (Canadian Sphagnum Peat 40%, processed pine bark and vermiculite) in a glasshouse at the University of Florida. Plants were arranged in the glasshouse following a partially balanced incomplete block design with three biological replicates, six incomplete benches per replicate and two nitrogen treatments. Within each replicate, a coordinate system with 30 rows and 26 columns, where each plant was located in a row × column intersection, was utilized to account for possible systematic sources of variation across the glasshouse in our statistical model (see below). The total number of plants in the experiment was 2376 (i.e. 396 genotypes × 2 nitrogen treatments × 3 biological replicates). After potting, all plants were grown for 6 wk with 5 mM NH₄NO₃ supplied with Hocking's complete nutrient solution (Hocking, 1971) in a flood irrigation system of ebb-and-flow benches. Benches were flooded twice a day for approximately 30 min. During the 5th week of growth, initial diameters and heights were measured. At the start of week six, one half of the benches in each replicate was flooded with Hocking's solution supplemented with 25 mM NH₄NO₃ and the other half was flooded with the same solution, but without any nitrogen supplement.

After growing for 4 wk with and without nitrogen, during the late summer and early fall of 2006 (a total of 10 wk after potting), plants were harvested and phenotyped for total height, stem diameter at 5 cm above the bottom of the cutting, number of sylleptic (lateral) branches and number of internodes. After these measurements were taken, the plants were dissected into roots, main stem, leaves and sylleptic branches with leaves attached. By peeling off the bark, the main stem was further separated into two tissues: xylem and bark + phloem. Tissue samples were placed into barcode-labeled paper envelopes and dried in a 65°C drying room or a freeze-drier (Freezezone 18L Bulk Tray Dryer, Labconco, Kansas City, MO, USA), depending on the tissue. Dried tissues were conditioned in the laboratory for more than 1 wk before being weighed with high-precision balances. Weights of individual organs and tissues were summed to estimate above-ground (shoot) and total biomasses. Biomass allocation into root and shoot was calculated by the ratio of above-ground to root weights.

Wood chemical composition was measured in two biological replicates of the experiment from the 5–10 cm of bottom xylem of each plant's main stem. The xylem was ground in a Wiley mill to pass through a 20 mesh screen and re-dried. Two subsamples (approximately 4 mg each) of milled wood from each plant were scooped into 80 µl stainless steel sample cups that were subsequently covered with glass fiber paper (type a/d). The cups were automatically loaded into a pyrolyzer coupled to a molecular beam mass spectrometer (py-MBMS) at the National Renewable Energy Laboratory of the US Department of Energy (NREL – DOE), Golden, CO, USA. Samples were separated into two blocks and completely randomized. To evaluate the consistency and accuracy of the instrument, one loblolly pine and three *Populus* wood samples, previously characterized with wet chemistry (Browning, 1967), were analyzed in the py-MBMS systematically after every 44 runs with our samples. Each subsample was pyrolyzed for 30 s at 500°C. Vapors from pyrolysis were rapidly expanded under vacuum through a 0.012 in crystal orifice, creating a molecular beam that was directed to the Extrel™ Model TQMS C50 mass spectrometer, yielding a spectrum ranging from 30 to 450 mass-to-charge (*m/z*) ratio. Data were normalized against differences in total ions of each pyrolyzed sample. Peaks associated with each wood chemistry component, based on previous literature (Evans & Milne, 1987), were summed to produce a single estimate, as indicated in Table 2 and described elsewhere (Sykes *et al.*, 2008).

Statistical analysis of phenotypic data

Before analyses of each phenotypic trait, the data were evaluated for the presence of outliers and removal or correction of measurements with recording errors. PROC INSIGHT (SAS Institute Inc. 9.1® 2004, Cary, NC, USA) was used to check the distribution of residuals. A square root transformation was

applied to the number of sylleptic branches because of the nonnormal distribution of residuals.

Univariate analyses of each trait were performed using the SAS® System for Mixed Models (Littell *et al.*, 1996) to separately account for the different sources of variation from our experiment. This analysis of variance allows an accurate estimation of the desired effects (i.e. clone and nitrogen treatment) by controlling the influence of undesired sources of variation (i.e. replicates, bench, row and column within replicate). The mixed model utilized was:

$$y_{ijklmno} = \mu + r_i + T_k + rt_{ik} + b_{j(i)} + c_l + rc_{il} + tc_{kl} + p_{m(i)} + q_{n(i)} + e_{ijklmno} \quad \text{Eqn 1}$$

$y_{ijklmno}$, response of the *o*th ramet of the *l*th clone in the *k*th treatment of the *j*th bench within the *i*th replication; μ , population mean; r_i , random effect of replication, ~ normally and independently distributed (NID) $(0, \sigma_r^2)$; T_k , fixed effect of the nitrogen treatment; rt_{ik} , effect of replication by treatment interaction, ~ NID $(0, \sigma_{rt}^2)$; $b_{j(i)}$, random effect of bench (incomplete block) within replication, ~ NID $(0, \sigma_{bc}^2)$; c_l , random effect of clone, ~ NID $(0, \sigma_c^2)$; rc_{il} , random effect of replication by clone interaction, ~ NID $(0, \sigma_{rc}^2)$; tc_{kl} , random effect of treatment by clone interaction, ~ NID $(0, \sigma_{tc}^2)$; $p_{m(i)}$, random effect of row within replication, ~ NID $(0, \sigma_p^2)$; $q_{n(i)}$, random effect of column within replication, ~ NID $(0, \sigma_q^2)$; $e_{ijklmno}$, random error effect within the experiment,

$$\sim \text{NID}(0, R) \text{ and } R = \begin{bmatrix} \sigma_{e_2}^2 & 0 & 0 \\ 0 & \sigma_{e_2}^2 & 0 \\ 0 & 0 & \sigma_{e_3}^2 \end{bmatrix}.$$

The genetic analyses were modified to include different error variances for each replication. $\sigma_r^2, \sigma_{rt}^2, \sigma_{bc}^2, \sigma_c^2, \sigma_{rc}^2, \sigma_{tc}^2, \sigma_p^2, \sigma_q^2$ and $\sigma_{e_1}^2, \sigma_{e_2}^2, \sigma_{e_3}^2$ are the variance components corresponding to replication, replication by treatment, bench within replication, clone, replication by clone, treatment by clone, row within replication, column within replication and residual effects of each replication, respectively.

Variance components and genetic parameters were estimated by restricted maximum likelihood, using ASReml (Gilmour *et al.*, 2002). Least-square means used in the QTL analysis were calculated by including both clone effect and its interaction with treatment as fixed effects in the model.

Clonal repeatability for each trait in the univariate analysis was calculated by ASReml with the estimates of variance components as follows: $H^2 = \sigma_c^2 / \sigma_c^2 + \sigma_{rc}^2 + \sigma_{tc}^2 + (\sum_1^3 \sigma_e^2 / 3)$ with $\sigma_c^2, \sigma_{rc}^2, \sigma_{tc}^2, \sigma_{e_1}^2, \sigma_{e_2}^2, \sigma_{e_3}^2$ as previously defined.

To estimate phenotypic and genetic correlations, pair-wise traits were analyzed with the following bivariate model equation:

$$y_i = X_i b_i + Z_1 a_i + Z_2 d_i + Z_3 b_i + Z_4 k_i + Z_5 l_i + Z_6 m_i + Z_7 n_i + e_i \quad \text{Eqn 2}$$

where y_i is the vector of observations for traits 1 (t_1) and 2 (t_2), b_i is the vector of fixed effects (i.e. means and treatments) associated with the incidence matrix X_i , a_i is the vector of random clonal effects associated with the incidence matrix Z_i and $a_{i-MVN}(0, G)$, with

$$G = \begin{bmatrix} \sigma_{c(t_1)}^2 & \sigma_{c(t_1 t_2)} \\ \sigma_{c(t_1 t_2)} & \sigma_{c(t_2)}^2 \end{bmatrix}$$

$d_i, h_i, k_i, l_i, m_i,$ and n_i are random effects corresponding to replication by treatment, bench within replication, replication by clone, treatment by clone, row within replication and column within replication associated with known incidence matrices Z_2, Z_3, Z_4, Z_5, Z_6 and Z_7 , respectively. All random effects have zero mean and variance structure similar to G . e_i is the vector of random error effects and $e_{i-MVN}(0, R)$, with

$$R = \begin{bmatrix} R_1 & 0 & 0 \\ 0 & R_2 & 0 \\ 0 & 0 & R_3 \end{bmatrix}$$

and

$$R_1 = \begin{bmatrix} \sigma_{e_1(t_1)}^2 & \sigma_{e_1(t_1 t_2)} \\ \sigma_{e_1(t_1 t_2)} & \sigma_{e_1(t_2)}^2 \end{bmatrix}, R_2 = \begin{bmatrix} \sigma_{e_2(t_1)}^2 & \sigma_{e_2(t_1 t_2)} \\ \sigma_{e_2(t_1 t_2)} & \sigma_{e_2(t_2)}^2 \end{bmatrix}$$

$$\text{and } R_3 = \begin{bmatrix} \sigma_{e_3(t_1)}^2 & \sigma_{e_3(t_1 t_2)} \\ \sigma_{e_3(t_1 t_2)} & \sigma_{e_3(t_2)}^2 \end{bmatrix}$$

where G and R are covariance–variance matrices corresponding to vectors a_i and e_i , respectively, and the R structure was associated with error effects of each replication. $\sigma_{c(t_1)}^2, \sigma_{c(t_2)}^2, \sigma_{c(t_1 t_2)}$ are clonal variance components for trait 1, trait 2 and the covariance component between trait 1 and trait 2. $\sigma_{e_1(t_1)}^2, \sigma_{e_1(t_2)}^2, \sigma_{e_1(t_1 t_2)}$ are error variance components and the covariance component, respectively, for trait 1 and trait 2 in replication 1. Replications 2 and 3 have similar descriptions of error variance and covariance components.

All traits were analyzed according to the full model (Eqn 2), but effects with no variation were dropped to fit the final model.

After estimating the variance and covariance components, genetic and phenotypic correlations were calculated for pairs of traits according to the following equation:

$$r_{t_1 t_2} = \frac{\sigma_{t_1 t_2}}{\sqrt{\sigma_{t_1}^2 \times \sigma_{t_2}^2}}$$

($r_{t_1 t_2}$, genetic or phenotypic correlation between trait 1 and trait 2; $\sigma_{t_1 t_2}$, clonal genetic covariance or phenotypic covariance between two traits; $\sigma_{t_1}^2, \sigma_{t_2}^2$, clonal genetic or phenotypic variances of trait 1 and trait 2.)

Genetic linkage map and QTL analysis

From a dense microsatellite and microarray-based genetic linkage map (Drost *et al.*, 2009), we selected 181 evenly distributed markers that segregate in the hybrid female parent. The selection favored microsatellite (simple sequence repeat, SSR) markers (163 selected) because they were genotyped in all 396 individuals of the progeny, whereas microarray-based markers were characterized in only 154. Eighteen microarray markers were included to expand map coverage towards the flanks of some linkage groups (LGs) and to fill a gap in LG6 where no SSR was genotyped. MapMaker 3.0 (Lander *et al.*, 1987) was utilized for the construction of the linkage map with a maximum recombination frequency of 45 cM using Kosambi's map function (Kosambi, 1944) and a minimum logarithm of the odds ratio (LOD) of three. Consistent with the haploid number of chromosomes of *P. trichocarpa* and *P. deltoides*, our map has 19 LGs, with a total length of 2889 cM and an average density of one marker every 16 cM. BLAST alignment of SSR primer and microarray probe sequences to the poplar genome sequence (JGI v.1.1) demonstrates that our map spans at least 85% of the assembled genome.

The linkage map was used for the identification of QTLs with composite interval mapping (Zeng, 1993) implemented in Windows QTL Cartographer v.2.5 (Wang *et al.*, 2007). We utilized the standard model 6 with default settings for the selection of cofactor markers to account for background variance not associated with the locus being tested. Least-square means estimates for each individual in each nitrogen treatment were used in the analysis. For each phenotypic trait, QTL analyses for 1000 permutations were performed, establishing a null distribution of genome-wide maximum LOD scores, where the 95th percentile was defined as the significance threshold (Churchill & Doerge, 1994).

Results

Genetic control of phenotypic traits and the effect of nitrogen treatments

For brevity, all growth and allocation traits depicted in Table 1 are referred to as phenotypic traits, whereas wood composition and partitioning traits depicted in Table 2 are called wood chemistry traits. Clonal repeatability (or within-family broad-sense heritability) was estimated for each trait across the entire dataset (i.e. all replicates and both nitrogen treatments). Estimates of clonal repeatability were moderate for all 12 phenotypic traits, ranging from 0.25 for sylleptic branch biomass to 0.38 for sylleptic branch count (Table 1). The effect of nitrogen fertilization was highly significant for all traits, increasing diameter, height, number of internodes, number of sylleptic branches and above-ground biomass, whereas root biomass was significantly decreased when

Table 1 Estimates of clonal repeatability and average trait value for each of 12 phenotypes measured in two nitrogen treatments

Trait	Acronym	Clonal repeatability	Nitrogen deficiency (0 mM)	High nitrogen (25 mM)	Prob > t
		$H^2 \pm SE$	Mean \pm SE	Mean \pm SE	
Diameter (cm)	diam	0.328 \pm 0.025	4.41 \pm 0.031	5.087 \pm 0.043	0
Height (cm)	ht	0.325 \pm 0.027	67.751 \pm 0.712	78.9 \pm 0.739	0
Height live crown (cm)	htlc	0.338 \pm 0.026	61.517 \pm 0.735	74.958 \pm 0.749	0
Internodes count	int	0.344 \pm 0.033	29.163 \pm 0.219	34.21 \pm 0.241	0
Leaf biomass (g)	tleaf	0.305 \pm 0.027	4.86 \pm 0.092	6.734 \pm 0.12	0
Stem biomass (g)	tstem	0.298 \pm 0.026	3.814 \pm 0.08	4.469 \pm 0.096	1.69E-07
Sylleptic branch count	sylf	0.382 \pm 0.028	1.819 \pm 0.08	4.097 \pm 0.171	0
Sylleptic branch biomass (g)	sylwt	0.249 \pm 0.04	0.8 \pm 0.057	2.053 \pm 0.097	0
Above-ground biomass (g)	abbio	0.314 \pm 0.025	9.064 \pm 0.186	12.462 \pm 0.244	0
Root biomass (g)	rootwt	0.303 \pm 0.025	2.204 \pm 0.057	1.265 \pm 0.033	4.14E-44
Ratio abbio/rootwt	rabbe	0.312 \pm 0.029	5.073 \pm 0.076	11.896 \pm 0.137	0
Total biomass (g)	tbio	0.31 \pm 0.026	11.267 \pm 0.241	13.742 \pm 0.278	2.26E-11

Standard error (SE) is depicted for each estimate. The last column contains *P* values of a *t*-test assessing the effect of nitrogen treatment in each phenotype.

Table 2 Estimates of clonal repeatability and average trait value for each of eight wood chemistry phenotypes estimated in two nitrogen treatments using a pyrolyzer coupled to a molecular beam mass spectrometer (py-MBMS)

Trait	Acronym	<i>m/z</i> peaks sum	Clonal repeatability	Nitrogen deficiency (0 mM)	High nitrogen (25 mM)	Prob > t
			$H^2 \pm SE$	Mean \pm SE	Mean \pm SE	
Five-carbon hemicellulose sugar	C5	57 + 73 + 85 + 96 + 114	0.163 \pm 0.039	25.678 \pm 0.039	27.019 \pm 0.078	0
Six-carbon cellulose sugar	C6	57 + 60 + 73 + 98 + 126 + 144	0.174 \pm 0.04	32.888 \pm 0.065	35.540 \pm 0.137	0
Ratio C6/C5	C6/C5		0.170 \pm 0.039	1.280 \pm 0.001	1.311 \pm 0.002	0
Syringyl lignin monomer	S-lignin	154 + 167 + 168 + 182 + 194 + 208 + 210	0.338 \pm 0.038	13.03 \pm 0.054	10.153 \pm 0.048	3.81E-280
Guaiacyl lignin monomer	G-lignin	124 + 137 + 138 + 150 + 164 + 178	0.150 \pm 0.039	13.156 \pm 0.029	11.160 \pm 0.039	9.44E-294
Ratio S-lignin/G-lignin	S/G		0.378 \pm 0.031	0.993 \pm 0.004	0.917 \pm 0.004	8.24E-41
Total lignin	Lignin	G-lignin + S-lignin + 120 + 152 + 180 + 181	0.234 \pm 0.039	21.690 \pm 0.06	17.382 \pm 0.063	0
Ratio C6/lignin	RatioCL		0.182 \pm 0.04	1.543 \pm 0.007	2.119 \pm 0.015	0

Standard error (SE) is depicted for each estimate. The last column contains *P* values of a *t*-test assessing the effect of nitrogen treatment in each phenotype.

compared with the nitrogen deficiency treatment (Table 1, Fig. 1). Carbon allocation favored shoot over root biomass, when nitrogen fertilization was applied. The average above- to below-ground biomass ratio increased significantly from 5.07 to 11.90 under 0 to 25 mM NH_4NO_3 treatments.

Estimates of clonal repeatability for wood chemistry traits ranged from 0.15 for G-lignin to 0.38 for partitioning between S- and G-lignin (S/G ratio). Most of the clonal repeatability estimates for wood chemistry traits were lower than those calculated for phenotypic traits, except for S-lignin (0.34) and the S/G ratio (0.38). The effect of nitrogen fertilization was highly significant for wood chemistry traits, increasing cellulose and hemicellulose and decreasing lignin when compared with plants grown under limiting nitrogen.

In response to nitrogen, the increase in carbohydrates was greater for cellulose compared with hemicellulose, as observed in the C6/C5 ratio. The decrease in mean lignin content in response to nitrogen was 7% greater for S-lignin than G-lignin, as observed in the S/G ratio (Table 2).

Analysis of genetic and phenotypic correlations among traits

Pair-wise phenotypic and genotypic correlations were estimated for all traits across the entire experiment, that is combining data from the two nitrogen treatments (Table 3; Table S1, see Supporting Information). Only genetic correlations are depicted in parentheses when a specific

Table 3 Pair-wise estimates of phenotypic (below diagonal) and genotypic (above diagonal) correlations between traits (see Tables 1, 2 for acronym description)

Trait	ht	diam	sylwt	rootwt	htlc	sylf	tleaf	tstem	tbio	abbio	rabbe	int	C6	C5	C6/C5	Lignin	S-lignin	G-lignin	S/G	RatioCL
ht	X	0.78	0.22	0.70	0.96	0.42	0.69	0.91	0.78	0.80	-0.13	0.67	0.26	0.36	0.00	-0.13	0.06	-0.50	0.27	0.20
diam	0.75	X	0.12	0.76	0.73	0.35	0.74	0.85	0.80	0.79	-0.26	0.50	0.11	0.18	-0.07	-0.03	0.10	-0.34	0.22	0.13
sylwt	0.15	0.19	X	0.43	0.40	0.80	0.35	0.26	0.46	0.45	-0.03	0.38	0.53	0.46	0.63	-0.60	-0.50	-0.45	-0.25	0.56
rootwt	0.70	0.74	0.49	X	0.74	0.43	0.86	0.81	0.90	0.86	-0.63	0.59	0.55	0.60	0.36	-0.37	-0.17	-0.61	0.07	0.53
htlc	0.94	0.70	0.25	0.72	X	0.56	0.73	0.89	0.83	0.84	-0.14	0.73	0.35	0.43	0.13	-0.23	-0.04	-0.51	0.18	0.33
sylf	0.26	0.31	0.70	0.42	0.39	X	0.34	0.40	0.50	0.50	0.04	0.58	0.46	0.42	0.46	-0.50	-0.41	-0.38	-0.27	0.48
tleaf	0.74	0.75	0.28	0.80	0.75	0.35	X	0.90	0.96	0.96	-0.22	0.57	0.50	0.53	0.33	-0.45	-0.29	-0.56	-0.03	0.52
tstem	0.86	0.81	0.23	0.76	0.83	0.32	0.90	X	0.93	0.94	-0.19	0.67	0.46	0.52	0.25	-0.27	-0.08	-0.53	0.14	0.45
tbio	0.76	0.77	0.44	0.84	0.78	0.46	0.95	0.92	X	1.00	-0.24	0.67	0.58	0.62	0.38	-0.48	-0.29	-0.61	-0.61	0.58
abbio	0.77	0.77	0.43	0.81	0.79	0.46	0.96	0.93	0.99	X	-0.15	0.67	0.53	0.57	0.36	-0.47	-0.29	-0.59	-0.59	0.54
rabbe	-0.03	-0.22	-0.15	-0.60	-0.05	-0.12	-0.23	-0.16	-0.29	-0.21	X	-0.05	-0.22	-0.25	-0.04	0.01	-0.09	0.30	-0.19	-0.15
int	0.70	0.54	0.32	0.61	0.71	0.44	0.62	0.64	0.65	0.66	-0.07	X	0.44	0.48	0.30	-0.43	-0.30	-0.46	-0.11	0.45
C6	0.23	0.16	0.31	0.43	0.31	0.42	0.42	0.31	0.43	0.43	-0.20	0.37	X	0.95	0.89	-1.00	-0.85	-0.72	-0.53	0.99
C5	0.30	0.22	0.32	0.47	0.35	0.39	0.44	0.36	0.45	0.45	-0.24	0.39	0.97	X	0.75	-0.87	-0.68	-0.77	-0.35	0.92
C6/C5	0.05	0.00	0.28	0.26	0.14	0.38	0.28	0.15	0.28	0.29	-0.11	0.24	0.81	0.59	X	-0.95	-0.93	-0.48	-0.81	0.97
Lignin	-0.23	-0.27	-0.45	-0.49	-0.33	-0.46	-0.47	-0.37	-0.48	-0.48	0.19	-0.42	-0.78	-0.71	-0.73	X	0.93	0.57	0.69	-0.98
S-lignin	-0.02	-0.07	-0.38	-0.27	-0.13	-0.41	-0.30	-0.16	-0.29	-0.30	0.07	-0.27	-0.63	-0.51	-0.72	0.90	X	0.23	0.91	-0.92
G-lignin	-0.46	-0.45	-0.29	-0.61	-0.49	-0.33	-0.55	-0.51	-0.55	-0.55	0.27	-0.46	-0.64	-0.68	-0.40	0.72	0.34	X	-0.27	-0.70
S/G	0.28	0.21	-0.20	0.11	0.18	-0.22	0.04	0.17	-0.55	-0.55	-0.08	0.01	-0.27	-0.11	-0.51	0.47	0.80	-0.19	X	-0.63
RatioCL	0.25	0.24	0.35	0.52	0.37	0.48	0.47	0.41	0.48	0.49	-0.22	0.41	0.96	0.89	0.83	-0.89	-0.75	-0.68	-0.35	X

Bold type shows pair-wise traits with correlation > |0.60|.

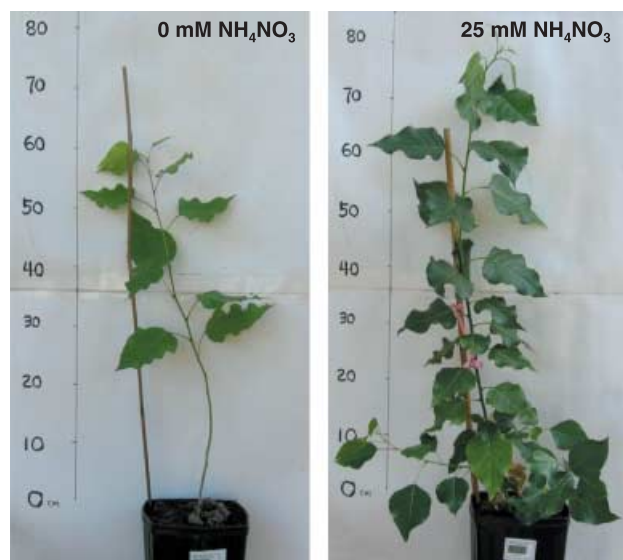


Fig. 1 Effect of nitrogen fertilization on shoot biomass accumulation on one genotype of the *Populus* pseudo-backcross population. After establishment, the plant on the left was grown under nitrogen deficiency (0 mM of NH_4NO_3), whereas the plant on the right was treated with 25 mM NH_4NO_3 . A height scale (in cm) is depicted in each photograph.

pair-wise relationship is described in the text. Most of the morphological and biomass traits were positively correlated phenotypically and genetically with each other – for example, height was strongly and positively correlated with diameter

(0.78), stem biomass (0.91), leaf biomass (0.69), above-ground biomass (0.79) and total biomass (0.78). All biomass traits were also positively correlated with each other. For example, root biomass was strongly genetically correlated with leaf (0.86), stem (0.81), above-ground (0.86) and total (0.90) biomass. As expected, plants growing more rapidly tended to accumulate biomass at higher rates in all vegetative tissues. The ratio between above- and below-ground biomass was the only trait that was not strongly correlated with any other phenotypic trait, except for its autocorrelation with root biomass (-0.63).

Wood chemistry traits were genetically and phenotypically correlated among themselves (Table 3). Cellulose (C6) levels were strongly positively correlated with the amount of hemicelluloses (C5) (0.95). By contrast, both C5 and C6 amounts were strongly negatively correlated with total lignin (-0.87 and -0.99), G-lignin (-0.77 and -0.71) and S-lignin (-0.68 and -0.85) contents. The almost perfect negative genetic correlation between cellulose and lignin content (-0.99) decreased to -0.78 at the phenotypic level. The ratio between wood carbohydrates (C6/C5) was negatively correlated with total lignin (-0.95) and S/G ratio (-0.81). This suggests that, when more carbon is partitioned into cellulose relative to hemicellulose, the total amount of lignin decreases and a disproportionate reduction in syringyl relative to guaiacyl monomers is observed. Lignin with a higher proportion of syringyl monomers contains less carbon-carbon cross-links and therefore is more easily extracted than guaiacyl-rich lignin (Chang & Sarkanen, 1973). Thus, in this population, carbon

partitioning into carbohydrates ($C6 > C5$) is associated with higher proportions of a less extractable type of lignin monomer. Variation in these traits was very high in both nitrogen treatments. The proportions of $C5$ and $C6$ carbohydrates were in the ranges 21–34% and 26–48%, respectively. For lignin syringyl monomers, the proportion ranged from 7 to 19%.

Biomass and wood chemistry were also strongly correlated (Table 3). In general, wood carbohydrate contents ($C5$ and $C6$) were positively correlated with the biomass of all vegetative tissues, whereas higher lignin was associated with lower levels of biomass accumulation. For example, total biomass was genetically correlated with $C6$ (0.57), $C5$ (0.62) and lignin (-0.48). The ratio between wood carbohydrates ($C6/C5$) was positively correlated with biomass traits, especially with sylleptic branches (0.63), but not correlated with height and diameter. This result indicates that, in the existing

experimental conditions, carbon partitioned to cellulose may lead to higher biomass accumulation than carbon partitioned to hemicelluloses. However, it is difficult to establish any relationships of cause and effect, and it is also possible that the higher cellulose accumulation is the result of superior growth ability. The ratio between lignin monomers (S/G) was negatively correlated with above-ground biomass (-0.59), but not genetically correlated with below-ground (root) biomass (0.06). Therefore, the higher the above-ground biomass, the lower the extractability of lignin in woody tissues.

QTL mapping in each nitrogen treatment

A total of 63 QTLs was identified in the two nitrogen treatments (0 and 25 mM) using $\alpha = 0.05$ as threshold defined with 1000 permutations (Table 4, Fig. 2). S-Lignin was the only trait for which no QTL was mapped in either

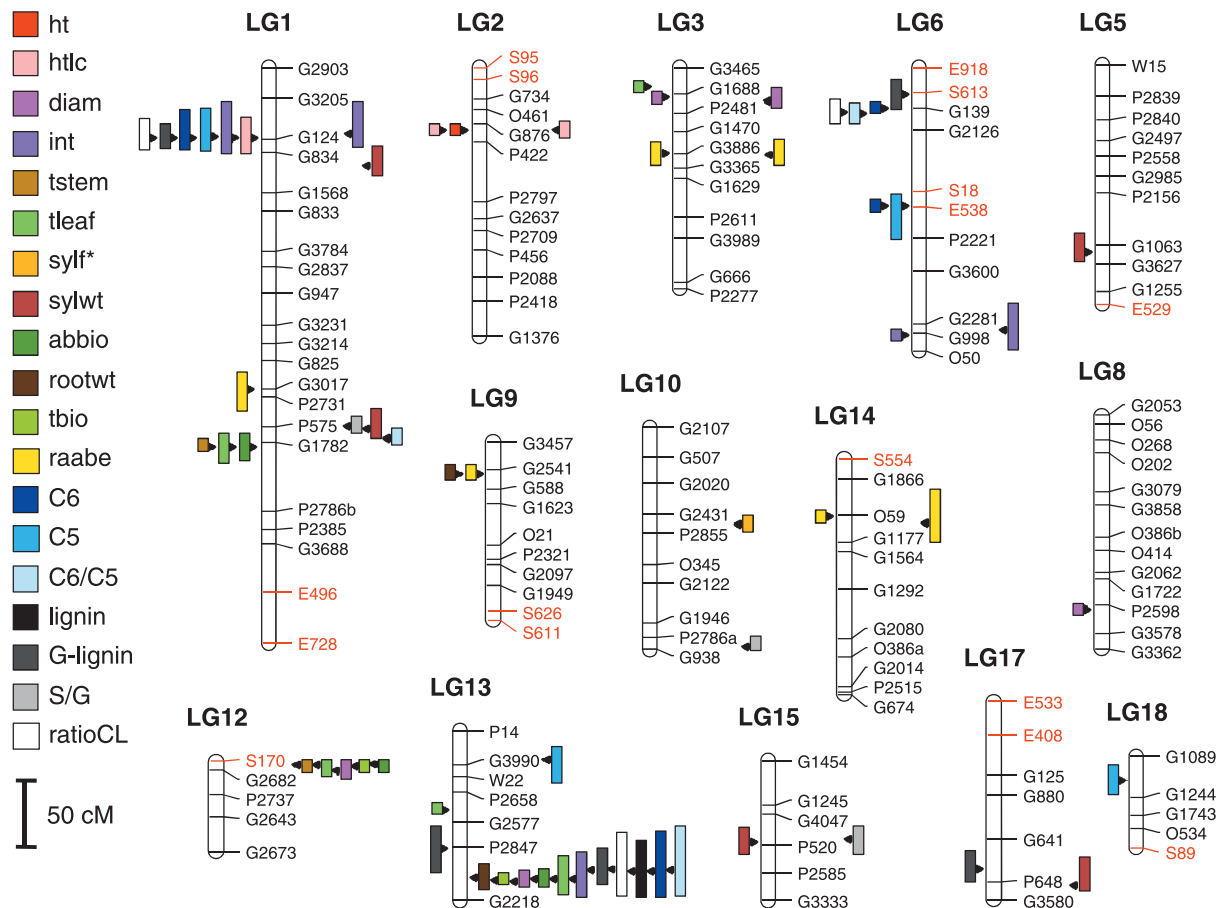


Fig. 2 Map location of all 63 quantitative trait loci (QTLs) identified in our experiment. QTLs mapped under nitrogen deficiency treatment are represented on the left of each linkage group, whereas QTLs mapped under high nitrogen are on the right. Colored bars indicate map regions in which the logarithm of the odds ratio (LOD) profile is above statistical threshold, and black arrows mark the position of the LOD peak for each QTL. QTLs spanning < 10 cM above threshold are represented by 10 cM bars. Microsatellite markers are identified in black and array-based markers in red. Linkage groups in which no QTLs were mapped are not shown. *QTL for sylf was mapped elsewhere (Ma *et al.*, 2008). (See Tables 1, 2 for trait acronyms.)

Table 4 Linkage group (LG) and flanking marker localization for the 63 quantitative trait loci (QTLs) identified under the two nitrogen treatments (H, high; D, deficiency)

QTL	Nitrogen treatment	Trait acronym	LG	Flanking markers		Origin of positive allele	LOD peak	Phenotypic variance explained
				Marker 1	Marker 2			
1	H	abbio	12	S170	P2737	<i>P. deltooides</i>	3.31	4.36%
2	H	abbio	13	P2847	G2218	<i>P. trichocarpa</i>	3.05	7.04%
3	H	diam	3	G3465	P2481	<i>P. deltooides</i>	3.83	5.32%
4	H	diam	12	S170	P2737	<i>P. deltooides</i>	4.20	5.25%
5	H	diam	13	P2847	G2218	<i>P. trichocarpa</i>	3.18	7.03%
6	H	htlc	2	O461	P422	<i>P. deltooides</i>	3.74	4.85%
7	H	int	1	G3205	G834	<i>P. trichocarpa</i>	4.72	7.26%
8	H	int	6	G3600	O50	<i>P. trichocarpa</i>	5.66	6.69%
9	H	int	13	P2847	G2218	<i>P. trichocarpa</i>	4.71	9.20%
10*	H	sylf	10	G2431	P2855	<i>P. trichocarpa</i>	3.01	7.20%
11	H	sylwt	1	G124	G1568	<i>P. trichocarpa</i>	4.98	5.70%
12	H	sylwt	1	P2731	G1782	<i>P. trichocarpa</i>	4.30	4.90%
13	H	sylwt	17	G641	G3580	<i>P. trichocarpa</i>	4.98	6.18%
14	H	rabbe	3	G1470	G3365	<i>P. deltooides</i>	4.97	5.55%
15	H	rabbe	14	G1866	G1177	<i>P. deltooides</i>	6.93	9.75%
16	H	rootwt	13	P2847	G2218	<i>P. trichocarpa</i>	3.11	7.35%
17	H	tbio	12	S170	P2737	<i>P. deltooides</i>	3.42	4.51%
18	H	tbio	13	P2847	G2218	<i>P. trichocarpa</i>	3.18	7.52%
19	H	tleaf	12	S170	P2737	<i>P. deltooides</i>	3.89	5.11%
20	H	tleaf	13	P2847	G2218	<i>P. trichocarpa</i>	3.86	9.52%
21	H	tstem	12	S170	G2682	<i>P. deltooides</i>	3.47	4.60%
22	H	C5	13	P14	P2658	<i>P. trichocarpa</i>	3.17	5.03%
23	H	C6	13	G2577	G2218	<i>P. trichocarpa</i>	4.78	11.24%
24	H	C6/C5	1	P575	G1782	<i>P. trichocarpa</i>	2.97	3.58%
25	H	C6/C5	13	G2577	G2218	<i>P. trichocarpa</i>	5.23	11.36%
26	H	G-lignin	13	P2847	G2218	<i>P. deltooides</i>	3.24	6.78%
27	H	Lignin	13	G2577	G2218	<i>P. deltooides</i>	4.51	9.61%
28	H	RatioCL	13	G2577	G2218	<i>P. trichocarpa</i>	4.69	10.17%
29	H	S/G	1	P2731	G1782	<i>P. deltooides</i>	3.11	3.88%
30	H	S/G	10	G1946	G938	<i>P. deltooides</i>	3.63	4.27%
31	H	S/G	15	G4047	P2585	<i>P. trichocarpa</i>	3.39	5.03%
32	D	abbio	1	P575	P2786b	<i>P. trichocarpa</i>	3.52	6.10%
33	D	diam	3	G3465	P2481	<i>P. deltooides</i>	3.36	4.61%
34	D	diam	8	P2598	G3578	<i>P. trichocarpa</i>	3.29	4.42%
35	D	ht	2	G876	P422	<i>P. deltooides</i>	3.21	4.50%
36	D	htlc	1	G3205	G834	<i>P. trichocarpa</i>	4.70	6.49%
37	D	htlc	2	G876	P422	<i>P. deltooides</i>	3.02	3.94%
38	D	int	1	G3205	G834	<i>P. trichocarpa</i>	6.33	9.91%
39	D	int	6	G2281	O50	<i>P. trichocarpa</i>	3.44	3.79%
40	D	sylwt	5	G1063	G3627	<i>P. trichocarpa</i>	3.58	5.10%
41	D	sylwt	15	G4047	P2585	<i>P. deltooides</i>	3.30	4.62%
42	D	rabbe	1	G825	P575	<i>P. trichocarpa</i>	3.30	4.24%
43	D	rabbe	3	G1470	G3365	<i>P. deltooides</i>	3.93	4.38%
44	D	rabbe	9	G3457	G588	<i>P. trichocarpa</i>	3.01	3.64%
45	D	rabbe	14	G1866	G1177	<i>P. deltooides</i>	2.95	3.95%
46	D	rootwt	9	G3457	G588	<i>P. deltooides</i>	3.33	4.34%
47	D	tleaf	1	P575	P2786b	<i>P. trichocarpa</i>	3.37	6.98%
48	D	tleaf	3	G3465	G1688	<i>P. deltooides</i>	3.11	4.90%
49	D	tleaf	13	P2658	G2577	<i>P. trichocarpa</i>	3.07	4.42%
50	D	tstem	1	P575	P2786b	<i>P. trichocarpa</i>	3.10	3.97%
51	D	C5	1	G3205	G834	<i>P. trichocarpa</i>	5.84	8.06%
52	D	C5	6	S18	P2221	<i>P. deltooides</i>	4.09	8.79%
53	D	C5	18	G1089	G1244	<i>P. trichocarpa</i>	3.63	6.85%
54	D	C6	1	G3205	G834	<i>P. trichocarpa</i>	5.80	7.96%
55	D	C6	6	S613	G2126	<i>P. deltooides</i>	2.94	3.85%
56	D	C6	6	S18	E538	<i>P. deltooides</i>	3.28	7.38%
57	D	C6/C5	6	S613	G2126	<i>P. deltooides</i>	3.58	4.73%

Table 4 continued

QTL	Nitrogen treatment	Trait acronym	LG	Flanking markers		Origin of positive allele	LOD peak	Phenotypic variance explained
				Marker 1	Marker 2			
58	D	G-lignin	1	G3205	G834	<i>P. deltoides</i>	3.90	4.95%
59	D	G-lignin	6	E918	G139	<i>P. trichocarpa</i>	3.58	6.97%
60	D	G-lignin	13	G2577	G2218	<i>P. deltoides</i>	4.01	7.51%
61	D	G-lignin	17	G641	P648	<i>P. deltoides</i>	3.62	6.24%
62	D	RatioCL	1	G3205	G834	<i>P. trichocarpa</i>	4.11	5.30%
63	D	RatioCL	6	S613	G2126	<i>P. deltoides</i>	3.35	4.42%

*QTL for sylf was mapped elsewhere (Ma *et al.*, 2008).

Also depicted for each QTL are the grand-parent origin of the allele with positive effect on the trait, logarithm of the odds ratio (LOD) score and percentage of the phenotypic variance explained.

nitrogen treatment. Because of the trait's heavy skewed distribution, QTL for sylleptic branch count (sylf) reported here was analyzed elsewhere with a Poisson statistical model (Ma *et al.*, 2008). Under high nitrogen, 31 QTLs were identified, 21 for 11 phenotypic traits and 10 for seven wood chemistry traits. Under the nitrogen deficiency treatment, 32 QTLs were detected, 19 for 10 phenotypic and 13 for five wood chemistry traits. The origin of the QTL alleles positively affecting the traits was balanced, with 34 coming from *P. trichocarpa* and 29 from *P. deltoides* grand-parents of the pedigree. Under high nitrogen, the most significant QTL (LOD = 6.93) was detected on LG14 for biomass allocation between above and below ground. In the nitrogen-limiting treatment, the most significant QTL (LOD = 6.33) was observed on LG1 for the number of internodes (Table 4). The percentage of the phenotypic variance explained by the QTLs ranged from 3.58% for the C6/C5 ratio to 11.36% for the same trait under high nitrogen treatment. Under high nitrogen treatment, LG13 had the most QTLs with 12, whereas, under nitrogen deficiency, LG1 had the most QTLs mapped, with loci controlling 10 different traits. On these two LGs, large fractions of the QTLs were detected in the same regions (Fig. 2). Five of the 19 LGs had no QTLs mapped in our study.

Phenotypic plasticity in response to nitrogen treatments

Only six QTLs for four phenotypic traits were mapped in the same genomic region under both nitrogen treatments (compare QTLs on both sides of each LG in Fig. 2). For the number of internodes, two QTLs (LG1 and LG6) were co-localized in both treatments. Similarly, for the ratio between above- and below-ground biomass, QTLs were detected on LG3 and LG14. Also mapped consistently under both nitrogen conditions were QTLs for the height of live crown (LG2) and diameter (LG3). The grand-parent origin of the positive allele was the same under both nitrogen treatments for all QTLs, indicating that the same genetic elements are likely to control the traits independent of nitrogen availability.

All 51 other QTLs identified in our pedigree were treatment specific, suggesting that, in *Populus*, a large fraction of the interspecific variance in growth, carbon allocation and carbon partitioning traits is highly responsive to the level of nitrogen available in the environment. For wood chemistry traits, none of the QTLs was mapped under both nitrogen treatments.

Co-localization of QTLs for different traits

In this interspecific *Populus* pedigree, we found eight genomic regions that controlled part of the variation for at least two different traits under the same nitrogen treatment. For example, under high nitrogen, there were two regions with QTLs co-localized for several traits – one on LG12 between markers S170 and G2682 and another on LG13 between P2847 and G2218 (Fig. 2). The traits with QTLs co-localized on LG12 were leaf, stem, above-ground and total biomass, as well as diameter. These traits were all positively, highly genetically correlated with each other and, as such, the QTL alleles had the same direction of effect in all traits – that is the *P. deltoides* QTL allele positively affected all traits. On LG13, there was a hot spot of co-localized QTLs for both phenotypic and wood chemistry traits. The traits with co-localized QTLs on LG13 were above-ground, below-ground, leaf and total biomass, diameter, number of internodes, cellulose, ratio between cellulose and hemicelluloses, lignin and ratio between cellulose and lignin. Consistent with the direction of correlations, the *P. trichocarpa* allele positively affected the total biomass (above- and below-ground), wood cellulose and hemicellulose, but negatively affected wood lignin content. Another co-localization of QTLs under high nitrogen occurred on LG1 for sylleptic branch biomass and S/G ratio.

Under nitrogen deficiency, five regions had co-localized QTLs for different traits (Fig. 2). Two of the regions occurred on LG1. The region between markers G3205 and G834 contained QTLs for height of the live crown, number of internodes, wood cellulose, hemicelluloses, guaiacyl lignin and ratio between cellulose and lignin, and the region between P575 and P2786b contained QTLs for above-ground, leaf

and stem biomass. As in the hot spot on LG13, the QTL alleles had opposite effects for wood carbohydrates and lignin, but the same direction of effect for height, number of internodes, biomass traits and wood carbohydrates. Between markers S613 and G2126 on LG6, QTLs were identified for the following wood chemistry traits: cellulose, guaiacyl lignin, ratio between cellulose and hemicellulose, and ratio between cellulose and lignin. Also on LG6, between markers S18 and E538, there were co-localized QTLs for wood cellulose and hemicellulose content. Co-localized QTLs for root weight and ratio between above- and below-ground biomass were mapped between markers G2541 and G588 of LG9.

Discussion

In this article, we report the widespread effects of nitrogen fertilization on the genetic regulation of growth and wood chemistry traits in the progeny of an interspecific pseudo-backcross of *P. deltoides* and *P. trichocarpa*. Nitrogen supply affected all growth and wood chemistry traits, confirming the extensive phenotypic plasticity of poplar in response to this essential nutrient. Studies have shown that nitrogen positively influences poplar tree height, diameter, number of leaves, number of sylleptic branches and shoot biomass (Pregitzer *et al.*, 1990; Ibrahim *et al.*, 1997; Kleiner *et al.*, 1998; Cooke *et al.*, 2005). However, contrary to our results, the same studies reported an increase in root biomass in response to nitrogen supply. Although statistical significance was either not observed (Cooke *et al.*, 2005) or not tested (Pregitzer *et al.*, 1990; Ibrahim *et al.*, 1997; Kleiner *et al.*, 1998), this disparity might reflect differences in experimental conditions. For example, in our trial, nutrients were supplied with a flood irrigation system in ebb-and-flow benches, whereas, in previous work, plants were fertilized with irrigation on the top of the pots. Despite differences in total root biomass, inarguably nitrogen fertilization caused significant changes in biomass allocation, decreasing root/shoot biomass ratio in poplar. Wood chemistry also responded to different nitrogen availability. Confirming previous studies with poplar trees (Pitre *et al.*, 2007a,b), we observed that nitrogen fertilization significantly increased wood cellulose content and decreased lignin content and its extractability, measured as the S/G ratio. We also found that nitrogen supply increased hemicelluloses at a significantly lower rate (5%) when compared with the increase in cellulose sugars (8%).

The extensive phenotypic plasticity in response to nitrogen availability observed in *Populus* may be triggered by changes in gene regulation (Sultan, 2000). Supporting this hypothesis in poplar, nitrogen significantly changed the transcript abundance of 52 cDNA clones identified by differential display (Cooke *et al.*, 2003). Consistent with a nitrogen-mediated decrease in wood lignin, two of the differentially expressed cDNA clones were from putative lignin biosynthesis genes, and were down-regulated in response to nitrogen. Similarly,

studies that assessed the effects of nitrogen fertilization on the whole transcriptome of *Arabidopsis* also demonstrated that nitrogen represses most of the genes from the phenylpropanoid pathway (Scheible *et al.*, 2004; Gutierrez *et al.*, 2008). Some genes involved in cell wall growth and modification, including expansins and xyloglucan:xyloglucosyl transferase, were induced in response to nitrogen. Nitrogen induction was also detected in most genes involved with photosynthesis, the Calvin cycle and photorespiration (Scheible *et al.*, 2004). Changes in gene expression in response to nitrogen fertilization may be the result of regulatory signals that balance carbon and nitrogen metabolism (Koch, 1997; Palenchar *et al.*, 2004; Weigelt *et al.*, 2008). The assimilation of nitrogen into amino acids depends on the availability of carbon skeletons, and carbon is stored in starch if nitrogen is deprived (Calenge *et al.*, 2006). When nitrogen is luxuriant (relatively low carbon to nitrogen ratio), plant metabolism shifts resources towards the absorption of carbon through photosynthesis and develops more shoot than root. Conversely, when nitrogen is limited (high carbon to nitrogen ratio), metabolism favors the allocation of biomass to root over shoot (Koch, 1997).

Consistent with the widespread phenotypic and gene expression plasticity of plants in response to nitrogen, our study detected extensive changes in the genetic control of carbon allocation and partitioning with nitrogen supply. Most of the QTLs identified in our study (51 of 63) are nitrogen treatment specific. In other plant species, significant QTL by nitrogen interactions have been reported for disease resistance and root traits in rice (Talukder *et al.*, 2005; MacMillan *et al.*, 2006), carbohydrate content and plant biomass in *Arabidopsis* (Rauh *et al.*, 2002; Calenge *et al.*, 2006), and grain yield and quality in wheat (Laperche *et al.*, 2007). Significant QTL by nitrogen treatment interaction indicates that fast- and slow-growing genotypes are not the same under limiting and luxuriant nitrogen conditions. Spearman correlation of phenotypic values under the two nitrogen treatments indicates extensive changes in rank among genotypes (Table S2, see Supporting Information). The even lower Spearman correlations between nitrogen treatments for wood chemistry traits indicate that most of these traits are more responsive to nitrogen supply than are biomass and growth traits. This agrees with the lower clonal repeatability observed for most wood components when compared with phenotypic traits (Tables 1, 2), which was not observed when clonal repeatability was estimated for each nitrogen treatment separately (Table S3, see Supporting Information). Supporting the idea that wood chemistry traits are more reactive to nitrogen availability, no QTLs for wood traits were consistently mapped in both nitrogen treatments, whereas six QTLs for phenotypic traits were mapped independently of nitrogen level.

Our results support the hypothesis that growth co-varies with wood chemistry traits in trees. As demonstrated previously

with mutants of *Populus* (Hu *et al.*, 1999) and *Pinus* (Wu *et al.*, 1999; Yu *et al.*, 2006), and in a segregating pedigree of *Eucalyptus* (Kirst *et al.*, 2004), we observed that fast-growing poplar trees have higher cellulose and lower lignin content when compared with slow-growing trees. To genetically dissect the association between growth and wood quality traits, we searched for common pleiotropic loci that regulate variation in both trait groups. We identified two common QTLs for growth and wood chemistry. One QTL cluster detected in our study occurs on LG13, between markers G2577 and G2218, and regulates growth, biomass and wood composition traits. Specifically, trees that inherited the *P. trichocarpa* allele at this locus grew faster, contained more cellulose and less lignin when compared with those that inherited the *P. deltoides* allele. Genomic regions in the vicinity of this LG13 QTL cluster control the abundance of metabolites derived from the phenylpropanoid pathway in an independent pedigree of *Populus* (Morreel *et al.*, 2006). The other QTL cluster coordinately controls the height of the live crown and wood composition under nitrogen deficiency and is located on LG1, between markers G3205 and G834. In this same region of LG1, QTLs have been mapped previously for height, stem circumference, stem volume, root density and root growth in family 331, which shares a common grand-parent (clone 93-968) with family 52-124 analyzed here (Rae *et al.*, 2007, 2008).

Other QTLs detected in our study were also detected in family 331. For example, both QTLs for the height of the live crown (LG1 and LG2), mapped under limiting nitrogen treatment, co-localized with the QTL for stem height identified by Rae *et al.* (2008). The QTL identified for sylleptic branch count under high nitrogen (LG10) from our experiment (Ma *et al.*, 2008) was also mapped in family 331 (Rae *et al.*, 2008). Two QTLs for above-ground biomass from our experiment (LG1 under limiting nitrogen and LG12 under high nitrogen) co-localized with QTLs for stem height and diameter in family 331 growing under elevated (LG1) or ambient (LG12) CO₂ (Rae *et al.*, 2007). Two of our QTLs for total leaf biomass (LG3 under limiting nitrogen and LG12 under luxuriant nitrogen) mapped in the same region of previously identified QTLs controlling leaf number (Rae *et al.*, 2006).

A number of interesting *a priori* candidate genes are physically positioned within the marker intervals flanking potential pleiotropic quantitative loci on LG1 and LG13. An immediately apparent candidate gene underlying the QTL cluster on LG13 is cinnamate-4-hydroxylase, encoding an enzyme that regulates the conversion of cinnamate to 4-coumarate in the phenylpropanoid biosynthesis pathway, and a transcript for which we previously identified a strong correlation with growth in the *Eucalyptus* segregating population (Kirst *et al.*, 2004). Similarly, the QTL cluster for growth and wood composition on LG1 harbors a cluster of three tandemly duplicated genes encoding putative cinnamoyl-

CoA reductases, which regulate the conversion of feruloyl-CoA to coniferaldehyde in the phenylpropanoid pathway. Additional components (chalcone synthase, 4-coumarate:CoA ligase) and potential regulators [*AtMYB12* homolog (Mehrtens *et al.*, 2005) and other uncharacterized R2R3-MYB-type transcription factors] of phenylpropanoid metabolism are also conceivable candidate genes within these intervals. Furthermore, several C5/C6 metabolic enzymes, including xyloglucan fucosyltransferase, glucose-6-phosphate-1-dehydrogenase, xyloglucan endotransglycosylase and cellulose synthase, are encoded by genes within these intervals. Finally, numerous genes of unknown function, or with no homology to known *Arabidopsis* or *Oryza* coding sequences, are prevalent among genes within the intervals, leaving open the possibility that some of these uncharacterized elements might coordinately regulate growth and biomass composition in woody plants. The gene models within marker intervals that flank most of our QTLs, including those clusters on LG1 and LG13, are available as Supporting Information (Table S4). Gene models within some QTL intervals (eight of 29) could not be found, because at least one of the flanking markers either did not have a BLAST hit in the genome or hit an unmapped scaffold.

The number of candidate genes within these QTLs is certainly too large to be tested with functional analysis – our average QTL interval is 31 cM wide and contains approximately 426 genes, 45% of which have unknown function (Table S4). Although a reduction in the size of the candidate gene pool is necessary, the traditional positional cloning-based isolation of the regulatory locus would be challenging given the breadth of the QTL intervals, low heritability of quantitative traits and the typical low resolution of maps in forest species. Nevertheless, the availability of the *P. trichocarpa* genome sequence (Tuskan *et al.*, 2006), allied to gene expression data that are being assayed with microarrays from three tissues (root, xylem and leaf) of our experiment, will form an excellent foundation to narrow down the candidate genes within QTLs. Integration of gene expression with genotypic and phenotypic data in a genetical genomics setting (Jansen & Nap, 2001) will uncover regions containing regulators of gene expression (eQTL) and of phenotypes. The identification of candidate genes for an eQTL/QTL hot spot can be achieved by elucidating genetic networks controlled by the region, as has been demonstrated in mammals (Mehrabian *et al.*, 2005; Meng *et al.*, 2007). Pinpointing genetic regulators linking tree growth to wood quality will ultimately enhance our ability to breed for and engineer genotypes improved for pulp, paper, below-ground carbon sequestration and cellulosic ethanol production.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Standard error for correlation estimates

Table S2 Spearman correlation (ρ) between high and deficient nitrogen treatments for each trait

Table S3 Comparison of clonal repeatability estimated with combined data from both nitrogen treatments and separately from plants grown under high and deficient nitrogen treatments

Table S4 Physical localization and annotation of gene models within each quantitative trait locus (QTL) interval

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