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Variations in bulk leaf carbon isotope discrimination, growth and related leaf traits among three *Populus nigra* L. populations

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The ongoing global change could be an additional threat to the establishment and the long-term survival of *Populus nigra* L., an emblematic European riparian species. With the general aim of gaining insights into the adaptive potential of this species, we (i) quantified variations within and among three French *P. nigra* populations for key physiological attributes, i.e., water-use efficiency (assessed from bulk leaf carbon isotope discrimination, $\Delta^{13}C$), growth performance and related leaf traits, (ii) examined genotype and population by environment interactions, and (iii) explored the relationship between $\Delta^{13}C$ and growth. Thirty genotypes were sampled in each of three naturally established populations and grown in two different sites, Orléans (ORL) and Guémené-Penfao (GMN). In ORL, two similar plots were established and different watering regimes were applied in order to test for the drought response. Significant variations were observed for all traits within and among populations irrespective of site and watering. Trait variation was larger within than among populations. The effect of drought was neither genotype- nor population-dependent, contrary to the effect of site. The population ranking was maintained in all sites and watering regimes for the two most complex traits: $\Delta^{13}C$ and growth. Moreover, these two traits were unrelated, which indicates that (i) water-use efficiency and growth are largely uncoupled in this species, and (ii) the environmental factors driving genetic structuration for $\Delta^{13}C$ and growth act independently. The large variations found within populations combined with the consistent differences among populations suggest a large adaptive potential for *P. nigra*.

Keywords: common gardens, European black poplar, phenotypic plasticity, population differentiation, water-use efficiency.

Introduction

European black poplar (*Populus nigra* L.) is a key pioneer tree species colonizing alluvial sediments along large rivers in temperate climate zones of Europe, northern Africa and western Asia (Dickmann and Kuzovkina 2008). Along with other riparian tree species such as willows, *P. nigra* plays a central role in the dynamics of riparian habitats since it is involved in various ecological processes such as the anchorage of sandy bars (Karrenberg et al. 2003), nutrient recycling and water purification (Ruffinoni et al. 2003), and the maintenance of faunistic biodiversity (Rotach 2004).

During the last few decades, modifications of European river systems have imposed significant threats to riparian communities. The two main factors currently threatening *P. nigra* are (i) the fragmentation and loss of its native habitat (Naiman et al. 2005), as well as (ii) hybridization with domestic poplars including the widespread Lombardy poplar (*P. nigra* cv. Italica) (Vanden Broeck et al. 2005). These two factors affect

population size and genetic diversity. Because the conservation of genetic diversity is a prerequisite to preserve the adaptive potential and the long-term survival of populations, programmes for the conservation of *P. nigra* genetic resources have been established in most European countries (European Forest Genetic Resources Network, EUFORGEN, Lefèvre et al. 2001, Villar and Forestier 2009).

The rise in the frequency of extreme events accompanying global change (IPCC 2007), especially drought and heat, is likely to cause more and more unexpected variations in river water tables as experienced in France during 2003. Changes in the patterns of flow regimes may affect seedling recruitment provided it occurs during spring seed dissemination (Stella and Battles 2010), but may also punctually affect the physiology of already established trees (Leffler and Evans 1999, Rood et al. 2003). An improved understanding of genotypic and phenotypic variations that occur within and among populations for key traits involved in carbon and water relations is therefore required to gain insights into the geographical structuration and adaptive potential of these populations. Water-use efficiency (WUE), defined at the whole-plant level as the ratio of biomass accumulation/water consumption, is likely to influence tree fitness and to therefore be a target for natural selection (Lauteri et al. 2004). Intrinsic WUE (WUE$_i$), which is the ratio of net CO_2 assimilation rate to stomatal conductance for water vapour, is a key component of whole-plant WUE. It can be recorded from its proxy, bulk leaf carbon isotope discrimination ($\Delta^{13}C$) (Farquhar and Richards 1984), including in poplar (Ripullone et al. 2004, Fichot et al. 2011). Significant variation for $\Delta^{13}C$, growth and related leaf traits (e.g., specific leaf area, leaf nitrogen content) has already been reported both within and among populations of different pure poplar species such as *Populus fremontii* Wats (Sparks and Ehleringer 1997, Leffler and Evans 2001), *Populus angustifolia* James (Sparks and Ehleringer 1997), *Populus davidiana* Dode (Zhang et al. 2004), *Populus trichocarpa* Torr. & Gray (Gornall and Guy 2007), *Populus cathayana* Rehd (Xu et al. 2008) and *Populus balsamifera* L. (Soolanayakanahally et al. 2009). Data related to the ecophysiology of *P. nigra* populations or genotypes remain rather limited, however (Ding et al. 2006, Regier et al. 2009, Cocozza et al. 2010, Centritto et al. 2011).

Many studies examining physiological differentiation within and among populations have been conducted in situ, and may therefore confound genetic and environmental (phenotypic) variations (e.g., Leffler and Evans 2001). Phenotypic plasticity, which is the ability of a genotype to express different phenotypes depending on the environment, is considered as the major means by which plant populations cope with environmental heterogeneity and may therefore confer adaptive potential (Bradshaw 1965, Nicotra et al. 2010). Common garden studies are required to separate genetic and environmental components of trait variation and allow the assessment of

genotype by environment (G × E) and population by environment interactions (P × E). Significant G × E and P × E interactions may indicate that the observed phenotypic plasticity is genotype- or population-dependent.

Here, we aimed at examining variations in physiological performances within and among three French *P. nigra* populations. Traits investigated included growth, $\Delta^{13}C$ and related leaf traits (specific leaf area, carbon and nitrogen contents). Our specific objectives were to (i) quantify within- and among-population variations, (ii) quantify phenotypic plasticity, i.e., precise genotype and P × E interactions, and (iii) explore the relationship between $\Delta^{13}C$ and growth performance. To answer these questions, 30 genotypes from three populations were grown in two sites. In one site, two different watering regimes were applied.

Materials and methods

Origin and sampling of plant material

Three French *P. nigra* populations, each occurring in Natural National Reserves and being part of the network of the French programmes of conservation of genetic resources of *P. nigra* L. (Villar and Forestier 2009), were retained. The Nohèdes population (NOH) originates from Southern France (Pyrénées Orientales) along the Nohèdes river on an alluvial soil with rocks. The Nohèdes river is very small (17 km), torrential type, with a natural flow regime varying from 0.05 m^3 s^{-1} in summer to 4.5 m^3 s^{-1} during peak periods (mean annual flow of 0.2 m^3 s^{-1}). The Ramières population (RAM) originates from South-Eastern France (Drôme) along the Drôme river, on a sandy alluvial soil (Regosol). The Drôme river is undammed along its 108 km and the flow regime can vary from 4 m^3 s^{-1} in summer to 900 m^3 s^{-1} during flooding periods (mean annual flow of 25 m^3 s^{-1}). The St-Pryvé St-Mesmin population (SPM) originates from Central France (Loiret) along the Loire river on an alluvial soil (Fluviosol). The Loire river is a long river (1020 km), partially regulated, with a flow regime ranging from 10 to 2000 m^3 s^{-1} (mean annual flow of 350 m^3 s^{-1}).

Climate data (1971–2000) of each source habitat were provided by Météo-France from the closest meteorological station (Table 1). Basic meteorological variables included mean annual temperature (MAT, °C), mean annual summer temperature (MAST, °C), mean annual precipitation (MAP, mm), mean annual summer precipitation (MASP, mm) and mean number of frost-free days (FFD, days); MAST and MASP data were derived from June-to-August data. The FFD is calculated based on the number of days when minimum temperature was above 0°C, and is used here as a proxy for season length (Soolanayakanahally et al. 2009). Also included were indices of annual and summer 'dryness' (ADI and SDI, respectively); these indices were derived as described in Soolanayakanahally et al. (2009) using MAT, MAP, MASP and the mean temperature

Table 1. Geoclimatic data for the three *P. nigra* populations.

Populations	LAT	LON	ELEV	MAT	MAST	MAP	MASP	FFD	ADI	SDI
NOH	42°37′24″N	2°16′36″E	1000	10.8	17.2	753.2	60.4	303	1.73	3.54
RAM	44°45′08″N	4°54′01″E	187	12.7	21.1	910.4	57.4	321	1.62	4.57
SPM	47°52′19″N	1°49′24″E	91	10.9	18.8	635.9	46.5	311	2.08	4.77

Data were retrieved from the meteorological stations located close to the three sites of origin.
NOH, Nohèdes (Pyrénées-Orientales); RAM, Ramières (Drôme); SPM, St-Pryvé St-Mesmin (Loiret); LAT, latitude; LON, longitude; ELEV, elevation (m); MAT, mean annual temperature (°C); MAST, mean annual summer temperature (°C); MAP, mean annual precipitation (mm); MASP, mean annual summer precipitation (mm); FFD, mean number of frost-free days based on the number of days when minimum temperature was above zero (days); ADI, annual dryness index; SDI, summer dryness index.

of the warmest month, higher ADI or SDI values being indicative of a drier habitat.

Branch cuttings were sampled on 30 sexually mature trees without a priori phenotypic selection in each population, along a linear distance depending on the size of the population (i.e., 1 km for NOH, 20 km for RAM and 2.3 km for SPM). The collection was done during January to March 2004 from the upper crowns of dormant trees. During sampling, attention was paid to reduce the possibility of sampling clones more than once by collecting well-separated individuals. Cuttings were then propagated and kept in a first common garden as a source of plant material until the plots were set up. A set of 11 nuclear simple sequence repeat markers actually revealed that all collected individuals were distinct genotypes (Jorge and Villar, unpublished data).

Experimental design and growth conditions

Three plots containing the three *P. nigra* populations were installed in France. The first two were located at the nursery of the INRA Research Unit of Orléans (Loiret, 47°49′40″N, 1°54′45″E, 110 m a.s.l.) but they differed in terms of water regime. A third one, which is the Forest State nursery of Guémené-Penfao (Loire-Atlantique, 47°37′46″N, 1°53′33″W, 20 m a.s.l.), was also included in the present study.

Plots at Orléans (ORL$_{WW}$ and ORL$_{WD}$)

Two plots were set up in June 2006, 15 m apart from each other, with five randomized complete blocks with one cutting of each genotype per block, on a loamy sand soil without fertilizer addition. Hardwood cuttings (0.25 m long) were planted at an initial spacing within and between rows of 0.75 × 1.2 m, respectively. One border row of the same *P. nigra* genotype ('Blanc de Garonne') was planted around each plot to minimize edge effects. All trees were cut back at the end of 2006 and 2007, and in early spring of each growing season only the most vigorous shoot of each stump was retained. Plantation management included mechanical and manual weed control, as well as fungicide (Horizon, tebuconazole) and insecticide (Decis, deltamethrine) treatments when required. During 2007, the two plots were irrigated with overhead sprinklers to meet evaporative demand (i.e., 4.5 mm of water was sprinkled every time cumula-

tive potential evapotranspiration reached 4 mm). During 2008, irrigation was withheld in one of the two plots from 18 June 2008 to the end of the growing season (ORL$_{WD}$), while the second one remained regularly irrigated (ORL$_{WW}$). Cumulative rainfall reached 126 mm from 18 June to 30 September 2008. Predawn leaf water potential (Ψ_{pd}, MPa) was used as a rough index of soil water availability (Améglio et al. 1999) and was monitored approximately once a week from 18 June to 18 August 2008 using a pressure chamber (PMS Instruments, Albany, OR, USA). Measurements were constrained to three genotypes (one per population) chosen at random at the beginning of the experiment, but performed over the five blocks of each plot to test for spatial homogeneity of Ψ_{pd} values ($n = 5$ per genotype and per plot). No spatial or genotypic differences were detected, and results indicated that from mid-June to mid-August Ψ_{pd} remained > -0.18 MPa on the control plot, while a progressive drop was recorded on the non-irrigated plot with a peak reaching -0.52 MPa on 24 July (see Supplementary Figure 1 available as Supplementary Data at *Tree Physiology* Online).

Plot at Guémené-Penfao (GMN)

This plot corresponds to the Forest State Nursery of Guémené-Penfao where poplar genotypes and provenances are conserved. The plot was set up in May 2005, with six randomized complete blocks with one cutting of each genotype per block, on a loamy sand soil with fertilizer addition. Additional fertilization was supplied as 150 and 100 kg ha^{-1} of N/P/K (10/20/20, v:v) during March and May 2006, respectively. Hardwood cuttings (0.25 m long) were planted at an initial spacing within and between rows of 0.75 × 1.2 m, respectively. One border row of the same *P. nigra* genotype ('Blanc de Garonne') was planted around each plot to minimize edge effects. Trees were cut back at the end of 2005 and only the most vigorous shoot of each stump was retained the following spring. Plantation management in terms of mechanical and manual weed control as well as fungicide and insecticide applications was comparable to the ORL site; however, irrigation was performed seldom during the growing season, though we do not have information regarding the cumulated amount of water supplied. GMN was included in the present study as an additional comparative plot,

though we acknowledge that GMN differed from ORL_WW and ORL_WD for more than one aspect, including experimental design, edaphic conditions, local climate, fertilization and year of collection.

Carbon isotope discrimination, related leaf traits and growth performance

The same traits were measured in the three plots for all genotypes, but the years of harvest differed with site. In Guémené-Penfao, leaves were collected on 8 August 2006 (1-year-old shoots, second growing season) from three randomly selected blocks out of the six; in Orléans, leaves were sampled on 12 September 2008 (1-year-old shoots, third growing season) from all blocks. At each sampling date, one mature and fully illuminated leaf was selected for each individual; six discs of leaf lamina ($2\ cm^2$ each) were then punched from each leaf, dried at 60°C for 48 h before being weighed and used to compute specific leaf area (SLA, $cm^2\ g^{-1}$). The same discs were then ground to a fine powder for the analysis of carbon isotope composition ($\delta^{13}C$). One milligram sub-samples of ground material were enclosed in tin capsules and combusted in an elemental analyser (Carlo Erba Instruments, Milan, Italy). The CO_2 produced by combustion was purified and the $^{13}CO_2/^{12}CO_2$ ratio was analysed with a Finnigan MAT Delta S isotope ratio mass spectrometer (IRMS) (Bremen, Germany). Carbon isotope composition was expressed relative to the Pee Dee Belemnite (PDB) standard and was calculated as in Craig (1957):

$$\delta^{13}C = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000 (‰)$$

where R_{sample} and $R_{standard}$ are the $^{13}CO_2/^{12}CO_2$ ratios of the sample and the standard, respectively (Farquhar et al. 1989). All analyses were performed at the Technical Platform of Functional Ecology (OC 081) at INRA-Nancy. The accuracy of $\delta^{13}C$ measurements was $\pm 0.03‰$ (standard deviation). The carbon isotope discrimination ($\Delta^{13}C$) between atmospheric CO_2 (δ_{air}) and plant material (δ_{plant}) was calculated according to Farquhar and Richards (1984) as

$$\Delta^{13}C = (\delta_{air} - \delta_{plant}) / (1 + (\delta_{plant} / 1000)) (‰)$$

assuming that δ_{air} was $-8‰$. Carbon and nitrogen contents of the same samples were expressed on a dry mass basis (C_M and N_M, mg g^{-1}).

Growth performance was estimated by measuring fresh mass of the annual shoot ($Biom_F$, g) in January 2007 (Guémené-Penfao) and January 2009 (Orléans), assuming that possible genotypic differences in water content were not sufficient to modify relative genotype rankings.

Statistical analyses

Statistical analyses were performed using the R software (Version 2.10.1, a language and environment for statistical computing and graphics). Means are presented with their standard error (SE) and all statistical tests were considered significant at $P < 0.05$. Data were found to meet the assumption of homoscedasticity and normal distribution of residuals; all analyses were therefore performed using analysis of variance (ANOVA) using the following models:

i. Block effects were first tested within each plot for all using a simple one-way ANOVA model. Whenever they were significant, individual values were adjusted to block effects by calculating the difference between the mean of each block and the general mean computed from all individuals.
ii. Population and genotype effects were tested within each plot for all variables using the following model:
$$var = Pop + Geno(Pop)$$
where 'var' refers to the tested variable, 'Pop' refers to the population effect considered as fixed and 'Geno(Pop)' refers to the genotype effect nested within population considered as random.
iii. Differences between plots were tested using the following model:

$$var = Plot + Pop + Geno(Pop) + Plot \times Pop$$
$$+ Plot \times Geno(Pop)$$

where 'var' refers to the tested variable, 'Plot' refers to the plot effect considered as fixed, 'Pop' refers to the population effect considered as fixed, 'Geno(Pop)' refers to the genotype effect nested within a population considered as random, 'Plot × Pop' refers to the plot by population interaction and 'Plot × Geno(Pop)' refers to the plot by genotype interaction. Note that these analyses were run as pairwise comparisons between plots (i.e., ORL_WW vs. ORL_WD, ORL_WW vs. GMN and ORL_WD vs. GMN; see Table 2).

For each variable, population and plot, genotypic and residual variance components (σ^2_G and σ^2_ε, respectively) were estimated using the restricted maximum likelihood method (REML). Broad-sense heritability (H^2) was then computed on an individual basis as $H^2_{individual} = \sigma^2_G/(\sigma^2_G + \sigma^2_\varepsilon)$ and standard errors associated with values of $H^2_{individual}$ were calculated as described by Singh et al. (1993). In the same way, the coefficient of genetic variation (CV_G) was calculated as $CV_G = (s \times 100)/\mu$, where s is the standard deviation and μ is the grand mean.

Relationships between pairs of continuous variables were analysed by linear regression analysis [Pearson's correlation coefficients (r_p)] on a genotypic mean basis, for each population and each plot. The stability of genotype ranking across plots was assessed on a genotypic mean basis for each variable and each population using Spearman's rank correlation coefficients (r_s).

Table 2. ANOVA results for pairwise comparisons between plots.

Plot comparisons	Variables	Plot	Pop	Geno(Pop)	Plot × Pop	Plot × Geno(Pop)
ORL_{WW} vs. ORL_{WD}	$\Delta^{13}C$	344.06***	35.83***	4.71***	0.56 ns	1.16ns
	SLA	58.80***	63.19***	4.08***	1.25ns	1.38*
	C_M	0.68ns	30.19***	1.72***	1.27ns	0.89ns
	N_M	14.52***	0.31 ns	4.53***	5.04**	1.17ns
	$Biom_F$	286.53***	17.58***	5.66***	1.91 ns	1.35*
ORL_{WW} vs. GMN	$\Delta^{13}C$	409.80***	109.33***	7.70***	16.20***	2.05***
	SLA	70.43***	20.20***	2.66***	8.38***	1.82***
	C_M	1479.17***	15.87***	1.60**	5.73**	0.89ns
	N_M	132.28***	12.03***	2.54***	12.30***	1.56**
	$Biom_F$	121.41***	14.96***	5.64***	0.64ns	2.09***
ORL_{WD} vs. GMN	$\Delta^{13}C$	0.07ns	58.07***	4.31***	16.30***	1.62**
	SLA	213.80***	21.89***	2.20***	12.00***	1.65***
	C_M	1212.73***	19.56***	1.32*	8.37***	1.11ns
	N_M	58.35***	6.27**	2.31***	15.35***	1.50**
	$Biom_F$	38.31***	21.37***	4.63***	1.33ns	2.43***

ORL_{WW}, irrigated plot at Orléans; ORL_{WD}, non-irrigated plot at Orléans; GMN, plot at Guémené-Penfao; $\Delta^{13}C$, bulk leaf carbon isotope discrimination (‰); SLA, specific leaf area (cm^2 g^{-1}); C_M, leaf carbon content (mg g^{-1}); N_M, leaf nitrogen content (mg g^{-1}); $Biom_F$, annual shoot fresh mass (g). Levels of significance are *P < 0.05; **P < 0.01; ***P < 0.001; ns, non-significant. Plot refers to the plot effect, Pop refers to the population effect, Geno(Pop) refers to the genotype effect nested within population, Plot × Pop refers to the plot by population interaction and Plot × Geno(Pop) refers to the plot by genotype interaction.

Results

Variations among populations, $P \times E$ interactions and geographical trends

In each of the three plots (ORL_{WW}, ORL_{WD} and GMN), ANOVA revealed significant differences between mean performance of the three populations for all studied traits, with the exception of N_M in ORL_{WW} (Figure 1). The difference between the two extreme population means at each site reached at least 0.55‰ for $\Delta^{13}C$, 6.3 cm^2 g^{-1} for SLA, 4.9 mg g^{-1} for C_M, 1.1 mg g^{-1} for N_M and 16.2 g for $Biom_F$.

Overall, most traits varied significantly from one plot to the other (Table 2, Figure 1). As expected, drought at ORL resulted in lower $\Delta^{13}C$, SLA and $Biom_F$ values as compared with the irrigated plot ORL_{WW} (Figure 1). Trees grown at GMN exhibited intermediate $Biom_F$ values, similar $\Delta^{13}C$ values as those grown at ORL_{WD} but higher SLA, C_M and N_M values than trees grown at ORL_{WW} or ORL_{WD} (Figure 1). The ANOVA model revealed no Plot × Pop interaction between ORL_{WW} and ORL_{WD}, except for N_M, so that population ranking was maintained for most of the variables (Table 2, Figure 1). In contrast, the comparison of GMN with ORL_{WW} or ORL_{WD} revealed a significant Plot × Pop interaction; however, it is worth noting that for $\Delta^{13}C$, although a significant interaction was observed, population ranking was maintained (Table 2, Figure 1).

Variations within populations and $G \times E$ interactions

ANOVA revealed significant differences between genotype performances for all studied traits, with the exception of C_M in ORL_{WD} (Table 2, Figure 1). Variations within populations were larger than among populations (Table 3); the range of variations between the two extreme genotypic means at each site reached at least 2.29‰ for $\Delta^{13}C$, 27.33 cm^2 g^{-1} for SLA, 23.47 mg g^{-1} for C_M, 7.85 mg g^{-1} for N_M and 104.14 g for $Biom_F$. The range of genotypic variation was similar among the three populations (Figure 1). The highest CV_G values were detected for $Biom_F$ (21.49–57.30%), although they differed markedly depending on population and plot (Table 4); lower or non-significant CV_G values were observed for the other variables (Table 4). Highly different heritabilities (0.08–0.77) were detected among traits when expressed on an individual basis ($H^2_{individual}$) (Table 4). The highest values were observed for $\Delta^{13}C$ and $Biom_F$ at GMN, and the lowest ones for C_M at ORL_{WW} and ORL_{WD} (Table 4).

The ANOVA model revealed a significant Plot × Geno(Pop) interaction for SLA and $Biom_F$ at Orléans (Table 2); Spearman's rank correlation coefficients (r_s) calculated for each population from genotypic means were, however, highly significant for most of the variables, indicating that genotype ranking was overall maintained between ORL_{WW} and ORL_{WD} (Table 5). In contrast, when comparing GMN with ORL_{WW} or ORL_{WD}, results of the ANOVA model revealed a significant Plot × Geno(Pop) interaction for all variables, except C_M (Table 2); r_s values calculated at the population level were not significant for most of the variables, except $\Delta^{13}C$, indicating that genotype ranking was not maintained for the other variables (Table 5).

Relationships between traits

The relationship between $\Delta^{13}C$ and $Biom_F$ was never significant, except in one case (Figure 2). Correlations between other variables were weak and mostly not significant, so that no clear relationship could be evidenced (see Supplementary Table 1, available as Supplementary Data at *Tree Physiology* Online).
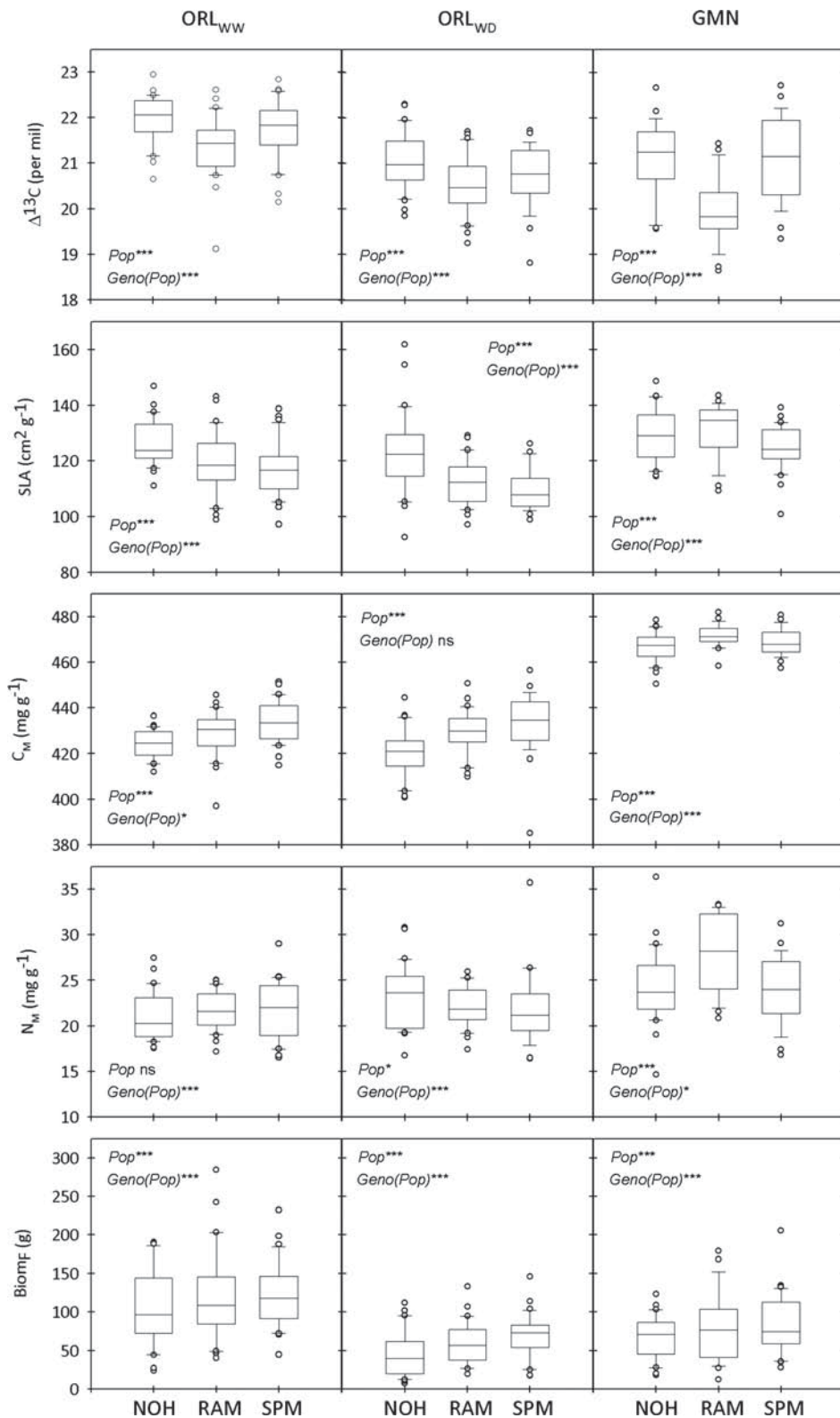
Figure 1. Box plots of genotypic means for each trait in each plot. Each box represents the quartile below (Q1) and above (Q3) the median value. Vertical bars represent minimum and maximum values except for genotypes away from 1.5 times from the top of the inter-quartile (Q3–Q1) range. Values beyond this range are represented as circles. Results of the ANOVA within each plot are indicated, where Pop is the population effect and Geno(Pop) is the genotype effect nested in the population. Levels of significance are *$P < 0.05$, ***$P < 0.001$, ns = non-significant. ORL_{WW}, irrigated plot at Orléans; ORL_{WD}, non-irrigated plot at Orléans; GMN, plot at Guémené-Penfao; NOH, Nohèdes; RAM, Ramières; SPM, St-Pryvé St-Mesmin; $\Delta^{13}C$, bulk leaf carbon isotope discrimination (‰); SLA, specific leaf area ($cm^2\ g^{-1}$), C_M, leaf carbon content ($mg\ g^{-1}$), N_M, leaf nitrogen content ($mg\ g^{-1}$); $Biom_F$, annual shoot fresh mass (g).

Table 3. Relative percentages of variance components in each plot and for each trait. Abbreviations are as in Table 2.

	Variance	$\Delta^{13}C$ (%)	SLA (%)	C_M (%)	N_M (%)	$Biom_F$ (%)
ORL_{WW}	Pop	10	11	8	0	1
	Geno(Pop)	38	28	7	30	41
	Residual	52	61	85	70	58
ORL_{WD}	Pop	6	19	12	1	6
	Geno(Pop)	21	20	4	30	25
	Residual	73	61	84	69	69
GMN	Pop	34	3	8	11	2
	Geno(Pop)	48	13	31	9	65
	Residual	18	84	61	80	33

Pooling values from the three populations did not modify these trends (data not shown).

Discussion

Populus nigra L. is an emblematic riparian species in Europe, for which we have, to date, only little information with respect to the diversity of functional traits. The present study revealed substantial variation in physiological performances within and among three French *P. nigra* populations originating from different geographic origins. The comparison among plots was valuable in assessing environmental effects on trait variations at population and genotype scales. Plant growth performance and WUE (here assessed through carbon isotope discrimination) are key physiological components integrating many structural and functional characters, e.g., photosynthetic capacity, stomatal function, and relative carbon allocations among roots, stems and leaves. Characterizing genetic variations for these traits and their variations in response to changes in the envi-

ronment may therefore provide insights into the adaptive potential of *P. nigra*.

Inter- and intra-population variations

Trait variation was larger within than among populations. In addition, the range of intra-population variation was quite comparable in the three populations, i.e., the range of within-population variations was not significantly influenced by the area of origin. The high number of representative genotypes per population (30) clearly favoured the expression of within-population variations, but it is unlikely that a higher number of populations would have changed the variance patterns. Other studies on phenotypic trait variations in tree species, including leaf morphological and physiological traits, have reported similar patterns regarding within- and among-population variations (Brendel et al. 2002, Ramírez-Valiente et al. 2009, Aranda et al. 2010, Keller et al. 2011). Forest tree species are generally characterized by large population sizes, high gene flow within population and therefore high genetic diversity that typically facilitates rapid adaptation (Savolainen et al. 2007). Furthermore, the maintenance of high levels of within-population variation may be a characteristic inherent to pioneering riparian species, ensuring successful establishment and adaptation under the highly fluctuating nature of the riparian habitat (Weber et al. 1985).

The measured phenotypic traits displayed significant heritabilities (H^2), although values were highly variable among plots and populations. This is not surprising when considering that H^2 values reflect the expression of genotypic variability for one specific population under one given environment (Lynch and Walsh 1998). In spite of this, it may be worth noting that the

Table 4. Broad-sense heritability ± standard error ($H^2_{individual}$ ± SE) and coefficient of genetic variation (CV_G, %) calculated for each trait for each population in each plot. Abbreviations are as in Tables 1 and 2.

Variables	Populations	$H^2_{individual}$ ± SE			CV_G		
		ORL_{WW}	ORL_{WD}	GMN	ORL_{WW}	ORL_{WD}	GMN
$\Delta^{13}C$	NOH	0.32 ± 0.08	0.23 ± 0.09	0.70 ± 0.13	1.96	2.09	3.41
	RAM	0.60 ± 0.07	0.28 ± 0.09	0.69 ± 0.13	2.89	2.35	3.45
	SPM	0.34 ± 0.08	0.18 ± 0.09	0.75 ± 0.16	2.50	2.16	3.93
SLA	NOH	0.12 ± 0.07	0.45 ± 0.07	0.16 ± 0.07	4.02	8.44	5.07
	RAM	0.46 ± 0.05	0.17 ± 0.08	0.13 ± 0.05	8.15	4.63	4.60
	SPM	0.40 ± 0.06	0.14 ± 0.08	0.10 ± 0.06	6.88	3.99	3.93
C_M	NOH	ns	ns	0.54 ± 0.05	ns	ns	1.23
	RAM	ns	ns	0.19 ± 0.10	ns	ns	0.64
	SPM	0.17 ± 0.08	0.08 ± 0.02	0.24 ± 0.10	1.41	1.31	0.86
N_M	NOH	0.25 ± 0.08	0.24 ± 0.09	0.21 ± 0.10	9.84	9.41	10.19
	RAM	0.15 ± 0.08	ns	ns	6.24	ns	ns
	SPM	0.45 ± 0.06	0.56 ± 0.06	ns	12.63	16.46	ns
$Biom_F$	NOH	0.50 ± 0.31	0.50 ± 0.04	0.43 ± 0.08	41.39	57.30	33.31
	RAM	0.45 ± 0.06	0.24 ± 0.08	0.77 ± 0.17	42.36	33.91	53.26
	SPM	0.28 ± 0.08	0.12 ± 0.08	0.69 ± 0.13	26.11	21.49	42.36

Table 5. Spearman's rank coefficients calculated from genotypic means of each population. Abbreviations and levels of significance are as in Table 2.

Variables	Populations	Spearman's rank coefficients		
		ORL$_{WW}$ vs. ORL$_{WD}$	ORL$_{WW}$ vs. GMN	ORL$_{WD}$ vs. GMN
$\Delta^{13}C$	NOH	0.51**	0.62***	0.46*
	RAM	0.71***	0.69***	0.41*
	SPM	0.51**	0.44*	0.39*
SLA	NOH	0.57**	ns	ns
	RAM	0.54**	0.43*	ns
	SPM	0.74***	ns	ns
C_M	NOH	ns	ns	ns
	RAM	0.36**	ns	ns
	SPM	0.63***	0.37*	ns
N_M	NOH	ns	0.43*	ns
	RAM	0.59**	ns	ns
	SPM	0.69***	ns	ns
Biom$_F$	NOH	0.48**	ns	ns
	RAM	0.79***	0.57**	0.57**
	SPM	0.58**	0.37*	ns

highest H^2 values were consistently observed for the two most complex traits, i.e., growth performance and $\Delta^{13}C$, with values sometimes reaching more than 0.75. H^2 values for growth were paralleled by high CV$_G$ values (21–57%), as already observed for a *Populus tremuloides* population (Kanaga et al. 2008). In contrast, H^2 values for $\Delta^{13}C$ were paralleled by a very low CV$_G$ value (always <4%), a pattern that seems to be common across tree species (Lauteri et al. 2004), including poplar (Kanaga et al. 2008, Monclus et al. 2009). Caution should be, however, exercised when interpreting genetic and physiological significances of the low $\Delta^{13}C$ variations. A CV$_G$ value of 4% referred to a general mean value of 21‰ (as observed when averaging data across populations and plots) would imply a variation of $\Delta^{13}C$ of about 0.85‰, supposedly translating into ~16.5% variation in intrinsic WUE based on Farquhar's model

(Farquhar et al. 1982). Such a variation is therefore physiologically relevant.

The extent of the period of active tree growth is known to be influenced by day length and/or temperature, and typically shows strong genetic differentiation resulting in locally adapted ecotypes (Luquez et al. 2008, Rohde et al. 2011). In agreement with this observation, growth performance proved to differentiate the three *P. nigra* populations in a consistent manner across the three plots, the population originating from altitude (NOH) exhibiting the lowest growth; however, considering the relatively low geoclimatic range accounted for in this study, the generalization of the patterns between growth potential and environment of origin remains speculative as compared with other studies reported on poplar (Gornall and Guy 2007, Soolanayakanahally et al. 2009, Messaoud and Chen 2011). Otherwise, mean $\Delta^{13}C$ values also differed among populations, and population ranking remained stable across plots, suggesting strong population differentiation for WUE in this species. Ding et al. (2006) reported an abundant variability of ~3.5‰ for $\Delta^{13}C$ when comparing 134 genotypes of *P. nigra* introduced in China from different areas of Europe; however, data on *P. nigra* natural populations remain so far not documented. Regarding other poplar species, a recent comparison across 21 provenances of *P. balsamifera* L. indicated a maximum interpopulation range of variation of 0.98‰ (Soolanayakanahally et al. 2009) while another study could not find significant differences among five provenances of *P. trichocarpa* Torr. & Gray (Gornall and Guy 2007). Water availability in the area of origin has been shown to drive tree population differentiation in WUE, populations from drier sources generally exhibiting lower $\Delta^{13}C$ (Lauteri et al. 2004, Zhang et al. 2004, Marchin et al. 2008, Aletà et al. 2009). Reverse patterns have, however, also been reported (Lauteri et al. 1997, Guy and Holowachuk 2001). In fact, the direction and strength of the relationship between WUE and climate are likely to depend on (i) which of photosynthetic capacity vs. stomatal conductance drives the variations
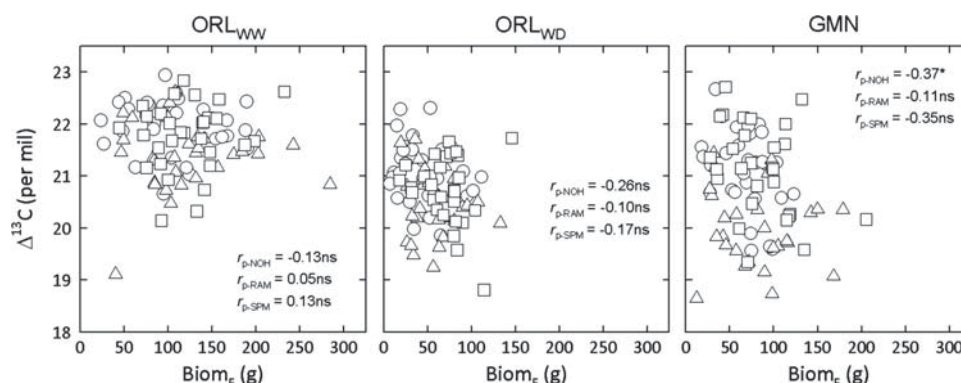

Figure 2. Relationship between bulk leaf carbon isotope discrimination ($\Delta^{13}C$, ‰) and annual shoot fresh mass (Biom$_F$, g) for each population (NOH circles, RAM triangles and SPM squares) in each plot. Linear regressions (Pearson's correlation coefficients, r_p) were computed on a genotypic mean basis for each population (r_{p-NOH}, r_{p-RAM} and r_{p-SPM}; $n = 30$). Levels of significance are *$P < 0.05$ and ns, non-significant. ORL$_{WW}$, irrigated plot at Orléans; ORL$_{WD}$, non-irrigated plot at Orléans; GMN, plot at Guémené-Penfao; NOH, Nohèdes; RAM, Ramières; SPM, St-Pryvé St-Mesmin.

in WUE_i and therefore in $\Delta^{13}C$, (ii) whether net assimilation rate is the main component of growth potential, and (iii) how growth is related to WUE. For instance, for species where photosynthetic rates are the main drivers of a higher WUE_i and growth, genotypes with the highest relative WUE often originate from provenances with a reduced likelihood of water stress, which makes sense given their higher inherent growth potential. In the case of riparian poplar species, their phreatophyte behaviour is expected to ensure adequate water supply to the leaves and to help buffer summer drought (Rood et al. 2003). This may partly explain why water availability would not be the main factor driving WUE differentiation in *P. nigra*. It is, however, interesting to note that the population from the warmest area (RAM) displayed a consistently lower $\Delta^{13}C$ than the other two in agreement with results reported for *P. trichocarpa* provenances (Gornall and Guy 2007). Higher air temperatures are expected to lead to higher air vapour pressure deficits, and differences in evaporative demand may be a more important driver of geographical differentiation than water availability in riparian poplars (Leffler and Evans 1999).

Environment-induced variations

Because of their tight dependency upon water availability, species of the *Populus* genus are generally considered as very sensitive to water deficit (Blake et al. 1996), and *P. nigra* is no exception (Regier et al. 2009, Cocozza et al. 2010, Centritto et al. 2011). Indeed, although drought was relatively moderate in our study ($\Delta\Psi_{pd}$ never dropped below −0.45 MPa as compared with the control plot), $\Delta^{13}C$, SLA and annual shoot fresh mass decreased on average by 4, 5 and 51%, respectively, while N_M and C_M remained approximately constant. Similar variations in terms of magnitude have already been reported in other poplar species/hybrids under comparable water deficit intensities (e.g., Zhang et al. 2004, Monclus et al. 2006, 2009, Fichot et al. 2010). The lower $\Delta^{13}C$ values are in agreement with the well-known observation that WUE generally increases with increasing evaporative demand/water limitation (Ehleringer et al. 2002) and illustrate the poplar water-saving strategy resulting from the high drought sensitivity of stomata (Braatne et al. 1992, Silim et al. 2009), including *P. nigra* (Regier et al. 2009). One of the most important results was that drought × genotype interactions were only seldom and weakly significant while Spearman's rank coefficients between genotypic means were positive, moderately high and highly significant for each population, resulting in the maintenance of a rather constant range of within-population variations; hence, although genotypes could punctually differ in their response amplitude, there was no systematic trade-off in genotypic physiological behaviour, regardless of the provenance. Further, drought × population interactions were also not significant (except for N_M), thereby highlighting the lack of differences in drought-induced plasticity between populations. Comparative

data are lacking in poplar, but uniformity in the drought response has already been reported at the population level for $\Delta^{13}C$ in *Pinus ponderosa* Dougl. ex Laws (Zhang et al. 1997, Olivas-García et al. 2000), *Castanea sativa* Mill. (Lauteri et al. 2004) and *Eucalyptus microtheca* F. Muell. (Li 2000), as well as for growth in *Pinus pinaster* Aiton (Aranda et al. 2010) or *Eucalyptus globulus* Labill. (Osório et al. 1998). In contrast, differences in drought sensitivity among populations have also been reported for $\Delta^{13}C$ in *P. pinaster* (Aranda et al. 2010) and for growth in *P. ponderosa* (Zhang et al. 1997).

In contrast to the drought effect, site-related variations were largely both genotype- and population-dependent, although they did not systematically translate into a different population ranking (see for $\Delta^{13}C$ and $Biom_F$). Therefore, we conclude that site-related variations were not predominantly explained by site differences in water availability. In fact, although the GMN site was not irrigated on a regular basis as in ORL_{WW}, water was punctually supplemented during the growing season, partly explaining why $Biom_F$ values at GMN were intermediate as compared with those obtained at ORL_{WW} and ORL_{WD}. In addition, the GMN site was fertilized, but not the others, a feature that was readily visible through the higher leaf N_M contents recorded at GMN. Fertilization has also been shown to improve WUE in poplar (Harvey and van den Driessche 1999, DesRochers et al. 2006), which is consistent with the low $\Delta^{13}C$ values recorded at GMN. Actually, fertilization may have exacerbated intrinsic differences between genotypes for nutrient-use efficiency and might be mainly responsible for the observed G × E and ultimately P × E interactions.

Relationship between $\Delta^{13}C$ and growth performance

We found no significant relationship between $\Delta^{13}C$ and $Biom_F$, suggesting that WUE and growth performance are to a large extent uncoupled in *P. nigra*, which is consistent with previous work on inter-specific hybrids such as *Populus deltoides × P. nigra* (Monclus et al. 2006, Fichot et al. 2010) and *P. trichocarpa × P. deltoides* (Rae et al. 2004). There was only one exception for the NOH population at the GMN site, for which a weak but significant negative relationship was observed. Another study on 134 *P. nigra* genotypes reported a negative relationship between $\Delta^{13}C$ and growth for only one out of the three common gardens investigated (Ding et al. 2006). It may be worth noting that in our study growth potential actually referred to annual realized growth. Yet, variation in annual realized growth among poplar genotypes is essentially driven by variation in bud break and most of all bud set (Dillen et al. 2009, Marron et al. 2010); the relationship between WUE and growth potential when considering a relative growth rate may therefore be different. Otherwise, differing patterns in the relationship between $\Delta^{13}C$ and growth lie in differences for the primary source of genotypic variation in WUE_i. Variations in $\Delta^{13}C$ are fundamentally influenced by the balance between

photosynthetic capacity and stomatal conductance; a negative relationship between $\Delta^{13}C$ and growth is generally interpreted as photosynthetic capacity driving WUE_i variations, while a lack of relationship between the two traits generally supports differences in stomatal conductance as the main driver for WUE_i (Farquhar et al. 1989). However, relative growth potential in poplar relies not only on photosynthetic capacity but also on leaf structure and relative carbon allocations between perennial compartments (Barigah et al. 1994), which may be also partly responsible for the lack of coupling of WUE and growth. Actually, previous work undertaken on *P. deltoides* × *P. nigra* hybrids, among which WUE and growth were not related, demonstrated both that (i) stomatal conductance was the main driver for WUE_i and $\Delta^{13}C$ genotypic variations, and (ii) photosynthetic capacity and net assimilation rate were not a significant determinant of growth performance (Monclus et al. 2006). Improved understanding of the physiological controls acting on WUE_i and growth is necessary and will provide the functional basis for the lack of relationship between these two complex traits.

Conclusion

To conclude, (i) this study evidenced variations in ecophysiological performance both within and among three French *P. nigra* populations; (ii) populations were consistently different for $\Delta^{13}C$ and growth although these two complex traits were not related to each other, ultimately indicating that the environmental factors driving genetic structuration for $\Delta^{13}C$ and growth act independently; (iii) drought-induced acclimation was neither genotype- nor population-dependent, while site-related variations were. Taken together, these results underline the significant adaptive potential of *P. nigra* under the currently changing environmental conditions. Further research on more numerous populations originating from all over Europe will help reveal local adaptations and elucidate the links between climate, water-use efficiency and growth performance for this emblematic riparian species.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* online.

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