# Tree aging does not affect the ranking for water use efficiency recorded from δ13C in three Populus deltoides x P. nigra genotypes

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# Tree aging does not affect the ranking for water use efficiency recorded from δ13C in three *Populus deltoides × P. nigra* genotypes

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A large variability of water use efficiency (assessed from the carbon isotopic discrimination in leaves and leaf soluble sugars) has been detected among poplar genotypes. Checking whether such differences detected in young trees (1-2 years old) remain stable with tree age is a prerequisite to use this trait with confidence for breeding purposes. In this study, a synchronic approach was used to test the age-related stability of the genotypic ranking of carbon isotopic discrimination in wood ($\Delta^{13}C$) until tree maturity. We sampled 376 trees between 4 and 20 years from three *Populus deltoides × P. nigra* genotypes growing in 41 common-garden trials across France. Carbon and nitrogen percentages along with $\delta^{13}C$ was measured in the bulk wood of the year 2009 and used to compute the $\Delta^{13}C$. Basal area increment between 2008 and 2009 was also measured. Results showed that $\Delta^{13}C$ increased (*i.e.*, water use efficiency decreased) between ages 4 to 6 and remained stable later on. Significant differences among genotypes were found but the ranking among genotypes remained stable with age during the assessed life span. Furthermore, basal area increment and $\Delta^{13}C$ were positively correlated interannually. This large-scale survey shows that despite crossing over in the temporal trend, water use efficiency remained stable with age across 3 poplar genotypes. However, further studies with a large number of genotypes are required to confirm whether this trait can be used to maintain or even improve productivity of poplar plantations, while lowering water consumption.

Keywords: Water Use Efficiency, Age, Wood, Tree Ring, *Populus × euramericana*, Basal Area Increment, Synchronic Approach

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## Introduction

In the context of climate change and fluctuating precipitation patterns (IPCC 2013), water use efficiency (*WUE*, ratio between biomass accumulated and water transpired) has become an interesting trait for breeding new genotypes (Condon et al. 2004, Richards et al. 2010, Vadez et al. 2014). Isotopic discrimination against $^{13}C$ ($\Delta^{13}C$) during $CO_2$ diffusion through stomata and photosynthesis is an indirect indicator of intrinsic water use efficiency (*iWUE*, ratio between net $CO_2$ assimilation rate, $A$, and stomatal conductance to water vapour, $g_s$ – Farquhar & Richards 1984, Farquhar et al. 1989, Ehleringer et al. 1993). Indeed, this trait was found to be under tight genetic control in several tree species like poplar, pines, chestnut and oaks among others (Lauteri et al. 1997, Brendel et al. 2002, 2008, Rasheed et al. 2015). Furthermore, given the lack of correlation observed between *WUE* and productivity or, in some instances, the positive correlation between both traits (Vadez et al. 2014), selecting tree species or genotypes based on their ability to express a high *WUE* is desirable. Such a strategy has been explored in some crops in a context of improving water management (Condon et al. 2004, Richards et al. 2010) and might thus be particularly suitable for sustaining wood production under the incertitude of water availability.

Poplar genotypes are known to display large differences in growth performance and biomass production (Rasheed et al. 2013). A large genotypic variability of $\Delta^{13}C$ was found among *Populus deltoides × P. nigra* genotypes in controlled environment (Marron et al. 2005) and open field experiments (Monclus et al. 2005, Dillen et al. 2008). Using direct measurements, this variability in $\Delta^{13}C$ is also found tightly related to variability in the whole plant water use efficiency in *Populus deltoides × P. nigra* and in *Populus nigra* (Rasheed et al. 2013, 2015). Interestingly, no correlation was found between productivity and $\Delta^{13}C$ (Monclus et al. 2005, Dillen et al. 2008), giving opportunity to breed for enhanced *WUE* without compromising productivity in poplar genotypes. Nevertheless, previous studies on poplar genotypes were conducted on seedlings of 1-2 years and there are few studies investigating the stability of genotype ranking for *WUE* at tree scale.

Previous studies has demonstrated that $\Delta^{13}C$ (recorded from tree ring cellulose and bulk wood) is severely affected by age, usually decreasing by a 1-3 per mil with increasing tree age (Francey & Farquhar 1982, Bert et al. 1997, Duquesnay et al. 1998, McCarroll & Pawellek 2001, Brienen et al. 2017). Such an age-related effect may be related to: (i) assimilation of respired $CO_2$ near the forest floor which is already depleted in $^{13}C$ (Schleser & Jayasekera

1985); (ii) re-assimilation of respired $CO_2$ retained within the plant canopy (Francey & Farquhar 1982); (iii) decreasing hydraulic conductivity of xylem conduits with age (Monserud & Marshall 2001); (iv) reduced total soil-to-leaf hydraulic conductance with tree size (McCarroll & Loader 2004); or (v) tree height and light availability (Brienen et al. 2017). Records of $\Delta^{13}C$ in leaves and phloem sap of poplars with different ages revealed a rather stable ranking of the genotypic differences with tree age (Bonhomme et al. 2008). More recently, using a diachronic approach, several genotypes of *Populus deltoides × P. nigra* growing in common gardens were compared and the effects of ageing (from 5 to 20 years) on the genotype ranking were studied using the $\Delta^{13}C$ signals in tree rings as a surrogate for *WUE* (Rasheed et al. 2011). In the latter study, small changes in $\Delta^{13}C$ with tree age and no significant interaction between genotype and tree age were found, reinforcing the pertinence of ranking genotypes using $\Delta^{13}C$. However, in a such diachronic study, the influence of long-term environmental variability and annual effects related to rainfall on $\Delta^{13}C$ is assumed similar as the sampled trees were grown in common gardens that experienced the same environmental conditions.

In the present study we used a synchronic approach where $\Delta^{13}C$ was recorded and compared in tree rings with same formation date, but from tree of different ages and contrasting environmental conditions. This sampling technique was used to minimise long-term environment effect and maximise age effect. Three different genotypes of *Populus deltoides × P. nigra* were used and carbon isotopic composition ($\delta^{13}C$) of the bulk wood from the year 2009 was measured and $\Delta^{13}C$ was computed. Since cellulose and lignin are the major components of bulk wood, and due to different biochemical pathways involved in their formation, the carbon isotopic signals of these two components are also different, *i.e.*, lignin is ~3‰ depleted than cellulose (Loader et al. 2003, McCarroll & Loader 2004). Any variation in cellulose:lignin ratio can potentially affect the overall $\delta^{13}C$ signals of whole wood. Therefore, the variation in carbon percentage in the whole wood was investigated, that potentially elucidates the variation of lignin in the bulk wood (Lamlom & Savidge 2003). Moreover, nitrogen percentage in the bulk wood that can give insight regarding the tree response to the variation of available soil nitrogen resource (Guerrieri et al. 2011, Billings et al. 2016) was also measured.

The main difficulty of the synchronic approach is the lack of large-scale common garden grouping a sufficient number of genotypes with different ages. We therefore used 41 small common gardens (Fig. 1) maintained in different locations in France by the *Institut du Développement Forestier* (IDF-CNPF) and gathered samples for three genotypes of *Populus deltoides × P. nigra*. Using this approach, we tested the hypotheses that: (i) water use efficiency estimated from $\Delta^{13}C$ in wood is affected by tree age; and (ii) genotype ranking based on $\Delta^{13}C$ does not change with tree age.

## Material and methods

The aim of this study was to evidence the effect of age on stability of genotype ranking for $\Delta^{13}C$. However, studying long-term physiological response in trees can be tricky because of age and environmental signals overlapping each other. Therefore, maximising age-related signals through appropriate sampling technique becomes a prerequisite. In this regard a synchronic technique, where carbon isotope discrimination was compared among tree rings with same formation date but from trees of different ages and environments, thereby maximising the age signal. A major constraint while applying such technique is to find a large number of poplar genotypes of different ages in a common garden. To obtain a satisfying sample of trees of different ages in different genotypes and environments, we sampled trees across a large number of plantations in France (Fig. 1). These small plantations were established by the *Centre National de la Propriété Forestière – Institut pour le Développement Forestier* (CNPF-IDF) to compare the local performance of genotypes.

### Study sites

The genotypes were planted in plots of 25 trees at a distance of 7 × 7 m, *i.e.*, at a density of 204 stems ha$^{-1}$. Forty-one sites were selected (Fig. 1) which were located all over France (see Tab. S1 in Supplementary material for site information and spatial coordinates). Sites covered a large range of climates with mean annual temperature from 10 to 14 °C and precipitation from 600 to 1200 mm. Sampling sites were subsequently categorised as water available (WA, annual rainfall from 1000 to 1200 mm) or water limiting (WL, annual rainfall from 600 to 800 mm; precipitation record maintained by the *Centre National de la Propriété Forestière – Institut pour le Développement Forestier*, CNPF-IDF).

### Sample collection and preparation

We focused on 3 *Populus deltoides × P. nigra* genotypes: *Koster, I-214* and *Dorskamp*, as these genotypes are known to display contrasting $\Delta^{13}C$ at leaf level (Monclus et al. 2005). Moreover, they were present in a large number of the surveyed plantations and covered the different age classes. The last annual ring corresponding to year 2009 was sampled in February 2010 with an increment borer (0.5 cm$^2$) on ~5 trees site$^{-1}$
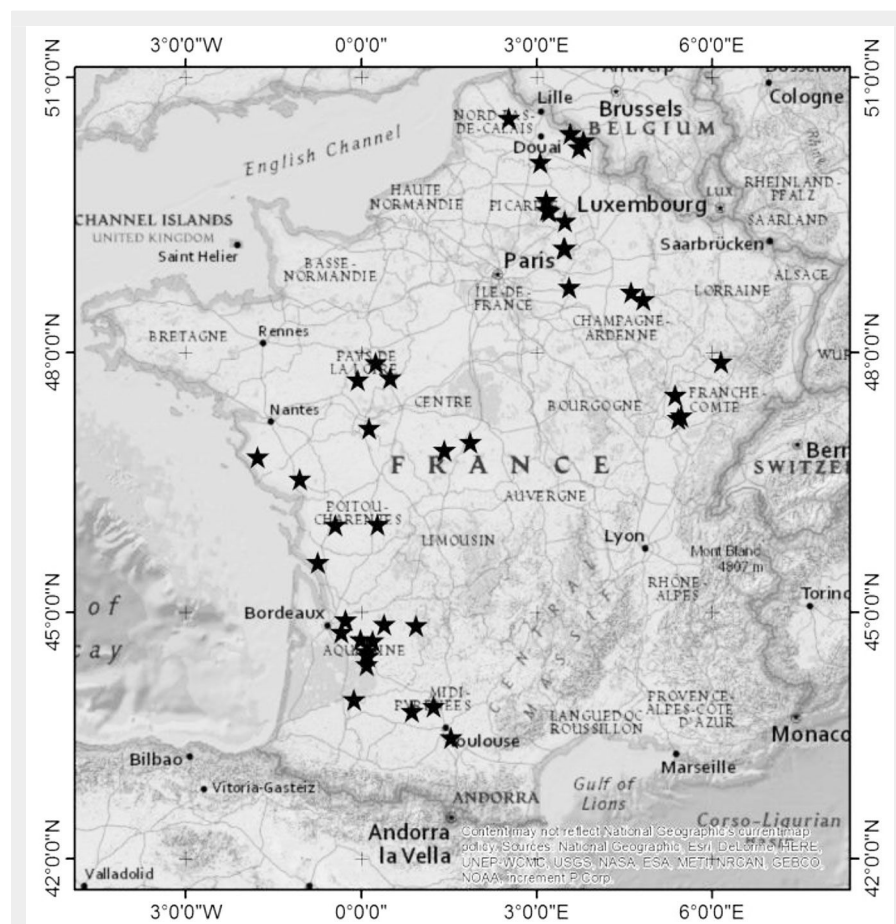


**Fig. 1** - Location of 41 sampling sites across France. Each site is represented by a closed star.

**Tab. 1** - Carbon and nitrogen percentage, and $\Delta^{13}C$ measured on the bulk wood from the year 2009 from three genotypes of *Populus deltoides* × *P. nigra* aged from 4 to 18 years. Data was analyzed using linear mixed model for the effects of genotype (G), age (A) and interaction (G × A). Table shows the mean values (± SE) combining all age classes. Different letters indicate significant genotype differences after multi-comparison test (Tukey HSD, P < 0.05).

| Variable | Clone | | | Effect (Prob.) | | |
|---|---|---|---|---|---|---|
| | *Dorskamp* | *I-214* | *Koster* | G | A | G × A |
| C % | 46.8 ± 0.28 [a] | 47.7 ± 0.097 [b] | 47.7 ± 0.10 [b] | <0.001 | 0.051 | 0.305 |
| N % | 0.154 ± 0.004 | 0.14 ± 0.007 | 0.168 ± 0.007 | 0.248 | <0.001 | 0.734 |
| $\Delta^{13}C$ (‰) | 20.3 ± 0.076 [a] | 19.9 ± 0.053 [b] | 19.4 ± 0.078 [c] | <0.001 | <0.001 | <0.001 |
| BAI (cm$^2$ yr$^{-1}$) | 88.4 ± 9.54 | 103 ± 11.0 | 107.3 ± 13.4 | 0.467 | 0.023 | 0.167 |

genotype$^{-1}$, yielding 376 samples. All cores were divided into seven age classes, *i.e.*, 4, 7, 9, 11, 13, 15 and 18 years. In order to minimise the environmental effect on $\Delta^{13}C$: (i) date effect was discarded as all cores corresponded to year 2009; and (ii) site effect was averaged in each age class by ensuring the representation of trees from both site types, *i.e.*, WA and WL sites.

The ring formed in 2009 was carefully separated from the adjacent one and from bark with a sharp razor blade. After drying at 70 °C for 48h, each ring was ground separately into fine homogeneous powder using a ring grinder (SODEMI, CEP Industries Department, Cergy-Pontoise. France). One mg of the resulting wood powder was weighed in tin capsules for $\delta^{13}C$ analysis.

### Carbon and nitrogen percentage and carbon isotope analysis

Wood powder was combusted at 1050 °C in sealed evacuated quartz tubes containing cobalt oxide and chromium oxide as catalyst, and an amount of pure oxygen. The gases produced during combustion, $CO_2$, $H_2O$ and $NO_x$ were passed through a reduction tube where $N_2$ was produced and excess of oxygen was removed. Water was trapped by using anhydrous magnesium perchlorate and after reversible absorption, carbon and nitrogen percentages were measured in an elemental analyser (NA 1500-NC®, Carlo Erba, Milan, Italy – Rasheed et al. 2011). Finally, combusted products were separated by gas chromatography and the $CO_2$ was delivered to an isotope ratio mass spectrometer (Delta-S®, Finnigan, Bremen, Germany). Carbon isotope composition was expressed as $\delta^{13}C$ (eqn. 1):

$$\delta^{13}C = (R_{sample} / R_{standard} - 1) \cdot 1000 \qquad (1)$$

where $R_{sample}$ and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in a sample and the standard (Vienna-Pee Dee Belemnite) respectively. Accuracy of the measurements was ± 0.1‰. Carbon isotope discrimination between atmosphere and wood was calculated as (eqn. 2):

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{wood}}{1 + \delta^{13}C_{wood}/1000} \qquad (2)$$

where $\delta^{13}C_{air}$ is the carbon isotope composition of $CO_2$ in the atmosphere and $\delta^{13}C_{wood}$ is the carbon isotope composition of the

wood powder. Given that $\delta^{13}C_{air}$ was -8.07‰ during 2003 and an annual decrease of 0.0281‰ (McCarroll & Loader 2004), $\delta^{13}C_{air}$ was estimated at -8.24‰ for 2009. We assumed that the mean value of $\delta^{13}C_{air}$ was similar across sites and did only marginally change with the season.

### Basal area increment

The data corresponding to annual circumference (cm) of each individual tree was acquired through the annual growth records from the *Centre National de la Propriété Forestière-Insitut pour le Développement Forestier*. Assuming trunks were circular in section, circumference was used to calculate the radius and eventually basal area for the years 2008 and 2009. BAI (cm$^2$ y$^{-1}$) for year 2009 was calculated as the difference between the total area corresponding to years 2009 and 2008.

### Statistical analysis

Normality and homoscedasticity of data were checked graphically with residual *vs.* predicted and normal quantile-to-quantile plots. The data set covering the seven age classes was analysed using linear mixed models fitted with genotype, age and their interaction as fixed effects and site as a random effect. Multiple comparison tests (*post-hoc* Tukey HSD test) were used to evaluate pairwise differences between age classes within each genotype. Further-

more, each age class was tested for genotype effect and *post-hoc* Tukey HSD test were used to evaluate pair-wise differences between genotypes within each age class. All tests were performed with R (R Core Team 2012) and R packages "nlme" (Pinheiro et al. 2012) and "multcomp" (Hothorn et al. 2008). All tests and correlations were declared significant at P < 0.05.

## Results

### Carbon and nitrogen percentage

Variance between sites was smaller than residual variance (intercept = 0.731; residuals = 1.84) and model $R^2$ resulted at 0.547 for carbon percentage. No genotype-age interaction was detected in the carbon percentage of bulk wood. It differed significantly among genotypes (P < 0.001 – for means, see Tab. 1). A non-significant trend with age was detected due to the slightly lower carbon percentage values at the age of 13 yrs. Nevertheless, carbon percentage did not display any significant trend related to age (Fig. 2). Variance between sites was smaller than residual variance (intercept = 0.009; residuals = 0.071) and model $R^2$ resulted at 0.525 for nitrogen percentage. For the nitrogen percentage, no interaction between genotype and age was found. Nitrogen percentage did not differ among genotypes but declined significantly with age (P < 0.001); it dropped from
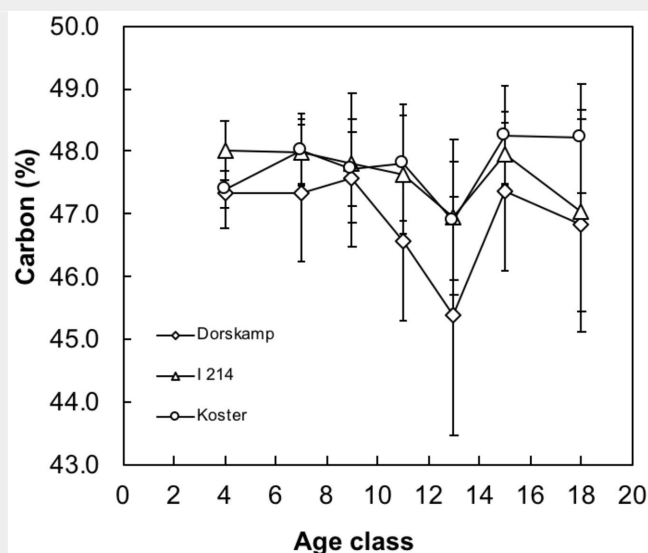
**Fig. 2** - Age-related variation of Carbon percentage in the bulk wood of the year 2009, sampled during winter 2010 in three *Populus deltoides* × *P. nigra* genotypes over seven age classes. Each point represents the mean of several sites. Error bars represent ± SE.

**Fig. 3** - Age-related variation of mean nitrogen percentage in the bulk wood of the year 2009, sampled during winter 2010. The curve represents the means of three *Populus deltoides × P. nigra* genotypes over seven age classes. Error bars represent ± SE and different letters indicate significant differences between age classes after Tukey HSD test (P < 0.05).
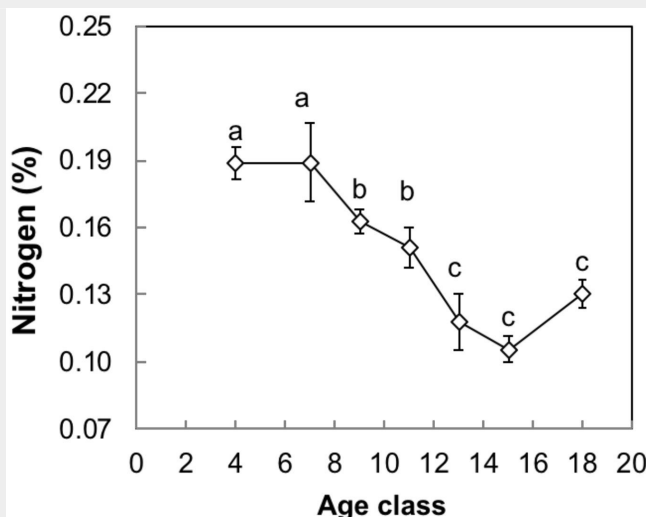
**Fig. 4** - Age-related variation in carbon isotope discrimination (Δ¹³C) in the 2009 year-ring for three *Populus deltoides × P. nigra* genotypes (a) *Dorskamp*, (b) *I 214* and (c) *Koster*. Each point represents a mean value from several sites. Error bars represent ± SE and different letters indicate significant differences between age classes for each genotype after Tukey HSD test (P < 0.05).
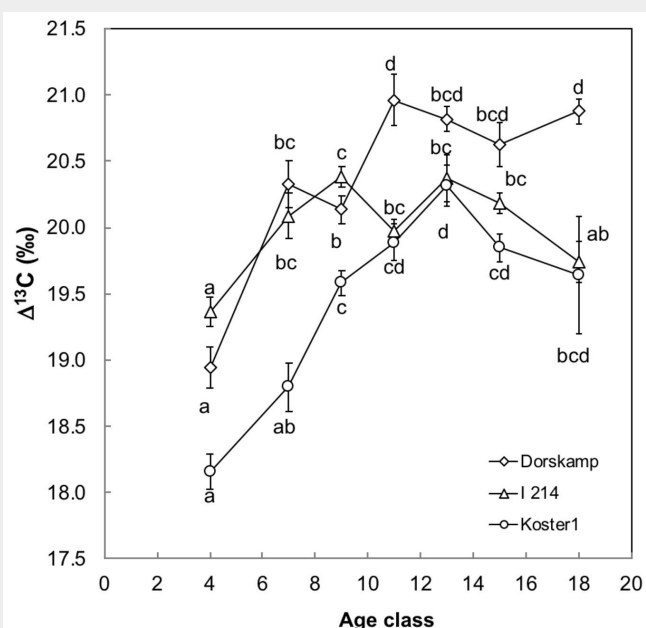
0.19 down to 0.11% over the tested period (Fig. 3).

### Age and genotype effects for Δ¹³C

Variance between sites was smaller than residual variance (intercept = 0.38; residuals = 0.51) and model $R^2$ resulted at 0.99.

Δ¹³C variation was significantly explained by an interaction between genotype and age (P < 0.001 – Tab. 1). A detailed *post-hoc* analysis revealed that an age effect was detectable in all three genotypes (Fig. 4). There was a significant increase of Δ¹³C by 2‰ from 4 to 9 yrs in *Dorskamp* and *Koster*

**Tab. 2** - Carbon isotopic discrimination (± SE) between atmosphere and bulk wood (Δ¹³C) in the year 2009 for three *Populus deltoides × P. nigra* genotypes (Dorskamp, I-214 and Koster). Each age class was tested for genotype effect (F-value and P-value) and different letters indicate a significant genotype difference for each age class after Tukey HSD test (P < 0.05).

| Age class (yrs) | df | *Dorskamp* | *I-214* | *Koster* | F-value | P-value |
|---|---|---|---|---|---|---|
| 4 | 43 | 18.9 ± 0.156 [a] | 19.3 ± 0.107 [a] | 18.1 ± 0.132 [b] | 22.3 | <0.001 |
| 7 | 47 | 20.3 ± 0.179 [a] | 20.0 ± 0.172 [a] | 18.7 ± 0.179 [b] | 18.4 | <0.001 |
| 9 | 88 | 20.1 ± 0.103 [a] | 20.3 ± 0.079 [a] | 19.5 ± 0.096 [b] | 17.9 | <0.001 |
| 11 | 80 | 20.9 ± 0.191 [a] | 19.9 ± 0.095 [b] | 19.8 ± 0.136 [b] | 13.8 | <0.001 |
| 13 | 24 | 20.8 ± 0.095 | 20.3 ± 0.175 | 20.3 ± 0.150 | 3.03 | 0.069 |
| 15 | 34 | 20.6 ± 0.161 [a] | 20.1 ± 0.077 [b] | 19.8 ± 0.103 [b] | 9.26 | <0.001 |
| 18 | 39 | 20.8 ± 0.092 [a] | 19.7 ± 0.151 [b] | 19.6 ± 0.443 [b] | 19.6 | <0.001 |

(Fig. 4a, Fig. 4c) and a less visible one in *I-214* (Fig. 4b) where it increased from 4 to 7 yrs and remained almost stable afterwards.

Genotype effect within each age group was found significant and was assessed with *post-hoc* Tukey HSD test (Tab. 2). In the young age classes (4 to 9 yrs), *Koster* displayed the lowest mean Δ¹³C followed by *Dorskamp* and *I-214* that displayed similar values. At higher ages, *Koster* and *I-214* displayed the lowest and *Dorskamp* displayed the highest mean Δ¹³C, with the exception of age class of 13 yrs, where no significant difference could be detected.

### Inter annual and genotype correlation between Δ¹³C and BAI

The average BAI of the year 2009 was homogenous among the three genotypes over the tested period (Tab. 1), and therefore the genotypic differences in Δ¹³C were independent from any difference in radial growth rate. The inter annual variation of BAI due to the age effect was positively correlated to Δ¹³C in the three genotypes (Fig. 5)

## Discussion

The present research was aimed to assess the stability of genotype differences in water use efficiency (*WUE*) with tree age; we compared three cultivars of *Populus deltoides × P. nigra* for this trait in plantation *in situ* across France and used a synchronic approach, based on Δ¹³C recorded from the year 2009 of individuals with different ages.

### Genotype and age effect on Δ¹³C and relationship with BAI

Whole wood consists of complex mixtures of molecules like cellulose, hemicellulose and lignin with variable isotopic signatures. Early studies used whole wood for the isotopic analysis on tree rings. However, the isotopic composition of individual wood components differs largely (Wilson & Grinsted 1977). Thus, we suspected that the observed significant genotypic difference in mean Δ¹³C could potentially be due to changes in cellulose *vs.* lignin ratios with contrasting isotopic signals. Although previous studies have shown that using whole wood is better than inducing extraction error while using cellulose to measure δ¹³C (Guy & Holowachuk 2001, Rasheed et al. 2011), in this study we minimised this effect by using the last annual ring (2009, with same cambial age) for isotope analysis.

Our results showed the existence of an age effect on Δ¹³C, where Δ¹³C increased to a larger extent during first age classes of 4-11 years in two genotypes (*Dorskamp* and *Koster*). This positive trend in Δ¹³C with age is contrary to several previous studies reporting age-related decreases in Δ¹³C using different dendrochronological approaches (Bert et al. 1997, Duquesnay et al. 1998, McCarroll & Pawellek 2001, Penuelas et al. 2008). Δ¹³C has been expected to be an estimator of water use efficiency (Farquhar

et al. 1989). Several studies have shown a clear negative correlation between $\Delta^{13}C$ and $A/g_s$ (intrinsic water use efficiency, *iWUE*) and *WUE* (whole-plant water use efficiency – Ripullone et al. 2004, Cernusak et al. 2008, Rasheed et al. 2013). Thus, increase in $\Delta^{13}C$ with age in our study reflects a decrease in *iWUE* with age. This decrease in *iWUE* ($A/g_s$) can either be due to decreased $CO_2$ assimilation rate ($A$) or increased stomatal conductance ($g_s$ – Farquhar & Richards 1984, Condon et al. 2004). Furthermore, $\Delta^{13}C$ and tree growth are often correlated depending upon the growth environment. This correlation was found to be positive for some species, *i.e.*, *Eucalyptus globulus* Labill (Osorio & Pereira 1994. Pita et al. 2001), *Fagus sylvatica* L. (Dupouey et al. 1993) and *Pinus radiata* (Rowell et al. 2008). On the contrary, some other conifer species displayed a negative correlation between $\Delta^{13}C$ and growth, *i.e.*, *Larix occidentalis* Nutt. (Zhang et al. 1994), *Pinus pinaster* (Nguyen-Queyrens et al. 1998, Brendel et al. 2002), *Picea mariana* Mill (Johnsen et al. 1999) and *Pinus caribaea* Morelet (Xu et al. 2000). However, no correlation between $\Delta^{13}C$ and tree growth was found in *P. × euramericana* in common garden (Monclus et al. 2005) and poplar plantation (Bonhomme et al. 2008). Within each genotype, we found a positive correlation between BAI and $\Delta^{13}C$ with age, which is in line with previous findings in different *Populus deltoides × P. nigra* genotypes (Rasheed et al. 2011). Similar positive correlation between BAI and $\Delta^{13}C$ has been reported in *Pinus radiata* (Rowell et al. 2008). The positive correlation between $\Delta^{13}C$ and BAI suggests that the inter-annual variation of $\Delta^{13}C$ is controlled to a larger extent by stomatal conductance than by photosynthetic capacity (Johnsen et al. 1999, Xu et al. 2000). In poplar, mostly variation in stomatal conductance rather than photosynthetic capacity seems to control carbon gain and growth (Ceulemans et al. 1987, Monclus et al. 2006, Rasheed et al. 2015). Therefore, based on the significant positive correlation between BAI and $\Delta^{13}C$ across tested genotypes, we may conclude that the observed increase in $\Delta^{13}C$ with age and inter-annual variation of $\Delta^{13}C$ was largely controlled by the variation in stomatal conductance rather photosynthetic capacity.

### Stability of the genotypic ranking for $\Delta^{13}C$

Checking genotype rank stability with age is of central importance for selecting genotypes for higher water use efficiency. Therefore, genotype ranking in *Populus deltoides × P. nigra* was tested for $\Delta^{13}C$ in controlled *vs.* open field conditions (Monclus et al. 2005) and subsequently, in well irrigated *vs.* water stress under field conditions (Monclus et al. 2006). In both studies, genotypic ranking remained stable with no correlation found between $\Delta^{13}C$ and productivity traits. However, these studies were done on young plants. Parallel to
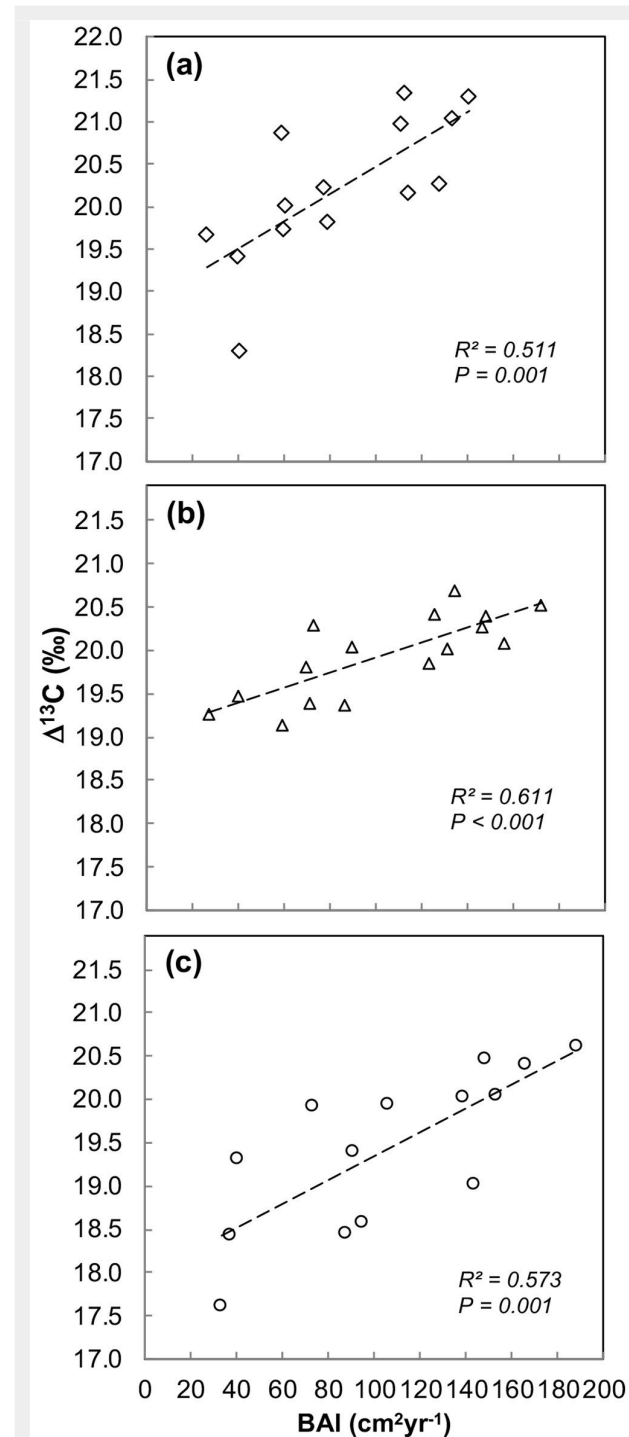


**Fig. 5** - Correlation between carbon isotopic discrimination ($\Delta^{13}C$) and basal area increment (BAI) in three *Populus deltoides × P. nigra* genotypes (a) *Dorskamp*: $\Delta^{13}C$ = 0.0161BAI + 18.9; (b) *I-214*: $\Delta^{13}C$ = 0.0087 BAI + 19.0; and (c) *Koster*: $\Delta^{13}C$ = 0.0138 BAI + 18.0. Each point represents values measured at a given site.

that, many previous studies evidenced an age effect on $\Delta^{13}C$ and demonstrated that duration and extent of age effect is variable according to many species (Leavitt 2010). In this context, genotype ranking made on young plants were susceptible to change with age. Our results showed that genotype ranking shuffled across first age class (4 years) to seventh age class (18 years), *i.e.*, *I-214* displayed highest and *Koster* the lowest $\Delta^{13}C$ values in the first age class (4 years), whereas *I-214* was ranking second in the seventh age class (18 years). In spite of rank shuffling with age, genotypic ranking for mean $\Delta^{13}C$ values matched with that found by Marron et al.

(2005) and Monclus et al. (2005), where *Koster* had the lowest $\Delta^{13}C$ and *Dorskamp* had the highest, and with Rasheed et al. (2011) in Begaar for *Dorskamp* and *I-214*. Thus, we may conclude that based on a relatively small number of cultivars, a stability of genotype ranking was observed with age in two cultivars, at least during the approx. 20 years from planting to harvest. This conclusion is based on a limited range of cultivars. Unfortunately, extension to a larger number of genotypes was impossible given the lack of suitable even-aged common garden plantation of poplar cultivars at suitable age, not to speak of common gardens with different tree ages.

## Conclusion

In this study, $\Delta^{13}C$ assessed from the year 2009 increased with tree age in the three genotypes, indicating a decrease in water use efficiency with age at whole tree level. Furthermore, inter-annual variation of $\Delta^{13}C$ was found positively correlated to BAI in all genotypes which shows that $\Delta^{13}C$ was largely controlled by stomatal conductance rather photosynthetic capacity. Significant genotypic effect was detected for mean $\Delta^{13}C$ over the tested period. The genotypic ranking of $\Delta^{13}C$ was: (i) maintained among the three *Populus deltoides* × *P. nigra* genotypes, despite of crossing detected with tree age; (ii) found consistent with the previous ranking for $\Delta^{13}C$. Finally, *Koster* genotype was found highly water use efficient and productive with age among the tested genotypes. However, further studies are required to test other genotypes as well.

## Acknowledgements

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## Supplementary Material

**Tab. S1** - Complete list of 376 tree cores sampled for three genotypes (*Dorskamp*, *I-214* and *Koster*) under each age class along with information of each site condition (Water available, WA and Water limiting, WL) and their GPS coordinates.

**Tab. S2** - Coefficients of predictor variable for Carbon, Nitrogen and along with variance explained by sites, which was taken as random factor.

**Tab. S3** - Coefficients of predictor variable for Nitrogen along with variance explained by sites, which was taken as random factor.

**Tab. S4** - Coefficients of predictor variable for Carbon isotope discrimination along with variance explained by sites which was taken as random factor.

**Link:** Rasheed_2896@suppl001.pdf