# Are differences among forest tree populations in carbon isotope composition an indication for adaptation to drought?

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1 **Are differences among forest tree populations in carbon isotope composition**
2 **an indication for adaptation to drought?**

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10 Running title: Population differences in carbon isotope composition
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## Population differences in water use efficiency and adaptation to drought

19    Plants have developed different strategies to cope with a reduced water availability
20 in the soil (Levitt 1980). Drought resistance of forest trees is mainly a question of
21 survival and competition within an ecosystem. However, in forestry, it is also a
22 question of maintaining growth. Functional traits can be termed "adaptive" to drought,
23 providing that their genetic variation can be linked to an increased fitness under soil
24 water deficit conditions. The observation of trees grown from seeds of different
25 populations in one or several common environments (e.g. provenance trials or
26 common garden experiments) is a means to control for environmental variation, and
27 therefore to detect genetic differences among these different populations and
28 potentially genotype x environment interactions, if trials are replicated at different
29 locations. Populations from different environments may differ in their mean values of
30 any functional trait in the sense that these mean values relate to the environmental
31 differences of the original population sites. This can be taken as a first indication that
32 these traits are involved in the adaptation of populations to the local conditions.
33 Studying population differences involves measuring the phenotypes of large numbers
34 of individual plants. In this context, the carbon isotope composition ($\delta^{13}$C) of plant
35 organic material has been widely used.

36    $\delta^{13}$C variations of plant organic matter reflect variations in intrinsic water use
37 efficiency ($W_i$, the ratio between A, the net $CO_2$ assimilation rate and $g_s$, the stomatal

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38 conductance to water vapour). This relationship has been explained by Farquhar et al.
39 (1989) using a mecanistic model of $CO_2$ fluxes in the leaf and isotopic fractionation
40 factors. Some of the model parameters — listed in Figure 1 — are likely to vary across
41 genotypes or populations and could therefore weaken the relationship between $\delta^{13}C$
42 and $W_i$. A number of publications on forest trees have nevertheless shown a strong
43 relationship between these two traits, either when comparing provenances
44 (Grossnickle et al. 2005, Ducrey et al. 2008, Kaluthota et al. 2015) or within family
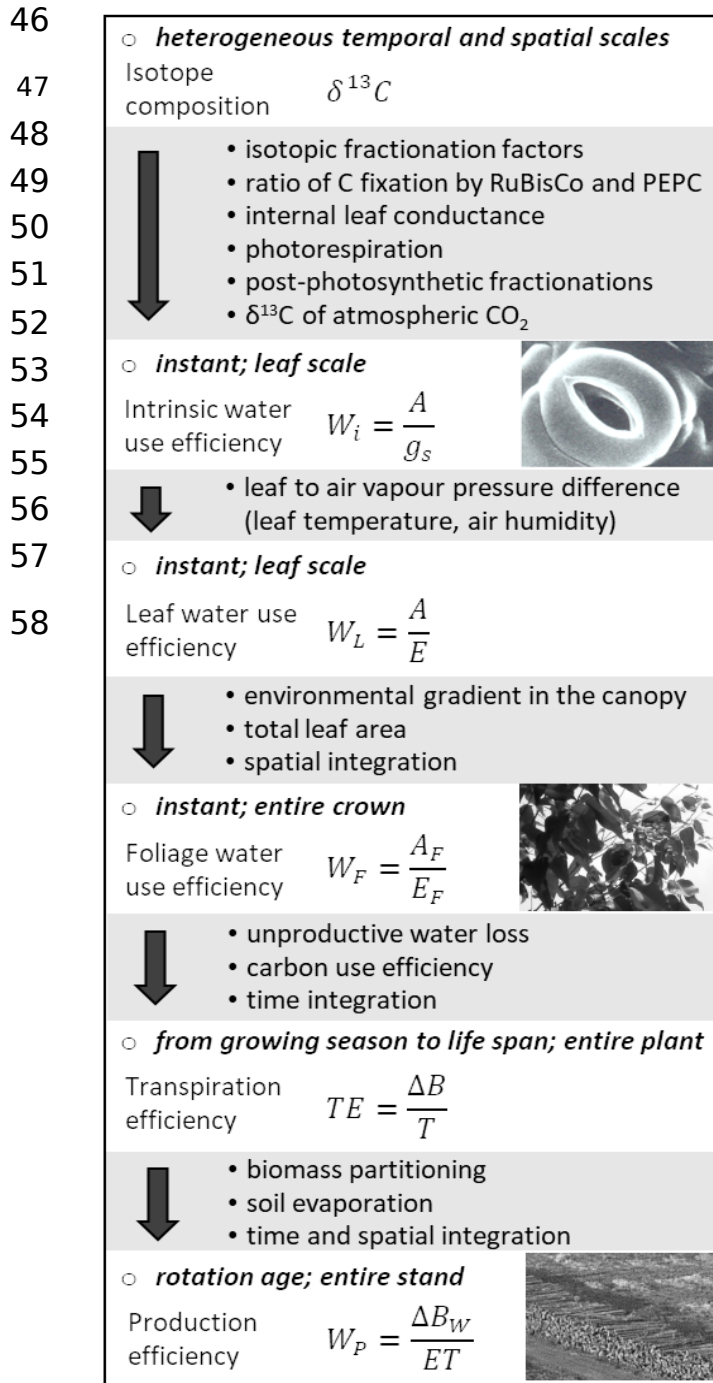45 variations (Roussel et al. 2009, Marguerit et al. 2014).

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○ *heterogeneous temporal and spatial scales*

Isotope composition $\delta^{13}C$

- isotopic fractionation factors
- ratio of C fixation by RuBisCo and PEPC
- internal leaf conductance
- photorespiration
- post-photosynthetic fractionations
- $\delta^{13}C$ of atmospheric $CO_2$

○ *instant; leaf scale*

Intrinsic water use efficiency $W_i = \dfrac{A}{g_s}$

- leaf to air vapour pressure difference (leaf temperature, air humidity)

○ *instant; leaf scale*

Leaf water use efficiency $W_L = \dfrac{A}{E}$

- environmental gradient in the canopy
- total leaf area
- spatial integration

○ *instant; entire crown*

Foliage water use efficiency $W_F = \dfrac{A_F}{E_F}$

- unproductive water loss
- carbon use efficiency
- time integration

○ *from growing season to life span; entire plant*

Transpiration efficiency $TE = \dfrac{\Delta B}{T}$

- biomass partitioning
- soil evaporation
- time and spatial integration

○ *rotation age; entire stand*

Production efficiency $W_P = \dfrac{\Delta B_W}{ET}$

Figure 1: Stable carbon isotope composition ($\delta^{13}C$) and water use efficiency at different time and spatial scales. Factors and traits involved at each change of scale are listed on a gray background. $\Delta B$ and $\Delta B_W$ are total tree biomass and harvested woody biomass, respectively; and T and ET are tree transpiration (or water use) and stand evapotranspiration, respectively.

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59    Hu et al. (2021) examined variations of $\delta^{13}C$ in leaves and stems of *Salix eriocephala*
60    individuals from 34 populations selected from within the large natural range of the
61    species across eastern and western Canada. They were able to show that $W_i$
62    (estimated from $\delta^{13}C$) varied among populations, and to relate these variations to the
63    geographical and climatic characteristics of the sites of origin. A large number of
64    studies have shown population differences in $W_i$ for forest trees. However, only a few
65    of these studies have actually related these differences to the variation in
66    environmental conditions among the original populations, thereby suggesting adaptive
67    differences.

68    As $W_i$ generally increases under drought stress, one hypothesis is that this would
69    also be the case with respect to adaptive differences among populations, so that
70    populations from dry environments would have a high $W_i$. As a first approximation, a
71    negative correlation between the mean $W_i$ of different populations and the
72    precipitation of their original environments would sustain this hypothesis, which has
73    been substantiated by a number of studies on tree species (e.g. Aitken et al. 1995, Li
74    et al. 2000, Cregg and Zhang 2001, Bekessy et al. 2002, Zhang et al. 2005, Aleta et al.
75    2009).

76    However, this hypothesis is not necessarily the only explanation. To elaborate on an
77    alternative hypothesis, we need to look at water use efficiency at the whole plant
78    level, where it is defined as the ratio of dry biomass accumulation per unit water
79    transpired, namely the transpiration efficiency (TE). Maximov (1929) suggested that
80    there was no direct proportionality between TE and the degree of drought resistance,
81    but that TE was an indicator for the ecological drought adaptation strategy of a plant.
82    He found both high and low TE plants among different herbaceous species that were
83    adapted to dry environments (xerophytes). He then demonstrated that plants with
84    high TE developed rapidly and had a large leaf area, whereas plants with low TE were
85    characterised by large root systems. Similarly, Ehleringer (1993) found high and low
86    $W_i$ individual plants within the species *Encelia farinosa*, a drought-deciduous desert
87    shrub. He hypothesized that such plants with low $W_i$ might allocate more carbon to the
88    root system than those with high $W_i$. Recent research on *Arabidopsis thaliana*
89    ecotypes has shown that different drought adaptation strategies can exist within one
90    species, where high $W_i$ was suggested to correspond to drought-sensitive and early
91    closing stomata and low $W_i$ to a drought escape strategy due to early flowering (Lovell
92    et al. 2013, Kenney et al. 2014, Campitelli et al. 2016, Lorts and Lasky 2020). These
93    works thus substantiate a second hypothesis, where populations from dry
94    environments would have a low $W_i$. The evolution of such a strategy would result in a

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95  negative relationship between $\delta^{13}C$ and the drought index of the populations, as
96  reported by Hu et al. (2021). Similar results have been found for other tree species,
97  where populations from low precipitation environments had low $W_i$ (Nguyen-Queyrens
98  et al. 1998) or low TE (Fan et al. 2008). But for trees, unlike annual plants, escaping
99  drought is not an option. Therefore, drought adapted trees with a low $W_i$, due to more
100 open stomata, could either correspond to a drought tolerance strategy, for example by
101 anatomical adaptations reducing vulnerability to cavitation, or to a drought
102 avoidance/water spender strategy through a wide soil exploration by roots. Both
103 strategies would allow stomata to remain open to some extent during drought.
104 Eriksson et al. (2005) demonstrated that European *Castanea sativa* populations from
105 the driest sites showed the lowest $W_i$, but also had the deepest rooting pattern
106 (Lauteri et al. (2004) and M. Lauteri, personal comm.). This response was similar to
107 that of low TE xerophytes in Maximov's study, and consistent with Ehleringer's
108 hypothesis. On the other hand, drought adapted trees with a high $W_i$ (the first
109 hypothesis above) could correspond to a drought avoidance/water saving strategy,
110 through drought-sensitive, early closing stomata.

111     Hu et al. (2021) included two drought indices in a canonical correlation analysis to
112 show that $W_i$ was more related to the duration and the temperature of the growing
113 seasons than water availability. The use of drought indices to characterise the dryness
114 of an environment is an improvement over the use of precipitation only, as these
115 indices take in account other atmospheric variables. However, drought indices often
116 do not directly reflect seasonal variation in precipitation and temperature. A generally
117 dry climate with low but regular precipitation does not correspond to the same
118 selection pressure as a highly seasonal climate with a strong drought (even if, the
119 overall net precipitation might be the same). The few studies that have taken into
120 account seasonality (Voltas et al. (2008) for *Pinus halepensis*; Soolanayakanahally et
121 al. (2009) for *Populus balsamifera*) could relate populations with a higher $W_i$ to sites
122 with a stronger seasonality of drought, compared with climates with less seasonal
123 variability. In addition, the variation of soil types between environments is only rarely
124 taken into account, even though the soil type has a strong impact on the long-term
125 soil water availability. Raddad and Luukkanen (2006) found a higher $W_i$ for *Acacia*
126 *senegal* populations from sandy soils with a low water holding capacity, compared
127 with soils with a higher clay content.

128     There is thus substantial evidence in the literature on forest trees which suggests
129 that population differences in $\delta^{13}C$, observed in a common environment, are linked to

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environmental differences in their original locations. In many cases, a higher $W_i$ is observed for populations from drier sites, but a few examples also show a lower $W_i$ at drier sites, supporting the interpretation of population differences in $W_i$ as an indicator of differences in ecological strategies. To facilitate the interpretation of variations in $W_i$ among populations in terms of adaptive strategies, a comprehensive characterisation of the strength and timing of the soil water deficit seems necessary. Moreover, adaptive strategies should also be explored by analysing the underlying functional causes of the observed diversity in $W_i$.

**How to interpret variations in $W_i$ in terms of underlying traits?**

Hu et al. (2021) found significant population differences for $\delta^{13}C$ and leaf nitrogen related traits in *Salix eriocephala*. They found higher mean $W_i$ for populations with a cooler, shorter growing season and suggested that $W_i$ differences were driven by photosynthetic capacity. This has also been found for *Alnus sinuata* and *Populus balsamifera* populations (Benowicz et al. 2000, Soolanayakanahally et al. 2009, respectively), whether using $W_i$ or through using $W_L$ (the ratio of A to leaf transpiration).

Photosynthetic capacity is one of the major drivers of variation in $W_i$; however, the resulting net $CO_2$ assimilation (A) is closely linked to stomatal conductance ($g_s$). During an ongoing, increasing soil water deficit, under most environmental circumstances and for most plants, $W_i$ increases due to the non-linear nature of the relationship between A and $g_s$ (e.g. Epron and Dreyer 1993). That is to say, during an increasing drought, A is reduced proportionally less than $g_s$, until respiration becomes the dominant component of A (Figure S1 available as Supplementary Data at Tree Physiology Online; Valladares and Sanchez-Gomez 2006). Because $W_i$ is the ratio of A/$g_s$, an observed genetic diversity in $W_i$ might be driven by a diversity in A, or in $g_s$. Even if a genetic diversity exists for photosynthetic capacity, it will be less expressed in an observable diversity of $W_i$ if A is constrained by a low $g_s$. However, the variation in A that is due to a variation in photosynthetic capacity increases with increasing $g_s$ (Figure S1; e.g. Marguerit et al. 2014). The resulting positive correlations between A and $W_i$ have been confirmed within species (e.g. Aranda et al. 2017). Under certain environmental conditions, a genetic diversity in photosynthetic capacity can therefore clearly play a role in observed differences in $W_i$ among populations.
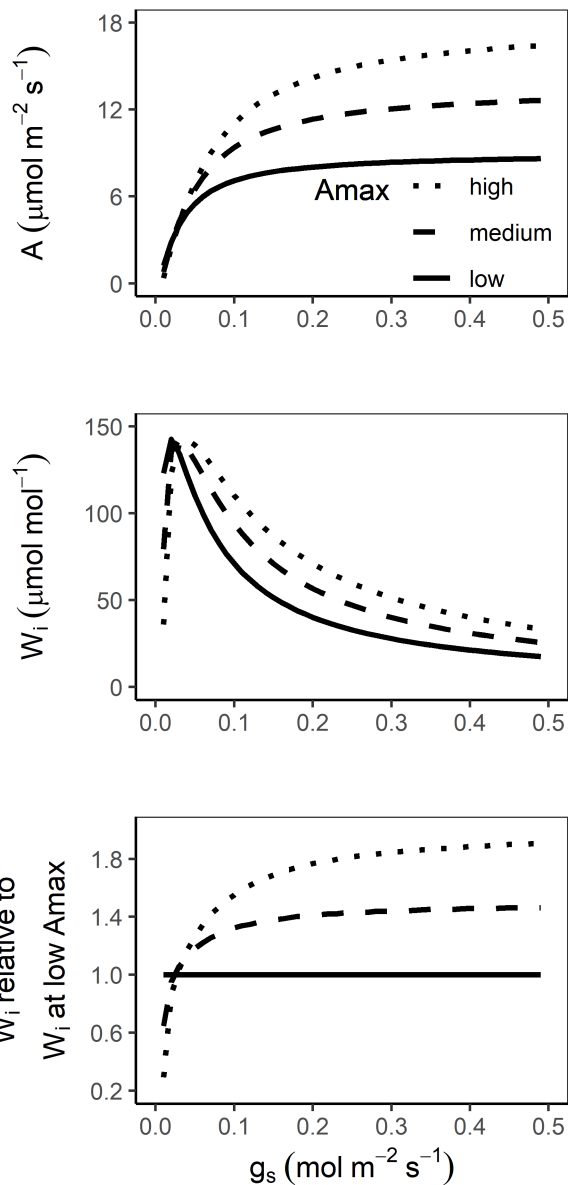
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Figure S1: Relationship between stomatal conductance to water vapour ($g_s$) and net $CO_2$ assimilation (A) and leaf intrinsic water use efficiency ($W_i$) for three different levels of photosynthetic capacity (Amax), and $W_i$ relative to its value at low Amax. We used a simple Michaelis-Menten kinetics equation with a competitive effect of $O_2$ with three different values of Amax (20, 30 and 40 $\mu$mol m$^{-2}$ s$^{-1}$). The Michaelis constant of the RuBisCO for $CO_2$ and the inhibition constant for $O_2$ were set to 270 ppm and 42% respectively. The ambient concentration of $CO_2$ and $O_2$, and the leaf to air vapour pressure difference were set to 400 ppm, 20% and 1 kPa respectively. In this calculation, the photon flux density was assumed not to limit A.

188    Hu et al. (2021) used leaf nitrogen content as a proxy for photosynthetic capacity.
189    Theoretical considerations demonstrate that compounds involved in photosynthesis
190    could account for well over three-quarters of total leaf nitrogen (Field and Mooney
191    1986) and for several plant species, photosynthetic capacity was found to correlate
192    with total leaf nitrogen (Evans 1989, Takashima et al. 2004). This is however not
193    always the case, especially at the within species level, when soil nitrogen does not
194    vary and is not limiting (Roussel et al. 2009). One reason for this might be that
195    changes in leaf structure, such as an increased leaf mass per area (LMA), can be
196    linked to a higher investment of nitrogen into cell walls (Takashima et al. 2004). This
197    results in lower photosynthetic capacity for a similar bulk leaf nitrogen content.

198 Furthermore, other leaf anatomical traits may influence photosynthetic capacity
199 (Niinemets 1999) and modulate the photosynthesis-nitrogen relationship (Reich et al.
200 1998). Therefore, an interpretation of bulk leaf nitrogen content as a proxy for
201 photosynthetic capacity could be improved if LMA, leaf thickness, and leaf tissue
202 density were measured simultaneously, on the same leaves. In addition,
203 measurements of stomatal density, size and depth as well as vein density would allow
204 for the calculation of a theoretical stomatal conductance (McElwain et al. 2016). Such
205 a comprehensive set of leaf traits improves the understanding of the evolution of
206 functional trait syndromes (Chapin III et al. 1993, Arntz and Delph 2001), and
207 therefore the interpretation in terms of ecological strategies.

208     Hu et al (2021) used the ratio of carbon to nitrogen in leaves (C/N) as an easily
209 accessible, time integrated estimator of nitrogen use efficiency (NUE; based on
210 Patterson et al. 1997). A more process-based version of this trait is the photosynthetic
211 nitrogen use efficiency (PNUE) - the ratio between photosynthetic capacity and leaf
212 nitrogen content (Chapin 1980). Because both efficiencies (water and nitrogen)
213 depend on photosynthesis, a relationship is expected. Field et al. (1983) observed a
214 trade-off between PNUE and $W_L$ among evergreen species from the central coast of
215 California. They concluded that species native to the driest sites tended to conserve
216 water, but showed a lower photosynthetic carbon gain per unit of leaf nitrogen,
217 whereas species native to wetter sites used more water but showed a greater carbon
218 gain per unit of leaf nitrogen. Reich et al. (1989) hypothesized, that plants would
219 maximize the use efficiency of the most limiting resource, resulting in the suggested
220 trade-off. Their data and others (Wang et al. 1998) support this hypothesis and show
221 that when water is limiting, $W_L$ increases and when nitrogen is limiting, PNUE
222 increases. Hu et al (2021) also found a negative correlation between $\delta^{13}$C and C/N
223 across all their individual measurements of heart-leaved willow, but not among
224 populations, even though both traits demonstrated significant population effects. This
225 could suggest that the trade-off between water and nitrogen use efficiency,
226 respectively approximated by $\delta^{13}$C and C/N, might not be associated with local
227 adaptation in heart-leaved willow, but rather with plant intrinsic functioning. However,
228 there are also examples in the literature where a significant correlation between $W_i$
229 and PNUE was found at the population level (Gornall and Guy 2007).

230 **Does diversity in $\delta^{13}$C reflect variation in whole plant water use efficiency?**

231     $\delta^{13}$C reflects $W_i$ at the leaf level. However, in the context of adaptation to drought,
232 some thoughts are necessary on the relationship between whole plant TE, which

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233   reflects whole plant carbon gain and water losses, and leaf bulk $\delta^{13}C$ (Figure 1), which
234   is used in the majority of diversity studies. Whole plant TE, which has been studied for
235   a long time (Brendel 2021), is highly relevant in agriculture (e.g. Tallec et al. 2013,
236   Vadez et al. 2014) and in forest ecology (e.g. Bates 1923, Zhang and Marshall 1994, Li
237   et al. 2000, Cernusak et al. 2007a).

238     A first aspect for consideration is the relationship between processes expressed per
239   leaf area (A, $g_s$), and the whole plant leaf area. The total leaf area is a major driver for
240   cumulative water loss through transpiration; however, it has been shown for *Populus*
241   *nigra* genotypes that a high total leaf area can be overcompensated by a low per leaf
242   area transpiration rate (Bogeat-Triboulot et al. 2019). Carbon allocation to different
243   plant organs matters for plant adaptive strategies, not only in terms of total leaf area,
244   but also in terms of carbon allocation to the root system. Moreover, from a forestry
245   point of view, the relationship between the carbon invested into roots and in the above
246   ground biomass is important, and the latter has been used to calculate an above-
247   ground TE ($W_P$ in Figure 1). Significant differences have been observed for the biomass
248   allocation to the roots compared with the shoots among *Pinus pinaster* families
249   (Fernandez et al. 2006) and also among *Cedrus libani* provenances (Ducrey et al.
250   2008), where provenances from dry sites had larger root systems than those from wet
251   sites. Allocating more biomass to the root system will reduce the available amount of
252   carbon for stem growth, but a larger root system could be a drought avoidance trait.
253   As highlighted by Blum (2009) for crop yield improvement under drought, an effective
254   use of water, even at the expense of a high water use efficiency, could be more
255   relevant for tree growth in dry areas.

256     Further, TE integrates whole plant functioning, whereas leaf bulk $\delta^{13}C$ represents the
257   functioning of one or only a few, often fully sunlit, leaves. But even though fully sunlit
258   leaves make up only a fraction of the photosynthesising and transpiring total leaf area
259   of a tree, they are likely a major contributor to the whole plant $CO_2$ assimilation or
260   transpiration, and therefore are, up to a certain point, representative of the total plant
261   functioning. In addition, it is clear that this aspect is even less crucial for young
262   seedlings grown hydroponically in a greenhouse, as in Hu et al. (2021), compared with
263   stands with closed canopies.

264     A second aspect are the traits that are involved in TE but not in leaf level $W_i$, such
265   as unproductive water losses, and carbon losses during the night and from non-
266   photosynthetic organs (Farquhar et al. 1989, Cernusak et al. 2008). For example, leaf
267   $\delta^{13}C$ captured well the variability of $W_i$ among several *Acacia* species, whereas

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268 differences in carbon use efficiency or the relative unproductive water losses disrupted
269 the relationship between TE and $\delta^{13}$C (Konate et al. 2016). Even though all of these
270 traits can also vary genetically among genotypes or populations, strong correlations
271 have nevertheless been observed between TE and $W_i$ (or $W_L$) within tree species,
272 either driven by environmental variations, such as different drought levels or soil
273 fertility (Osorio and Pereira 1994, Cernusak et al. 2007, respectively), or by within
274 species genetic diversity (Zhang et al. 1994, Sun et al. 1996, Roupsard et al. 1998, Li
275 2000). However, it is clear that more research is needed on the genetic co-variation of
276 the traits underlying TE and their relationship to the plant's ecological strategy.

277 **Is $\delta^{13}$C a promising physiological proxy to improve forest genetic resources?**

278    Hu et al. (2021) reported an absence of a trade-off between water and nitrogen use
279 efficiencies and suggested that it is therefore possible to capture additive genetic
280 variation of both. Because $\delta^{13}$C can be easily measured on a large number of samples,
281 it is tempting to use $\delta^{13}$C for tree breeding to select genotypes with high water use
282 efficiency, especially when positive genetic gains can be achieved without
283 compromising growth, wood quality and other adaptive and economic traits (Cumbie
284 et al. 2011, Marguerit et al. 2014, Bartholomé et al. 2015, Bouvet et al. 2020).
285 Furthermore, $\delta^{13}$C is a promising trait for the characterisation of within population
286 functional diversity to foster forest resilience (Messier et al. 2019), especially for the
287 Mediterranean area, where the introduction of less water demanding ecotypes, in the
288 frame of an assisted migration program, could increase resilience (Bussotti et al.
289 2015). It is however important to ensure that variation in $\delta^{13}$C corresponds to variation
290 in TE, and therefore accounts for an adaptive strategy. Other than the possibility of
291 increasing drought, also the rising $CO_2$ concentration in the decades to come would
292 need to be taken into account to predict the water use efficiency of different genetic
293 backgrounds. Up to now, the rising $CO_2$ concentration has increased water use
294 efficiency at a global level (Cernusak et al. 2019), because of its impact on
295 photosynthesis and stomatal conductance. However this trend might slow in future
296 due to saturation of photosynthesis. Also, the introduction of high TE and high growth
297 genotypes, which might use more water overall, into existing ecosystems or
298 plantations needs to be regarded with caution. This is because it has important
299 economic and political implications, especially in areas where water resources are
300 critical.

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302 **Supplementary Data**

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303 Supplementary Data for this article are available at Tree Physiology Online.

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