Mixing beech with fir or pubescent oak does not help mitigate drought exposure at the limit of its climatic range

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Abstract:

In the context of climate change, it remains unclear whether mixed-species forests will help mitigate the impacts of future droughts and, if so, through which processes. As European beech (*Fagus sylvatica*) is one of the major European species, it is crucial to evaluate its response to drought when mixed with species with contrasted functional traits and in contrasted climatic conditions, particularly at the limit of its climatic range. This study aimed to (i) characterize the effects of tree species interactions on the drought exposure of beech in south-eastern France, and (ii) determine whether belowground water uptake complementarity underlies these effects. We focused on beech-silver fir (*Abies alba*) and beech-pubescent oak (*Quercus pubescens*) forests across six sites in the French pre-Alps, a region at the limit of the climatic range for beech. We used a triplet approach to compare the tree-ring carbon isotope composition (δ^{13}C) of these species in pure and two-species mixed stands during a period of very dry years, and used water hydrogen isotope composition (δ^2H) in the xylem to identify water uptake sources. Overall, we found no clear mixture effect pattern on beech physiological functioning among sites and triplets. In beech-fir sites, mixing beech with fir had no effect on beech δ^{13}C values during dry years. In beech-oak sites, mixture effects on beech were mostly neutral, although sometimes beech suffered from a stronger exposure to drought in mixed stands. Our study emphasizes the impact of the tree sampling design on the outcome of studies on forest biodiversity-ecosystem functioning relationships. Limiting tree sampling to dominant trees when analyzing stand-level relationships may bias these outcomes. We evidenced differences

46 in water uptake sources between beech and fir, but not between beech and oak during a dry

47 summer. However, these patterns did not help explain the lack of species mixture effects, or

48 existence thereof, at the triplet scale. Our study demonstrates that managing beech in mixed

49 stands with silver fir or pubescent oak at the limit of beech climatic range does not buffer

50 drought impacts on beech during dry years. In the long term, with more frequent extreme

51 droughts, promoting beech-fir mixtures will not be detrimental to beech drought response, while

52 beech may suffer in mixtures with pubescent oak.

1. Introduction

European forest ecosystems are expected to be highly impacted by ongoing climate change (Hanewinkel et al., 2013) through an increase in temperatures and more frequent and intense droughts (IPCC, 2014; Lehner et al., 2017). Drought-induced stress can lead to a loss of vitality and to mortality for trees, caused by hydraulic failure and carbon starvation (McDowell et al., 2008; Hartmann et al., 2018). This will be especially true in southern parts of the European temperate and Mediterranean regions (Lindner et al., 2010). There is therefore a strong need for new management strategies to help forest owners mitigate current and future drought impacts (Bolte et al., 2009).

European beech (*Fagus sylvatica* L.) is an essential species in European forests: its natural distribution covers most of continental Europe and it is the most abundant broad-leaved forest tree in Central Europe (Dittmar et al., 2003). Beech is a drought-sensitive species and is especially vulnerable when the soil water deficit is severe and prolonged (Gessler et al., 2007). Model-based projections predict that climate change will likely induce a reduction in beech-dominated forests in southern Europe (Cheaib et al., 2012; Hanewinkel et al., 2013). Growth declines for beech have already been reported in recent decades (Charru et al., 2017), especially at its southern range limits (Jump et al., 2006).

Managing temperate forests with higher tree species diversity has been identified as one management strategy that can improve forest productivity (e.g. Toïgo et al., 2015; Liang et al., 2016; Ammer, 2019) and other ecosystem functions (Brockerhoff et al., 2017), such as resistance to disturbances (Jactel et al., 2017). In particular, it has been shown that beech productivity improves in mixed forests compared to pure ones, particularly when beech is admixed with silver fir (*Abies alba* Mill.) or other coniferous species (e.g. Toïgo et al., 2015; Pretzsch and Forrester, 2017). However, it is still not clear whether mixed-species forests also

77   contribute to mitigating drought impacts (Grossiord, 2019), which in turn would have an effect

78   on forest vitality and productivity. In the case of beech, contrasted effects of species interactions

79   have been found under drought conditions. Some studies demonstrate lower drought exposure

80   of beech in mixed stands (e.g. Pretzsch et al., 2013; Metz et al., 2016), but others conclude that

81   the mixture effect is neutral (e.g. Schwarz and Bauhus, 2019). Furthermore, contrasted results

82   were found when comparing the effects of the same species mixture, including beech, among

83   different sites (Schäfer et al., 2017; González de Andrés et al., 2018; Jourdan et al., 2019b).

84   Two factors have been identified as major drivers for the variability in the outcome of mixture

85   effects on the mitigation of drought impacts: the importance of functional variability among

86   species and the effect of site climatic conditions (Forrester, 2014; Forrester and Bauhus, 2016).

87   First, the outcomes are species-dependent: species with more contrasted functional traits in

88   terms of physiology, phenology or morphology (e.g. root depth, stomatal control, physical traits

89   involved in xylem resistance to embolism), are more likely to show complementarity effects,

90   especially for drought responses (Grossiord, 2019). Secondly, even if the given species mixture

91   potentially mitigates drought impacts, the degree of the effect may vary with site-level

92   environmental conditions (Forrester and Bauhus, 2016). Forrester and Bauhus (2016) suggested

93   a theoretical framework, as a generalization of the stress gradient hypothesis (Bertness and

94   Callaway, 1994), in which positive mixture effects are expected to be stronger when resources

95   are scarce, for example along a declining precipitation gradient or during severe drought events.

96   In accordance with this framework, Grossiord et al. (2014c, 2014b) found negative or neutral

97   effects in more humid sites and positive effects in drier ones (Grossiord et al., 2014b, 2014c).

98   However, the relationship between mixture effects and a resource gradient may not be linear

99   but may rather emerge from threshold points of resource availability (de Streel et al., 2020).

100   Such patterns could be explained by the fact that positive mixture effects may not be strong

101   enough to compensate for the increasing environmental constraints in extremely dry sites and

102 that positive mixture effects on drought response could disappear with strong water stress (e.g.

103 de Streel et al., 2020).

104 The outcome of studies on the effects of species mixing may also depend on how the trees were

105 sampled. To date the typical approach has been to limit the sampling design to dominant trees

106 when examining mixture effects on the response of trees to drought, particularly as measured

107 by carbon isotope composition ($\delta^{13}C$) (Grossiord et al., 2014c; Metz et al., 2016; Schwarz and

108 Bauhus, 2019). However, not including light-suppressed trees in these analyses could bias the

109 overall patterns of mixture effects, as the drought sensitivity of suppressed and dominant trees

110 may differ (Mérian and Lebourgeois, 2011). Including all trees for a more complete

111 representation of the forest stand could help in understanding the variability of mixture effects

112 on drought responses.

113 The processes behind mixture effects on drought exposure are linked to spatial or temporal

114 resource partitioning among species due to competition, facilitation, or niche differentiation for

115 resource acquisition and use (Ammer, 2019; Grossiord, 2019). At aboveground level, this may

116 result in, for example, differences in canopy packing and shading effects (Ishii and Asano,

117 2010; Jucker et al., 2015; Grossiord 2019). At belowground level, complementarity for water

118 uptake and niche differentiation among species could result in a lesser exposure to decreased

119 soil water content during extreme drought events (Forrester, 2014), but these processes have

120 rarely been experimentally tested in the field (Grossiord et al., 2014a; Goisser et al., 2016).

121 Further studies for species diversity effects on beech drought exposure are therefore necessary

122 to identify whether or not tree-species mixing might mitigate drought impacts and maintain

123 beech productivity, particularly along the southern margins of beech distribution in Europe

124 where climate change is occurring the fastest. To address these questions, we studied the effect

125 of species mixture on beech drought exposure during a period with severe summer droughts, in

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forest plots of different species compositions distributed across six sites in south-eastern France with contrasting climate conditions. We studied two widespread species mixtures with beech in mountainous areas of south-eastern France: beech associated with silver fir (*Abies alba* Mill.) in the northern part of the region, and beech associated with pubescent oak (*Quercus pubescens* Willd.) in the southern part. These two accompanying species are likely to show complementarity effects with beech as they have contrasted physiological functioning, i.e. contrasted water transport systems and phenology between beech (deciduous species) and fir (evergreen conifer), and contrasted drought and shade tolerance between beech and pubescent oak (Niinemets and Valladares, 2006). We sampled all trees in the stands, including both dominant and light suppressed trees. We used stable carbon isotope composition ($\delta^{13}C$) in tree rings from dry years to examine the effect of species composition on tree exposition to drought (Grossiord et al., 2014c; Metz et al., 2016). Tree ring analysis provided us with an integrative measurement of past drought effects on tree functioning (McCarroll and Loader, 2004). Tree-ring $\delta^{13}C$ is expressed as a negative value that increases (less negative values) when a tree is exposed to increased water stress (Saurer et al., 1995). It indirectly reflects the compromise between carbon and water acquisition and use at leaf and plant level (Farquhar et al., 1982). Finally, to assess potential water-use complementarity mechanisms, we looked at differences in water uptake sources between species. To do so, we measured the stable hydrogen isotope composition (δ^2H) in branch xylem water (Dawson, 1993; West et al., 2006).

We specifically addressed the following questions:

1. Is beech physiological functioning ($\delta^{13}C$) during dry years influenced by site-specific environmental conditions?

2. Is beech $\delta^{13}C$ during dry years different between pure and mixed stands?

3. Can potential mixture effects be explained by complementarity in species water uptake sources?

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2. Material and Methods

2.1. Field design

We selected six sites in the French pre-Alps on limestone bedrock (Fig. 1a, see also Jourdan et al., 2019b) spanning a wide range of climatic conditions (Fig. 2). At each site, we used a triplet approach, i.e. an ensemble of three plots: one plot in pure stands of each studied species and one plot in a mixed stand of the same two species (Fig. 1b). All the triplets were located on north-facing slopes. The three plots within each triplet were as close to each other as possible and with a similar slope (Table A1). The soil textures and gravel contents were similar within a triplet. At each site, two sets of such triplets were selected in order to cover local variability in environmental conditions and stand structure, resulting in six plots per site (Fig. 1b). Plot elevations ranged from 700 m to 1400 m (Table A1).

Figure 1 HERE; size: 2 columns; B&W

European beech (*Fagus sylvatica* L.) was present at all six sites and was mixed with either silver fir (*Abies alba* Mill.) in the three northern sites (S1 to S3) or with pubescent oak (*Quercus pubescens* Willd.) in the three southern ones (S4 to S6) (Fig. 1a). In pure plots, the focal species accounted for at least 80% of the total basal area and in the mixed plots each focal species accounted for at least 40% of the total basal area (Table A2). Other tree species present in the plots represented less than 20% of the total basal area and were mostly in the understory of the canopy (Table 1, Table A2). The trees from both focal species in the mixed stands were mixed on a stemwise intermingling pattern (del Río et al., 2018). Sites S1 to S4 were located in forests with continuous-cover management whereas sites S5 and S6 had limited management. To limit direct management effects, we only worked in stands where no thinning operations had been conducted for at least the last two decades. All the stands were located in mature forests with an uneven-aged high forest structure, except for S5, which was in a coppice forest. Individual

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175   plots were circular with a radius of 17.5 m (area = 962 m^2) and consisted of a central area (10

176   m radius, area = 314 m^2) where the measurements were conducted, plus a buffer zone (7.5 m

177   radius) to ensure the plot was surrounded by homogeneous conditions (Fig. 1c).

178   Table 1 HERE; size: 1.5 columns

179   *2.2. Climate data*

180   Climatic variables were extracted from the mesoscale SAFRAN reanalysis of the Météo France

181   national observation network data (Quintana-Seguí et al., 2008). As SAFRAN was initially

182   designed for mountainous areas, it was the best-estimated source of daily climatic data for our

183   study region. The precision of the climate data was relevant at the site scale, but not at the triplet

184   scale, as the SAFRAN analysis covers France with an 8 km × 8 km grid and is calculated for

185   the average elevation of the corresponding grid. We used the SAFRAN long-term climate data

186   to estimate the average cumulative annual precipitation and the mean annual temperature per

187   site over a 20-year period (1994-2013) (Fig. 2; Table A3).

188   Figure 2 HERE; size: 1 column; B&W

189   *2.3. Choice and characterization of dry years*

190   Jourdan et al. (2019b) calculated the standardized precipitation evapotranspiration index (SPEI,

191   Vicente-Serrano et al., 2010) for each of the six sites for the months of February to July, i.e. the

192   growth period, of each year from 1994 to 2013 (see Fig. 2 in Jourdan et al., 2019b). SPEI is a

193   standardized index that makes it possible to identify dry years for a given site, but not to

194   compare drought intensity among sites. This enabled us to choose the period of dry years

195   relative to each site within the 1994-2013 period for tree-ring carbon isotope composition (δ^{13}C)

196   analyses. We targeted the 2003-2007 period because it was representative of a series of severe

197   dry years for all sites. Indeed, this period was on average much drier in terms of precipitation

198 across sites than the 20-year average for 1994-2013 (Fig. 2), but was also hotter during the

199 summer months (Table A3).

200 To characterize the water stress during these dry years, we used the daily forest water balance

201 model BILJOU© (Granier et al., 1999). We estimated the daily relative extractable water

202 (REW, unitless) for each site based on daily climate data over the 1994-2013 period. REW is

203 the ratio between available soil water and the maximum extractable water (water holding

204 capacity) and varies from field capacity (REW=1) to permanent wilting point (REW=0). Water

205 stress is assumed to occur when REW drops below a threshold of 0.4 under which stomatal

206 conductance, and therefore leaf gas exchange, is gradually reduced (Granier et al., 1999). The

207 BILJOU© model has a daily time step and requires daily climatic input variables (mean air

208 temperature, precipitation, wind speed, global radiation, and humidity) as well as soil water

209 holding capacity and leaf area index (LAI). We used SAFRAN daily climatic variables at the

210 site scale. We fixed soil water holding capacity at 100 mm since we were not able to

211 characterize the plot soil profiles precisely. Nevertheless, we tested the BILJOU© model with

212 different soil water holding capacity values but this did not change the ranking of the sites in

213 terms of water stress (data not shown). Similarly, we used a single LAI value (i.e. 7.0 $m^2 m^{-2}$)

214 for all the sites as a standard value for mature forests. Adapting this value to the different sites

215 did not change the ranking of the sites. For each year at each site, an annual number of days

216 when REW is below 0.4 was calculated, which allowed to quantify water stress levels (Table

217 A3).

218   *2.4. Tree measurements*

219   *2.4.1. Dendrometry*

220 In 2014, in the central area of each of the 36 plots, Jourdan et al. (2019b) surveyed all of the

221 trees with a diameter at breast height (DBH, cm) greater than 7.5 cm and recorded their DBH

222 and total height. They also cored the trunk of all trees with a DBH greater than 7.5 cm in the

223 plots at a height of 1.3 m with a Pressler increment borer (Haglöf, Sweden) in 2014-2015. In

224 coppice stands, they only cored the largest stem of each clump of coppice shoots (individual

225 tree). Some cores were too difficult to date accurately and were discarded from further analyses,

226 leaving a total of 792 trees sampled (368 beech, 222 fir and 202 pubescent oak trees),

227 representing all the diameter classes and different strata in the canopy. On these cores, Jourdan

228 et al. (2019b) measured tree-ring width for the 1994-2013 period and performed cross-dating

229 for each species according to specific pointer years.

230   *2.4.2. Carbon isotope composition ($\delta^{13}C$)*

231 We used these tree cores to analyse the stable carbon isotope composition ($\delta^{13}C$) of each tree

232 in the tree-rings of the selected period (2003-2007). Using a scalpel under a stereomicroscope,

233 we extracted the tree-rings (including earlywood and latewood) that corresponded to the target

234 period (2003-2007) as one pooled block for all five years. We used whole wood tissue for our

235 analyses as it shows the same trends as cellulose-only isotopic measurements and allowed us to

236 analyze a larger number of trees (McCarroll and Loader, 2004). For each tree, the tree-rings of

237 the 2003-2007 period were kept as one sample. We ground them into a fine powder, then

238 weighed around 1 mg of powder for each sample in tin capsules for the isotopic analysis. We

239 used a different technique when the dry mass of the 2003-2007 tree-ring sample was less than

240 6 mg (15 samples out of 792) to avoid any loss of matter in the grinding process. These 15

241 samples were cut into four quarters perpendicularly to the tree-ring axis with a scalpel instead

242 of being ground. For each of these samples, we then analyzed separately three out of four

243 subsamples for isotopic composition and used the average of these three $\delta^{13}C$ values. The fourth

244 subsample was kept as a spare in case further analyses should be needed on these samples.

The $^{13}C/^{12}C$ ratios of each wood sample were analyzed with an isotope-ratio mass spectrometer (IRMS, Isoprime 100, Isoprime Ltd., Cheadle Hulme, UK) coupled with an elemental analyzer (EA, Elementar vario, ISOTOPE cube, Elementar Analysen Systeme GmbH, Hanau, Germany) at the SILVATECH platform (Silvatech, INRAE, 2018. Structural and functional analysis of tree and wood Facility, Nancy, France). The results were expressed as carbon isotope composition $\delta^{13}C$ (‰) relative to the international standard Vienna Pee Dee Bee Belemnite (V-PDB) (IAEA, 1995) following Eq. 1:

$$\delta^{13}C\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000 \tag{1}$$

where R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ ratios of the sample and the V-PDB standard, respectively (Farquhar et al., 1982). The mass spectrometer had a precision of ± 0.2 ‰ for $\delta^{13}C$ values.

In this study, we used two sets of $\delta^{13}C$ data for our statistical analyses. First, we used all the $\delta^{13}C$ values to characterize the response of all the trees, individually or at the stand level, hereafter called "all trees". Second, we used a subsample of pre-selected dominant trees in each plot, hereafter called "dominant-tree subsample", to analyze the effect of mixture on the $\delta^{13}C$ of the trees without any confounding factor related to light availability in the canopy (McCarroll and Loader, 2004). These dominant trees were selected according to the following criteria: (i) they were in the biggest diameter class in each plot, (ii) they had a crown illumination index (Dawkins index) of 4 or 5, meaning that they received direct vertical light (Clark and Clark, 1992), (iii) they were well mixed with the other species on a stem-to-stem basis in the mixed stands, and (iv) they were suitable for branch sampling by climbers (see $\delta^{2}H$ section). We then randomly chose between four and seven of these dominant trees per species in each plot, leading to a total of 240 dominant trees (beech = 121, fir = 57, pubescent oak = 62) out of a total of 792 trees measured for $\delta^{13}C$.

269   One caveat should be noted concerning our $\delta^{13}C$ measurements: we were able to compare $\delta^{13}C$

270   functioning during dry years between pure and mixed plots, but we were not able to use the

271   difference in $\delta^{13}C$ between a reference wet year and a dry year to estimate tree drought

272   resistance, as some studies have done to detect mixture effects (e.g., Grossiord et al., 2014c;

273   Schwarz and Bauhus, 2019). Indeed, during the period from 1994 to 2013, we were not able to

274   find any years that were wet enough to serve as a reference across all sites. Moreover, as growth

275   is very slow at some of the sites with a Mediterranean influence, we would not have been able

276   to cut out single yearly tree-rings precisely enough. Other studies have also used absolute values

277   of $\delta^{13}C$, as we did, albeit usually over a longer-term chronology (González de Andrés et al.,

278   2018; de Streel et al., 2020). Moreover, in a study looking at tree diversity effects on drought

279   responses using $\delta^{13}C$, the same trends were found with differences in $\delta^{13}C$ between a wet and a

280   dry year (Grossiord et al., 2014c) than with absolute $\delta^{13}C$ values for the dry years only

281   (Grossiord, 2014, p.48). We therefore focused our analyses on mixture effects on tree

282   functioning during dry years by comparing values between pure and mixed stands, expecting

283   more negative $\delta^{13}C$ values for beech in mixed stands as compared to pure ones if the presence

284   of oak or fir in the mixture induced less competition for water resources.

285   *2.4.3. Xylem water δ^2H*

286   To estimate among-tree differences in water uptake sources, we used a method that relies on

287   the natural abundance of stable hydrogen isotopes in the xylem water from branches (Dawson,

288   1993). During dry spells, an isotopic gradient for hydrogen is established in the top layers of

289   the soil (Clark and Fritz, 1997). The hydrogen isotope composition (δ^2H, ‰) of water flowing

290   in the xylem, which is the raw sap coming up from the roots, represents the mean signature of

291   all the water sources for the tree's rooting system. Due to very rocky shallow soils, we were not

292   able to obtain a full soil profile at all of our sites. To address niche partitioning in water uptake

13

293   sources, we therefore analyzed differences in δ^2H values within triplets, but we were not able

294   to quantitatively estimate a depth of water uptake by trees.

295   We sampled the same trees selected for the dominant-tree subsample of $\delta^{13}C$ analyses. To

296   determine the δ^2H of the xylem water, we sampled a 30 to 40-cm-long branch from each

297   dominant tree in the summer of 2018 during a dry spell at five sites, for a total of 200 samples.

298   We could not visit S4 that year for technical reasons, and therefore we do not have δ^2H values

299   for that site. Professional climbers cut the branches out of the upper third of the tree crown. We

300   removed bark tissue immediately with a knife to prevent phloem sap from mixing with xylem

301   water. Five-centimeter-long samples were then cut from the branches and placed into closed

302   airtight glass vials and stored in cool conditions.

303   Once in the lab, we stored the branch samples in cool chambers at 4°C to reduce the risk of

304   evaporation. We then extracted water from the branch samples through cold trapping with a

305   custom-made static vacuum cryogenic distillation system (West et al., 2006). The water from

306   the branch samples was evaporated by heating the sampling tube in a water bath (65°C), then

307   condensed with liquid nitrogen for 90 minutes in a collection tube. The extracted water was

308   analyzed for $^2H/^1H$ ratios with an elemental analyzer (EA, EuroPyrOH; EuroVector, Milano,

309   Italy) coupled to an isotope ratio mass spectrometer (Isoprime IRMS; Elementar, Manchester,

310   UK) at the SILVATECH platform (Silvatech, INRAE, 2018. Structural and functional analysis

311   of tree and wood Facility, Nancy, France). The $^2H/^1H$ ratio of each water sample was

312   determined from six subsamples of 0.2 μL of extracted water and only the last three

313   measurements were kept, then averaged for each sample. The first three measurements were

314   discarded to avoid any bias related to a potential "memory effect", meaning a contamination

315   from the previous sample analyzed. The results were expressed as δ^2H relatively to the

316   international Vienna-standard mean ocean water standard (V-SMOW) following Eq. 2:

$$\delta^2 H \ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000 \tag{2}$$

where R_{sample} and $R_{standard}$ represent the $^2H/^1H$ ratios of the sample and of the V-SMOW standard (IAEA, 1995). The mass spectrometer used in this study had a precision of $\pm$ 2.0 ‰ for δ^2H values.

We weighed the branch samples after cryo-distillation and placed them in a drying oven for 48 h at 65°C to check if all the water had been extracted. If part of the water had not been completely extracted, we applied a correction to the δ^2H values since the extracted water had less of the heavier isotope (^2H) than the water remaining in the branch. We calculated the theoretical fractionation of the hydrogen isotopes with Eq. 3 to obtain a corrected δ^2H value (Araguás-Araguás et al., 1995):

$$\delta^2 H_{corr} = \delta^2 H_{measured} - \left(\frac{F^{(1/\alpha)} - F}{F - 1} * 1000 \right) \tag{3}$$

where $\delta^2H_{measured}$ is the known measured value, δ^2H_{corr} the expected real value if there had been no fractionation during extraction, F the fraction of water remaining in the branch, and α a fixed factor specific to the isotope measured (^2H) and to the extraction temperature (65°C). Factor α is calculated from Eq. 4 (Majoube, 1971):

$$10^3 * \ln(\alpha) = a * \left(\frac{10^6}{T_K^2} \right) + b * \left(\frac{10^3}{T_K} \right) + c \tag{4}$$

where T_K is the extraction temperature in degrees Kelvin, and a, b, and c are fixed factors for ^2H defined in Majoube (1971) with a = 24.844, b = -76.248, and c = 52.612 (Clark and Fritz, 1997). We applied this correction to all the samples with a water-extraction percentage less than 100%.

2.5. Data analysis

338 All data analyses were conducted including either all trees or those in the dominant-tree
339 subsample.

2.5.1. Variability of $\delta^{13}C$ values in pure stands

341 To assess the variability of δ^{13}C values among sites and species, we compared δ^{13}C values in

342 the pure stands only. First, for site effects, we studied the differences in δ^{13}C values among the

343 six pure beech stands, and among the three stands of pure fir and pure pubescent oak, following

344 the model:

$$\delta^{13}C \sim \text{Site} + \text{Triplet} + \text{Site:Triplet} \tag{5}$$

346 We used a linear model for each species with *Site* (S1 to S6) and *Triplet* (1 or 2) as factor

347 variables. We further tested the interaction between *Site* and *Triplet* to reveal the differences

348 between the two triplets at each site.

349 Second, we tested for differences in δ^{13}C values between the two species (beech and fir, or

350 beech and oak) in pure stands, following the model:

$$\delta^{13}C \sim \log(\text{DBH}) + \text{Species} + \log(\text{DBH}):\text{Species} + 1|\text{Triplet ID} \tag{6}$$

352 We used a linear mixed model with *Species* as a two-level factor (beech vs. fir, or beech vs.

353 oak) and *log(DBH)* of the individual tree as a continuous variable. The interaction between

354 *log(DBH)* and *Species* was also included in the model. *Triplet ID* was used as a random factor

355 (*Triplet ID* is a six-level factor for each type of mixture, identified by site and triplet number,

356 e.g. S1:2 for site 1, triplet 2). The introduction of individual tree DBH as a covariate was used

357 as a proxy for light availability, as the biggest trees within triplets are the ones with the most

358 access to light while smaller trees represent the understory trees. This approach allowed us to

359 take into account the confounding factor related to light availability in the canopy and its effect

16

360 on $\delta^{13}C$ (McCarroll and Loader, 2004). We used the log transformation of DBH to obtain a

361 linear relationship between *log(DBH)* and $\delta^{13}C$ values .

362 *2.5.2. Mixture effects on $\delta^{13}C$*

363 We analyzed the effect of species mixture on $\delta^{13}C$ values separately by type of mixture (beech-

364 fir or beech-oak) with three different types of analyses that made it possible to test for a mixture

365 effect at different levels. First, we tested the mixture effect on individual $\delta^{13}C$ values by species

366 separately for the beech-fir and beech-oak sites with all six triplets together following the linear

367 mixed-effect model:

$$\delta^{13}C \sim \log(DBH) + \text{Mixture} + \log(DBH) : \text{Mixture} + 1|\text{Triplet ID} \qquad (7)$$

369 with *Mixture* (pure vs. mixed stands) as a fixed two-level factor, *log(DBH)* of each tree as a

370 continuous variable, and *Triplet ID* as a random factor (*Triplet ID* is a six-level factor for each

371 type of mixture, identified by site and triplet number, e.g. S1:2 for site 1, triplet 2). The

372 interaction between *log(DBH)* and *Mixture* was also included in the model.

373 Second, we tested the same effect separately for each triplet with the following linear model for

374 each species and triplet:

$$\delta^{13}C \sim \log(DBH) + \text{Mixture} + \log(DBH) : \text{Mixture} \qquad (8)$$

376 with *Mixture* as a two-level factor (pure vs. mixed stands), *log(DBH)* as a covariate. The

377 interaction between *log(DBH)* and *Mixture* was also included in the model.

378 Finally, to evaluate any effect of mixture on stand-level functioning, we used a metric called

379 "Net Biodiversity Effect" (NBE, ‰), adapted for complex traits such as $\delta^{13}C$ by Grossiord et

380 al. (2013) from Loreau and Hector (2001). We calculated NBE for the dominant-tree subsample

381 only (NBE_{dom}) because with all trees we would not have been able to separate the effects of

382 light availability vs. water availability.

17

383   NBE_{dom} on the average $\delta^{13}C$ of a given mixture of species is the difference between the observed

384   averaged $\delta^{13}C$ in the mixture ($\delta^{13}C_O$, ‰) and the expected averaged $\delta^{13}C$ in the mixture ($\delta^{13}C_E$,

385   ‰) calculated with species values in pure stands:

$$NBE_{dom} = \delta^{13}C_O - \delta^{13}C_E = \sum_{i=1}^{2}(\delta^{13}C_{Oi} * P_{O\,i}) + \sum_{i=1}^{2}(\delta^{13}C_{Ei} * P_{Oi}) \qquad (9)$$

387   where $\delta^{13}C_{Oi}$ is the observed average $\delta^{13}C$ value for dominant trees of species i in mixed stands,

388     $\delta^{13}C_{Ei}$ is the average $\delta^{13}C$ value for dominant trees of species i in its respective pure stand and

389   P_{Oi} is the proportion of species i in the mixed plot in terms of basal area.

390   We calculated NBE_{dom} values for each triplet. Based on the precision of the mass spectrometer

391   used for the isotopic measurements in this study ($\pm$ 0.2 ‰), NBE_{dom} values were considered

392   different from 0.0 ‰ when the difference between observed and expected mixed stand values

393   was greater than $\pm$ 0.4 ‰. A positive difference means that the observed mixed stand had a

394   higher $\delta^{13}C$ value than expected based on values from the corresponding pure stands. A negative

395   difference means that the observed mixed stand had a lower $\delta^{13}C$ value than expected from

396   pure-stand values. Since there was only one value per triplet, no statistical analyses were

397   possible for this variable, though we were able to compare results across triplets.

398   *2.5.3. δ^2H analysis*

399   To test for differences in water uptake sources between species and between pure and mixed

400   stands, we analyzed δ^2H data separately by type of mixture (beech-fir or beech-oak). We used

401   a linear mixed model including *Triplet ID* as a random factor to test for differences between

402   species and pure or mixed stands. We used the following model:

403           $\delta^2H \sim$ Species + Mixture + Species: Mixture + 1|Triplet ID   (10)

404   with *Species* a two-level factor (beech and fir, or beech and oak), *Mixture* a two-level factor

405   (pure or mixed), and *Triplet ID* a random factor (*Triplet ID* is a six-level factor for each type of

406   mixture, identified by site and triplet number, e.g. S1:2 for site 1, triplet 2).

407   2.5.4. *Statistical analyses*

408   All analyses were performed with R software version 3.6.2 (R Core Team, 2019). Linear mixed

409   effect models were conducted with the lme function of the NLME package (Pinheiro et al.,

410   2019). Analyses of variance following linear models or linear mixed models were conducted

411   with the Anova function (Type II SS) from the CAR package (Fox and Weisberg, 2019) and

412   post-hoc multiple comparisons were made with the glht function (Tukey contrasts) in the

413   MULTCOMP package (Hothorn et al., 2008). We graphically assessed normality and

414   homoscedasticity of all model residuals. For mixed effect models, we used a pseudo R-squared

415   in the R package MUMIN (Barton, 2019) to determine the variance explained by both the fixed

416   terms only (marginal coefficient of determination, R^2_m) and the entire model including the

417   random terms (conditional coefficient of determination, R^2_c).

418

3. Results

3.1. Site and species patterns of $\delta^{13}C$ in pure stands

3.1.1. Site and triplet effects on $\delta^{13}C$ within species

In pure beech stands, mean $\delta^{13}C$ values for the selected dry years for all sampled trees ranged from -27.9 ± 0.8 ‰ at S1:1 to -25.7 ± 0.5 ‰ at S2:2 (Fig. 3a). There was a significant site effect on $\delta^{13}C$ values (Eq. 5, $P < 0.001$): the trees at S1 had significantly lower $\delta^{13}C$ values than at the other five sites (Fig. 3a). Mean $\delta^{13}C$ values differed significantly between the two triplets of a site at S6 only (Fig 3a). The range for the dominant-tree subsample was similar to the range for all trees, but the $\delta^{13}C$ values were higher, with beech mean $\delta^{13}C$ values ranging from -27.6 ± 0.4 ‰ at S1:2 to -25.4 ± 0.4 ‰ at S4:2 (Fig. 4a). As for all trees, there was a significant site effect on $\delta^{13}C$ values (Eq. 5, $P < 0.001$): the trees at S1 had significantly lower $\delta^{13}C$ values than the trees at the other five sites (Fig. 4a).

In pure fir stands, mean $\delta^{13}C$ values for all sampled trees ranged from -28.0 ± 0.6 ‰ at S1:2 to -25.2 ± 0.3 ‰ at S3:1 (Fig. 3b). There was a significant site effect on $\delta^{13}C$ values (Eq. 5, $P < 0.001$): the trees at S1 had significantly lower $\delta^{13}C$ values than the trees at S2 and S3 (Fig. 3b). Mean $\delta^{13}C$ values differed significantly between the two triplets of a site at S3 only (Fig 3b). For the dominant-tree subsample, the range was narrower and the $\delta^{13}C$ values were higher, with fir mean $\delta^{13}C$ values ranging from -26.9 ± 0.7 ‰ at S1:2 to -24.8 ± 0.3 ‰ at S3:2 (Fig. 4b). As for all trees, there was a significant site effect on $\delta^{13}C$ values (Eq. 5, $P < 0.001$): the trees at S1 had significantly lower $\delta^{13}C$ values than the trees at the other five sites (Fig. 4b).

In pure pubescent oak stands, mean $\delta^{13}C$ values for all sampled trees ranged from -26.1 ± 0.3 ‰ at S6:1 to -24.8 ± 0.4 ‰ at S4:2 (Fig. 3c). There was a significant site effect on $\delta^{13}C$ values (Eq. 6, $P < 0.001$): all three sites had significantly different $\delta^{13}C$ values (Fig. 3c). For the dominant-tree subsample, the range was narrower, but the mean $\delta^{13}C$ values were similar to all

443   trees: oak mean $\delta^{13}C$ values ranged from -25.9 ± 0.7 ‰ at S6:1 to -24.5 ± 0.4 ‰ at S4:2 (Fig.

444   4c). We observed no significant differences among the three sites or between triplets within

445   sites (Fig. 4c).

446   Figure 3 HERE; size: 2 columns; Colour online, B&W in print

447   Figure 4 HERE; size: 2 columns; Colour online, B&W in print

448   *3.1.2. Species differences in $\delta^{13}C$*

449   For pure stands including data for all the trees, species effect was not significant between beech

450   and fir in the more northern sites (P = 0.38), but was significant between beech and oak in the

451   more southern sites (P < 0.001). When including *log(DBH)* as a covariate in the model (Eq. 6),

452   *log(DBH)* was always significant. Beech had significantly lower $\delta^{13}C$ values than fir in the

453   northern sites or than oak in the southern sites (Table B1). The interaction between *log(DBH)*

454   and $\delta^{13}C$ was significant with all trees (Table B1), meaning that the slope of the relationship

455   between *log(DBH)* and $\delta^{13}C$ values differed between species, although it was always positive.

456   In the northern sites, fir had a stronger positive slope (b=1.76) than beech (b=0.85). In the

457   southern sites, beech had a stronger positive slope (b=2.23) than oak (b=0.85).

458   For pure stands with only the dominant-tree subsample, species effect was significant for beech-

459   fir sites (P < 0.001) and for beech-oak sites (P < 0.01) with or without log(DBH) in the model.

460   When including *log(DBH)* in the models (Eq. 6), *log(DBH)* was not significant, nor was the

461   interaction between *Species* and *log(DBH)* (Table B1). Beech had significantly lower $\delta^{13}C$

462   values than fir in the northern sites or than oak in the southern sites (Table B1). Mean species

463    $\delta^{13}C$ values of the dominant-tree subsample was -26.5 ± 0.4 ‰ for beech and -25.8 ± 0.3 ‰ for

464   fir at the northern sites, whereas it was -25.8 ± 0.2 ‰ for beech and -25.3 ± 0.3 ‰ for oak at

465   the southern sites.

466   *3.2. Mixture effects on $\delta^{13}C$*

467   *3.2.1. Effect of dbh*

468 Before analyzing the mixture effects on $\delta^{13}C$ values, we assessed the effect of *log(DBH)* in both

469 the global (Eq. 7) and triplet-level (Eq. 8) models for each species. The effect of *log(DBH)* was

470 significant for all four global models when all the sampled trees were included (Table 2). At

471 the triplet-level, *log(DBH)* was significant for beech, except at S1:2 and S2:2. It was significant

472 for all fir triplets and for half of the oak triplets (Table 3). The positive relationships between

473 $\delta^{13}C$ values and *log(DBH)* are consistent with expectations from the literature and with the

474 impact of canopy light gradients on leaf or needle $\delta^{13}C$ (McCarroll and Loader, 2004). When

475 only the dominant-tree subsample was used in the models, the effect of *log(DBH)* on $\delta^{13}C$ was

476 not significant in the global models nor in most triplets, except at S4:2 for beech and S2:2 for

477 fir (Table 2, Table 3). The absence of significant effects for the dominant-tree subsample was

478 probably the consequence of our sampling design: this subsample only included trees that

479 received direct vertical light and for which the possible impact of canopy light gradients on

480 $\delta^{13}C$ (McCarroll and Loader, 2004) was not expected.

481 Table 2 HERE; size: 2 columns

482 Table 3 HERE; size: 2 columns

483   *3.2.2. Beech-fir sites*

484 At the tree level for the global model, there were no significant mixture effects on $\delta^{13}C$ values

485 during the selected dry years, either for all trees or the dominant-tree subsample (Table 2). This

486 result indicates that beech and fir trees in the mixed stands had statistically similar $\delta^{13}C$ values

487 to their values in pure stands for a given DBH (Table 2). The interaction between *log(DBH)*

488 and *Mixture* was significant for fir with all trees: both pure and mixed stands had a positive

489 relationship between *log(DBH)* and $\delta^{13}C$ values, but pure stands had a stronger slope (b=1.69)

22

490 than mixed stands (b=1.35). Despite this difference in the slopes of the relationship, it did not

491 result in a statistical difference between pure and mixed stands. We also included the interaction

492 term for the dominant-tree subsample alone, although we did not expect any interaction between

493 *log(DBH)* and *Mixture* as (i) trees of this subsample should receive the same levels of light and

494 (ii) because none of the triplets, except one for fir, had a significant *log(DBH)* effect. There was

495 a small interaction effect for fir in the global model with dominant trees (Table 2).

496 At the triplet level (Table 3, Fig. 5a, 6a), there were no significant mixture effects for beech in

497 any of the triplets, either for all trees or the dominant-tree subsample. For fir, at the triplet level

498 for all trees, a significant mixture effect was found for triplets S1:1 and S2:1 only. In both these

499 triplets, the models predicted higher $\delta^{13}C$ values in mixed stands than in pure stands (Table 3).

500 For fir, only triplet S3:2 had a significant interaction between *log(DBH)* and *Mixture*: the pure

501 stand had a steeper slope (b=2.22) than the mixed stand (b=1.33). There were no significant

502 interactions between *log(DBH)* and *Mixture* for beech. There was no mixture effect for fir with

503 the dominant-tree subsample except for triplet S2:2 (Table 3). Triplet S1:1 for fir had a

504 significant interaction effect when including all trees (Table 3), but this was probably driven by

505 the differences in DBH of the dominant trees between stands (Table 1).

506 Three triplets had NBE_{dom} values close to 0.0 ‰ and the three other triplets had values below -

507 0.4 ‰ (Fig. 7).

508   *3.2.3. Beech-oak sites*

509 When including all trees in the global model, we found a significant mixture effect on beech

510 $\delta^{13}C$, with higher $\delta^{13}C$ values in mixed stands compared to pure ones (Fig. 5b, Table 2). There

511 was no significant effect for oak (Fig. 5b, Table 2). We no longer observed the significant effect

512 for beech when we restricted the analysis to the dominant-tree subsample (Fig. 6b, Table 2).

513 The interaction between *log(DBH)* and *Mixture* was not significant for either species (Table 2).

23

514 At the triplet level, we observed significant mixture effects for both beech and oak (Table 3).

515 For beech, when including all the trees, $\delta^{13}C$ values were higher in mixed stands than in pure

516 stands in triplets S4:2 and S5:2 (Table 3). This result held true for the beech dominant-tree

517 subsample at S4:2, but not at S5:2 (Table 3). S4:2 was the only triplet with a significant

518 interaction effects between *log(DBH)* and *Mixture* for beech (Table 3), with a steeper slope for

519 mixed stands (b= 3.78) than pure stands (b=1.69). However, this did not change the mixture

520 effect for this triplet. For oak, when all the trees were included, $\delta^{13}C$ values were significantly

521 lower in mixed stands at S4:2 and significantly higher at S5:1 (Table 3). We did not observe

522 these effects for oak when testing the dominant-tree subsample only (Table 3). There were no

523 interaction effects between *log(DBH)* and *Mixture* for oak (Table 3).

524 In this region, there was a trend toward the lack of a net biodiversity effect: most NBE_{dom} values

525 were close to 0.0 ‰ (Fig.7), except in triplet S6:1 where it was below -0.4 ‰.

526 Figure 5 HERE; size: 1 column; Colour online, B&W in print

527 Figure 6 HERE; size: 1 column; Colour online, B&W in print

528 Figure 7 HERE; size: 1.5 column; Colour online, B&W in print

529   *3.3. Xylem-water δ^2H*

530 At the beech-fir sites, we observed a significant interaction effect between *Species* and *Mixture*

531 (P=0.002) (Table B2). There was a significant mixture effect on the δ^2H values for beech, but

532 not for fir. More specifically, beech δ^2H values were lower in mixed stands than in pure stands,

533 except for triplet S3:2 (Fig. 8). There was also a significant difference between species for δ^2H

534 values in both pure and mixed stands ($P<0.001$) (Table B2): beech δ^2H values were lower than

535 those of fir, except in triplet S1:1 (Fig. 8). The difference between beech and fir was larger in

536 mixed stands (estimate: 15.3 ± 1.4 ‰) than in pure stands (estimate: 9.1 ± 1.3 ‰).

537   In beech-oak sites, we observed no mixture effect on δ^2H values for either beech or oak (P=0.33)

538   (Fig. 8), no differences between species (P=0.71), and no interaction effect (P=0.16) (Table

539   B2).

540   Figure 8 HERE; size: 2 columns; Colour online, B&W in print

4. Discussion

4.1. Variability of $\delta^{13}C$ values in pure stands

4.1.1. Site effect for beech

Our range of mean $\delta^{13}C$ values in beech tree-rings for pure stands was close to those reported in the literature (Fig. 3a, 4a). For instance, in Germany, at the heart of beech's distribution range, mean beech $\delta^{13}C$ value was -26.7 ‰ during the very dry year of 2003 (Schwarz and Bauhus, 2019). In northern Spain, the southern-most area of beech distribution, mean $\delta^{13}C$ value was -26.5 ‰ at a drier lower-elevation site and -27.5 ‰ at a wetter higher-elevation site for the period 2000 to 2003 (Peñuelas et al., 2008). We therefore recorded one of the least negative mean $\delta^{13}C$ values for beech published to date, i.e. -25.4 ± 0.4 ‰ at S4:2 for the dominant-tree subsample (Fig.4a). The origin of the high $\delta^{13}C$ values observed in our study can be explained (i) by our choice to select, by design, a range of years characterized by severe summer droughts (2003-2007), which undoubtedly triggered high tree water use efficiency (Saurer et al., 1995), and (ii) by the fact that some of our sites were at the range limit of precipitation and temperature conditions for beech in Europe (Fig. 2).

We found that the mean $\delta^{13}C$ values for beech in pure stands did not differ among sites except at S1, either for all trees or for the dominant-tree subsample (Fig. 3a, 4a), though we had expected stronger differences due to the wide range of climatic conditions across our sites (Fig. 2). Plant tissue $\delta^{13}C$ is a functional trait that is highly sensitive to differences in environmental conditions (Farquhar et al., 1989). However, different environmental conditions can have opposite effects on $\delta^{13}C$ values, and the lack of difference in $\delta^{13}C$ among the five sites (S2 to S6) could be explained by a combination of conditions acting in opposite directions. We explain the significantly lower mean $\delta^{13}C$ values for beech at S1 (Fig. 3a, 4b) by the fact that S1 has the wettest and coolest climate among the six sites (Fig.2). Site S1 also had relatively low water

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565 stress and was the least affected by water deficit during the period 2003-2007 covered by our

566 study (Table A3).

567   *4.1.2. Species differences*

568 The pure beech stands had significantly lower $\delta^{13}C$ values than did fir or oak in the respective

569 sites (Table B1). This indicated lower mean water use efficiency for beech, even though the

570 magnitude of the differences between species remained low. For beech and fir, lower $\delta^{13}C$

571 values for beech are consistent with the study by Schwarz and Bauhus (2019) during the 2003

572 drought in Germany and Croatia. For beech and pubescent oak, to our knowledge, no studies

573 have compared $\delta^{13}C$ values of mature pubescent oak with other species. Differences in $\delta^{13}C$

574 values among species can be explained by physiological and morphological characteristics

575 (McCarroll and Loader, 2004) or by differences in canopy structure in pure stands. Yet, we

576 included a dendrometric variable (*log(DBH)*) in the statistical tests to take these characteristics

577 into account when testing for species differences. Species effects remained significant in the

578 models with the dominant-tree subsample while the effect of *log(DBH)* did not (Table B1). We

579 can therefore conclude that species differences in $\delta^{13}C$ were mainly related to intrinsic

580 differences among species in water use efficiency, and not just to canopy structure factors.

581 As expected, a large part of the variability in $\delta^{13}C$ values was explained by the *log(DBH)*

582 variable when we included all trees (Table B1). The relationship between $\delta^{13}C$ values and

583 *log(DBH)* was always positive. This is consistent with the fact that gradients in light availability

584 in the canopy strongly influence $\delta^{13}C$ values (McCarroll and Loader, 2004). The slopes of this

585 relationship differed between species: fir always had a stronger slope than beech in northern

586 sites, indicating that small fir trees were more suppressed for light than small beech trees in

587 their respective pure stands. As both species have high shade tolerance (Niinemets and

588 Valladares, 2006), this could simply be due to the canopy structure of fir trees leading to lower

589   light availability for dominated fir trees. Beech had a stronger slope than oak in the southern

590   sites, indicating that small beech trees were more suppressed for light than small oak trees in

591   pure stands. As beech is more shade tolerant than oak (Niinemets and Valladares, 2006), this

592   could be explained by the fact that small beech trees are growing in light-suppressed

593   environments whereas oak trees, even when small, seem to only grow in environments with a

594   higher light availability leading to less variability in $\delta^{13}C$ values. These results show that in

595   addition to intrinsic species differences in water use efficiency, canopy structure and light

596   interception also influenced water and carbon functioning in pure stands .

597   *4.2. Mixture effects on $\delta^{13}C$*

598   *4.2.1. Beech-fir sites*

599   For beech, there was no mixture effect on $\delta^{13}C$ values during dry years when mixed with fir

600   (Table 2 & 3, Fig. 5a & 6a), despite strong differences in functional traits between these two

601   species. The lack of mixture effect is consistent with several studies on beech-fir mixtures. For

602   instance, at the same sites, Jourdan et al. (2019a) reported no effect of the proportion of fir on

603   the drought resistance of beech, estimated with growth data at the neighborhood tree-to-tree

604   level. In another study in Romania, no mixture effect was observed for beech on $\delta^{13}C$

605   differences between a wet and a dry year, either at the species level (Forrester et al., 2016) or

606   at the stand level (Grossiord et al., 2014c). Recently, Schwarz & Bauhus (2019) looked at the

607   resistance to a drought event for four sites with beech-fir mixtures in Germany and Croatia and

608   found no mixture effect on $\delta^{13}C$, even though they observed long-term positive effects of

609   mixture on radial growth.

610   For fir, the influence of mixture on $\delta^{13}C$ values during dry years was more variable (Table 2 &

611   3, Fig. 5a & 6a). We observed that mixture effects for fir differed among sites, between triplets

612   within a given site, or according to the type of dataset we used. This suggests that local

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613 environmental conditions and stand structure strongly influenced the outcome of the tests for

614 fir and that mixture effects may play a minor role in explaining observed differences.

615 Consequently, we cannot conclude that including fir in mixtures with beech will mitigate fir

616 exposure to drought. The existing literature has highlighted contrasted patterns for fir in

617 mixtures with beech. Some studies observed that the sensitivity of fir to drought was reduced

618 when mixed with beech in drier sites (Lebourgeois et al., 2013; Gazol and Camarero, 2016),

619 while other studies found no effect (Forrester et al., 2016; Schwarz and Bauhus, 2019), or even

620 a negative effect (Jourdan et al., 2019a). Differences in site conditions could explain

621 discrepancies in the observed patterns between our results and previous studies, as mixture

622 effects may change along resource gradients (Forrester and Bauhus, 2016). The interaction

623 effects detected for fir, with all trees, between *log(DBH)* and stand composition suggest that

624 there was less variability in $\delta^{13}C$ values due to light availability in mixed stands. In pure stands,

625 small fir trees had lower light availability (more negative $\delta^{13}C$ values) than in mixed ones. This

626 could be explained by the fact that fir trees in mixed stands received more light than in pure

627 stands due to canopy opening with the presence of beech and to phenological differences

628 between fir and beech. However, these interactions did not impact the detected mixture effects.

629 At the stand level with both species, there was no single mixture effect pattern among sites and

630 triplets for beech-fir sites (Fig. 7). NBE_{dom} was either neutral or negative, meaning that mixtures

631 had more negative $\delta^{13}C$ values, and potentially lower water stress, than expected from pure

632 stands. Within each site, triplet NBE_{dom} values were often neutral for one triplet and negative

633 for the other one. As a reminder, these triplets were not selected as replicates, but were designed

634 to cover local variations in environmental conditions and stand structure; within-site variability

635 was therefore not surprising. The neutral or negative NBE_{dom} values could be explained by

636 differences in above-ground features, such as stand structure, proportion of shaded subcanopy

637 or canopy packing levels, or in below-ground processes, such as complementarity or facilitation

638 for water acquisition (Grossiord, 2019). The species-interaction effects between beech and fir

639 for some triplets may be attenuated or even masked by local differences in environmental

640 conditions, which could not be controlled for in these stands (Metz et al., 2016). Furthermore,

641 opposite processes that may strongly influence photosynthesis (e.g. light gradients) or water

642 use (e.g. depth of water uptake) in mixed forests may actually cancel each other out and result

643 in the absence of overall mixture effects (Forrester and Bauhus, 2016). However, the overall

644 result of NBE_{dom} for mixed beech-fir stands is that $\delta^{13}C$ values were never less negative than

645 expected, suggesting a potentially lower water stress in mixtures. These results differ from the

646 mixture effects tested for each species separately. Differences between species-level and stand-

647 level results have been observed before (Forrester et al., 2016) and can result from opposite

648 trends of mixture effects between the species (Toïgo et al., 2015). For the overall stand $\delta^{13}C$

649 values, beech-fir mixtures were not detrimental in terms of functioning during dry years. We

650 will discuss potential differences in water sources among species and stand composition below;

651 however, we were not able to characterize above-ground influences in the current study.

652   *4.2.2. Beech-oak sites*

653 The presence of pubescent oak did not decrease beech exposure to drought. In fact, with all

654 trees in the global model (Table 2) and for two individual triplets (Table 3), the interaction with

655 pubescent oak even led to higher beech $\delta^{13}C$ values, indicating a higher drought exposure of

656 beech in mixed stands compared to pure ones. In the global model (Table 2) and in one of these

657 two triplets (S5:2, Table 3), the absence of any mixture effect for the dominant-tree subsample

658 suggested that these effects might be related to the social status of the trees, even though we

659 were not able to demonstrate this. The interaction effect between *log(DBH)* and $\delta^{13}C$ values in

660 S4:2 for beech was opposite to what was observed for fir: there was less variability in $\delta^{13}C$

661 values due to light availability in the pure stand than in the mixed one. However, this effect was

662 mostly driven by a few bigger trees in the pure stand (Table 1) and did not affect the observed

663   mixture effect. Comparing these results with previous work on beech-pubescent oak mixtures

664   is impossible as this is the first study to look at beech $\delta^{13}C$ response in interaction with

665   pubescent oak. Beech $\delta^{13}C$ responses when mixed with another oak species (sessile oak,

666   *Quercus petraea*) in German forests indicated that it was less exposed to drought than in pure

667   stands (Forrester et al., 2016). Compared to sessile oak, pubescent oak is more drought tolerant

668   (Niinemets and Valladares, 2006) and could be more competitive than sessile oak when mixed

669   with beech. Furthermore, when looking at growth response patterns to drought in mixture with

670   different oak species, beech showed very inconsistent results so far, with positive (Pretzsch et

671   al., 2013; *Quercus petraea*), negative (Vanhellemont et al., 2019; *Quercus robur*) or neutral

672   (Jourdan et al., 2019a; *Quercus pubescens*) patterns. Our results underline the current

673   uncertainty on the choice of silvicultural practices that forest managers must make today to

674   mitigate drought impact on beech productivity along its southern margins under future climate

675   conditions.

676   The presence of beech did not influence the physiological functioning of pubescent oak during

677   dry summers in this region (Fig. 5b, 6b, Table 2, 3). As with beech, we were not able to reveal

678   any general trend for beech-oak interactions under severe drought conditions. Our study

679   suggests that, at least in this region, managing pubescent oak with beech does not lead to clear

680   mitigation of climate change impacts on oak response to drought.

681   *4.2.3. Impact of sampling design and use of absolute $\delta^{13}C$ values*

682   Our results emphasize how the sampling design (all trees or dominant ones only) and level of

683   analysis (tree or stand) of diversity-ecosystem functioning studies may influence the outcome

684   of mixture effect tests. Limiting the sampling design to dominant trees when looking at mixture

685   effects on the $\delta^{13}C$ response of trees to drought has been the typical approach to date (Grossiord

686   et al., 2014c; Metz et al., 2016; Schwarz and Bauhus, 2019). However, we show that this choice

687 may bias the outcome of these mixture effect tests. Selecting dominant trees only is, indeed,

688 pertinent with regards to $\delta^{13}C$, as dominant trees are those with the greatest sunlit leaf areas and

689 therefore potential carbon (photosynthesis) and water (transpiration) exchange with the

690 atmosphere. However, not including suppressed trees in these analyses may bias the stand-level

691 patterns of mixture effects, as the sensitivity to drought of suppressed and dominant trees in

692 mixed stands may differ (Mérian and Lebourgeois, 2011). This highlights the need for a

693 standardization of protocols in mixed-species forest studies in accordance with the goal of the

694 study (e.g. ecophysiological or forest management questions).

695 A limitation of this study, as mentioned in the methods, was the use of absolute $\delta^{13}C$ values for

696 dry years instead of the difference in $\delta^{13}C$ between wet and dry years. Calculating a difference

697 between the $\delta^{13}C$ of a wet and a dry year would allow analyzing a direct response of trees to

698 drought and compare this response in pure and mixed stands independently of the micro-

699 environmental variability among stands within a given triplet. However, in a study looking at

700 diversity effects on drought responses of $\delta^{13}C$, the same trends were found with $\delta^{13}C$ differences

701 between wet and dry years (Grossiord et al., 2014c) than with absolute $\delta^{13}C$ values for the dry

702 years only (Grossiord, 2014, p.48). Then, this methodological caveat should not prevent us from

703 interpreting mixture effects, especially since we took precautions to avoid over-interpreting

704 these effects at the triplet scale.

705   *4.3. Complementarity in water uptake sources*

706   *4.3.1. Beech-fir sites*

707 At the more northern beech-fir sites, during the 2018 summer drought, we observed a plasticity

708 in the depth of water uptake for beech at these sites between pure and mixed stands (Fig. 8),

709 which is consistent with the known plasticity of the species in terms of rooting traits and depth

710 of water uptake (Brinkmann et al., 2018). When competing with fir, beech seemed to have a

711   deeper mean depth of water uptake than when competing with beech only. These results are in

712   accordance with previous studies which show a shift of beech fine roots down to deeper soil

713   horizons when competing with coniferous tree species (Bolte and Villanueva, 2006; Grossiord

714   et al., 2014a). Plasticity in functional traits can be the result of abiotic or biotic interactions

715   (Valladares et al., 2007). Therefore, the shift in beech water uptake depth could be explained

716   either by a response to the competition with fir roots in the same soil horizons, or by a response

717   to a depletion in soil water availability in the topsoil (Brinkmann et al., 2018).

718   In the mixed stands, xylem δ^2H values showed that beech tended to uptake water from deeper

719   soil layers than fir did (Table B2, Fig 8), suggesting niche complementarity for water uptake

720   between the two species. This kind of complementarity between species in water use is assumed

721   to partly explain positive biodiversity-ecosystem functioning relationships (Grossiord, 2019).

722   However, it has rarely been linked to mixture effects on plant growth (Mueller et al., 2013;

723   Bachmann et al., 2015) or functional traits under drought (Verheyen et al., 2008; Goisser et al.,

724   2016). In our study, the absence of a clear mixture effect on δ^{13}C for both beech and fir, despite

725   a complementarity in water uptake, may be explained by several factors. First, our results did

726   not allow us to quantify the real gain in soil water availability during these dry periods for

727   beech. Indeed, the shift in δ^2H between pure and mixed stands was slight (10 ‰ on average)

728   and may indicate only a small difference in water uptake depth and soil water content. Second,

729   below-ground processes may not be the main drivers of the mixture effects for these species in

730   the study region. Above-ground processes could well be the major drivers, but they were not

731   tested in this study. Third, as discussed by Goisser et al. (2016), the impact of species mixture

732   on soil water availability may vary across seasons. The time scale of the processes associated

733   with our δ^2H or δ^{13}C approaches were different: δ^2H gave a one-shot image of the mean

734   signature of soil water extracted during the summer, whereas the δ^{13}C values gave an integrated

735   measurement of carbon and water acquisition and use over five years with dry summers.

4.3.2. Beech-oak sites

737   For the more southern beech-oak sites, there were no signs of complementarity or niche

738   differentiation in water acquisition for either species (Table B2, Fig. 8). This result is consistent

739   with the absence of mixture effects on $\delta^{13}C$ for beech and pubescent oak in most triplets. The

740   absence of plasticity in water uptake for beech when mixed with pubescent oak is interesting to

741   note in comparison to the beech-fir sites. It suggests that the presence of pubescent oak did not

742   induce any changes in beech water uptake compared to pure beech stands. The shallow soils in

743   our sites (especially S5) might be one reason for the absence of differences in rooting depth

744   between the species. We did not find data in the literature on rooting depth of pubescent oak.

745   However, Lebourgeois and Jabiol (2002) have shown that beech rooting depth, compared to

746   two other oak species (*Quercus petraea* and *Quercus robur*), is more sensitive to constraints

747   but also that in the absence of strong constraints, beech and oak species rooting profiles are

748   similar. In this part of the beech distribution area, during extreme dry summers, beech and

749   pubescent oak displayed a functional redundancy in their below-ground water uptake. Mixing

750   these two species to improve beech access to soil water in southern France may not be an

751   appropriate forest management strategy.

752

5. Conclusion

Our study was designed to help decision-making for beech forest management strategies adapted to more frequent and extreme droughts in the future, particularly at the limits of the distribution area of beech. Overall, we found no major mixture effect on drought exposure for beech, although there was a high variability in the outcome of mixture effects at the triplet scale. In the northern part of our study, mixing beech with fir had no effect on beech $\delta^{13}C$ values during dry years. This result is in accordance with a growing body of literature showing the lack of a species-interaction effect on beech drought resistance. Managing mixed stands of beech and fir does not seem to help mitigate drought impacts for either of the two species, despite an observed complementarity in water uptake. In the southern part of our study, mixture effects on beech were mostly neutral, although beech seemed to be more exposed to drought in mixed stands with pubescent oak at some sites. In a region at the limits of the distribution area of beech, this study allowed us to conclude that managing beech in mixed stands with silver fir or pubescent oak does not buffer drought impacts during dry years. Yet, in the long term, as extreme droughts will become more frequent, beech-fir mixtures should not be detrimental to beech response to drought, while it might be in mixtures with pubescent oak. In the southernmost distribution regions of beech, an overall assessment of multi-criteria potential benefits for each type of mixture should then be conducted in order to take management decisions.

CRediT authorship contribution statement

Soline Martin-Blangy: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Marie Charru**: Conceptualization, Methodology, Investigation, Writing - original draft, Supervision. **Sylvain Gérard**: Investigation, Writing - review & editing. **Hervé Jactel**: Conceptualization, Resources, Project administration, Funding acquisition, Writing - review & editing. **Marion Jourdan**: Resources, Investigation, Writing - review & editing. **Xavier Morin**: Conceptualization, Investigation, Resources, Writing - review & editing. **Damien Bonal**: Conceptualization, Methodology, Investigation, Writing - original draft, Supervision.

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1062   **Tables**

1063   **Table 1** Plot characteristics: mean DBH (cm) and range by species for all trees in each site,

1064   triplet, and plot.

Site	Triplet	Plot	DBH (cm)			
			Beech	Fir	Oak	Other sp.
S1	1	Pure beech	41.9 (8-105)	_	_	19.1 (13.4-24.2)
S1	1	Pure fir	8.3 (8.3-8.3)	37 (8.6-92.9)	_	21.5 (10.5-49.3)
S1	1	Mixed	39 (28.3-49.7)	22.9 (7.6-38.2)	_	18.5 (9.2-34.4)
S1	2	Pure beech	47 (28.3-59.8)	14.1 (5.7-28.6)	_	_
S1	2	Pure fir	_	32.6 (8-79.9)	_	26.7 (13.1-50)
S1	2	Mixed	42.4 (9.5-76.4)	28.3 (7.3-91.7)	_	45.2 (45.2-45.2)
S2	1	Pure beech	16.5 (8.3-46.8)	_	_	19.4 (14.3-26.7)
S2	1	Pure fir	_	24.2 (8-42)	_	23.6 (23.6-23.6)
S2	1	Mixed	14.2 (7.3-40.1)	19.6 (7.6-57.3)	_	10.2 (8.6-14.3)
S2	2	Pure beech	47.4 (35.3-58.3)	17.3 (12.7-23.9)	_	_
S2	2	Pure fir	15.3 (15.3-15.3)	42.4 (15.6-57.3)	_	_
S2	2	Mixed	36.8 (30.2-44.2)	27.7 (12.7-57.6)	_	_
S3	1	Pure beech	25.3 (9.2-38.8)	_	_	_
S3	1	Pure fir	18.8 (18.1-19.4)	35.9 (15-47.7)	_	_
S3	1	Mixed	14.9 (7.6-23.9)	44.8 (9.5-59.8)	_	_
S3	2	Pure beech	29.9 (16.6-53.5)	20.4 (20.4-20.4)	_	_
S3	2	Pure fir	19.2 (8-30.2)	17.1 (7.3-41.7)	_	15 (10.8-19.1)
S3	2	Mixed	24 (16.2-32.1)	15.2 (7.3-60.5)	_	30.6 (28.6-32.5)
S4	1	Pure beech	24.9 (7.6-68.1)	_	_	13.3 (11.8-14.3)
S4	1	Pure oak	11.4 (7.6-15.3)	_	18.3 (7.6-42.7)	10.7 (7.6-15.9)
S4	1	Mixed	16.1 (8.6-24.5)	_	20.7 (11.1-27.4)	18.9 (8.3-39.8)
S4	2	Pure beech	22.6 (8.6-64.3)	_	11.1 (11.1-11.1)	_
S4	2	Pure oak	13.4 (13.4-13.4)	_	15.2 (8.9-24.8)	9.7 (9.2-10.2)
S4	2	Mixed	14.4 (7-27.7)	_	17.1 (12.1-22.9)	13.8 (12.4-15.3)
S5	1	Pure beech	17.1 (7.3-28.6)	_	12.9 (8.9-16.9)	13.7 (8-19.4)
S5	1	Pure oak	_	_	18.7 (10.5-32.5)	18.6 (9.2-29)
S5	1	Mixed	14.5 (8-22)	_	14.7 (7.6-21)	10.4 (7.3-16.2)
S5	2	Pure beech	16.6 (7-27.1)	_	_	13.8 (8.3-23.6)
S5	2	Pure oak	_	_	15.6 (10.8-22.9)	10.1 (7-13.4)
S5	2	Mixed	13.7 (7.3-23.6)	_	15.9 (7.6-20.7)	9.2 (7-15.3)
S6	1	Pure beech	40.4 (29.6-53.2)	_	_	16 (9.9-29)
S6	1	Pure oak	_	_	36.1 (28-45.5)	12.8 (7-22.3)
S6	1	Mixed	30.6 (9.5-45.2)	_	34.6 (20.4-42.3)	15.5 (7.3-34.4)
S6	2	Pure beech	19.6 (7.6-35.7)	_	17.2 (14-19.4)	13.3 (7.6-25.5)
S6	2	Pure oak	_	_	19 (9.9-38.2)	8.2 (7-9.9)
S6	2	Mixed	21.3 (8.6-31.8)	_	18 (8.3-26.7)	11.3 (7.6-15.9)

1065

1066   *Note*: Other tree species represent less than 20% of the total basal area of the plots (12% on average). Most

1067   common species are *Acer pseudoplatanus, Acer campestre, Ilex aquifolium*, *Sorbus torminalis* and *Taxus*

1068   *baccata*.

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1070

Table 2 Linear mixed model output for *Mixture* effects and *log(DBH)* on δ¹³C by type of

mixture and species (Eq. 7).

	Beech-fir sites						Beech-oak sites					
	Beech			**Fir**			**Beech**			**Oak**		
	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*
All trees	154			213			191			189		
log(DBH)	27	1	<.001***	268	1	<.001***	185	1	<.001***	21	1	<.001***
Mixture	0	1	0.77	2	1	0.13	26	1	<.001*** $\delta^{13}C_m > \delta^{13}C_p$	1	1	0.35
log(DBH):Mixture	0	1	0.52	4	1	0.04*	1	1	0.24	1	1	0.30
$R^2_m (R^2_c)$		*0.16 (0.65)*			*0.40 (0.76)*			*0.41 (0.68)*			*0.10 (0.46)*	
Dominant-tree subsample	52			48			50			58		
log(DBH)	0	1	0.58	3	1	0.07	0	1	0.90	1	1	0.27
Mixture	2	1	0.21	1	1	0.25	0	1	0.49	2	1	0.15
log(DBH):Mixture	2	1	0.14	5	1	0.02*	4	1	0.05	1	1	0.35
$R^2_m (R^2_c)$		*0.03 (0.52)*			*0.10 (0.62)*			*0.06 (0.26)*			*0.05 (0.32)*	

1073

Note: Stars indicate significant *log(DBH)*, *Mixture* and interaction effects. *Mixture* is a two-level factor (pure vs.

mixed). For significant *Mixture* effects, the direction of the estimate is indicated by $\delta^{13}C_p > \delta^{13}C_m$ if pure stands

have a higher δ¹³C estimate than mixed stands (i.e. mixture improves response to water stress compared to pure

stands), and $\delta^{13}C_m > \delta^{13}C_p$ if mixed stands have a higher δ¹³C estimate than pure stands. *χ2*: chi square test results

for type "II" ANOVA. *df*: degrees of freedom. *P*: p-value for fixed terms. *Triplet ID* is used as a random factor in

all eight models. A pseudo R-squared for mixed effect models was used to determine the variance explained by

the two fixed terms only (marginal coefficient of determination, R^2_m) and the entire model including the random

terms (conditional coefficient of determination, R^2_c).

1082

**Table 3** Model p-values for *Mixture* effects, *log(DBH)* on $\delta^{13}C$ by triplet and species (Eq. 8).

Site+Triplet	Beech			Fir			Oak		
	P - log(DBH)	*P - Mixture*	*P - log(DBH): Mixture*	*P - log(DBH)*	*P - Mixture*	*P - log(DBH): Mixture*	*P - log(DBH)*	*P - Mixture*	*P - log(DBH): Mixture*
All trees									
S1:1	0.03*	0.13	0.67	<0.001***	<0.01** $\delta^{13}C_m > \delta^{13}C_p$	0.7	–	–	–
S1:2	0.18	0.24	0.31	<0.001***	0.94	0.15	–	–	–
S2:1	<0.001***	0.62	0.45	<0.001***	<0.01** $\delta^{13}C_m > \delta^{13}C_p$	0.35	–	–	–
S2:2	0.42	0.85	0.61	<0.001***	0.66	0.09	–	–	–
S3:1	0.03*	0.44	0.62	<0.01**	0.09	0.77	–	–	–
S3:2	<0.01**	0.54	0.82	<0.001***	0.87	0.04*	–	–	–
S4:1	<0.001***	0.06	0.6	–	–	–	0.04*	0.47	0.49
S4:2	<0.001***	<0.001*** $\delta^{13}C_m > \delta^{13}C_p$	<0.01**	–	–	–	0.02*	0.02* $\delta^{13}C_p > \delta^{13}C_m$	0.75
S5:1	<0.001***	0.32	0.39	–	–	–	0.06	<0.01** $\delta^{13}C_m > \delta^{13}C_p$	0.83
S5:2	<0.001***	<0.001*** $\delta^{13}C_m > \delta^{13}C_p$	0.15	–	–	–	0.81	0.09	0.67
S6:1	<0.01**	0.12	0.19	–	–	–	0.21	0.53	0.56
S6:2	<0.001***	0.22	0.95	–	–	–	<0.01**	0.55	0.88
Dominant-tree subsample									
S1:1	0.88	0.97	0.53	0.48	0.93	0.02*	–	–	–
S1:2	0.17	0.09	0.17	0.09	0.68	0.46	–	–	–
S2:1	0.95	0.54	0.59	0.10	0.57	0.61	–	–	–
S2:2	0.51	0.52	0.51	<0.01**	0.04* $\delta^{13}C_p > \delta^{13}C_m$	0.05	–	–	–
S3:1	0.45	0.48	0.86	0.40	0.34	0.28	–	–	–
S3:2	0.30	0.98	0.07	0.87	0.06	0.24	–	–	–
S4:1	0.76	0.47	0.55	–	–	–	0.22	0.37	0.65
S4:2	<0.001***	<0.001*** $\delta^{13}C_m > \delta^{13}C_p$	<0.001***	–	–	–	0.63	0.18	0.47
S5:1	0.62	0.53	0.78	–	–	–	0.48	0.62	0.43
S5:2	0.51	0.06	0.7	–	–	–	0.36	0.21	0.43
S6:1	0.19	0.19	0.93	–	–	–	0.31	0.96	0.09
S6:2	0.98	0.18	0.42	–	–	–	0.81	0.44	0.44

Note: Stars indicate significant *log(DBH)*, *Mixture* or interaction effects. *Mixture* is a two-level factor (pure vs. mixed). For significant *Mixture* effects, the direction of the estimate is indicated by $\delta^{13}C_p > \delta^{13}C_m$ if pure stands have a higher $\delta^{13}C$ estimate than mixed stands (i.e. mixture improves response to water stress compared to pure stands), and $\delta^{13}C_m > \delta^{13}C_p$ if mixed stands have a higher $\delta^{13}C$ estimate than pure stands.
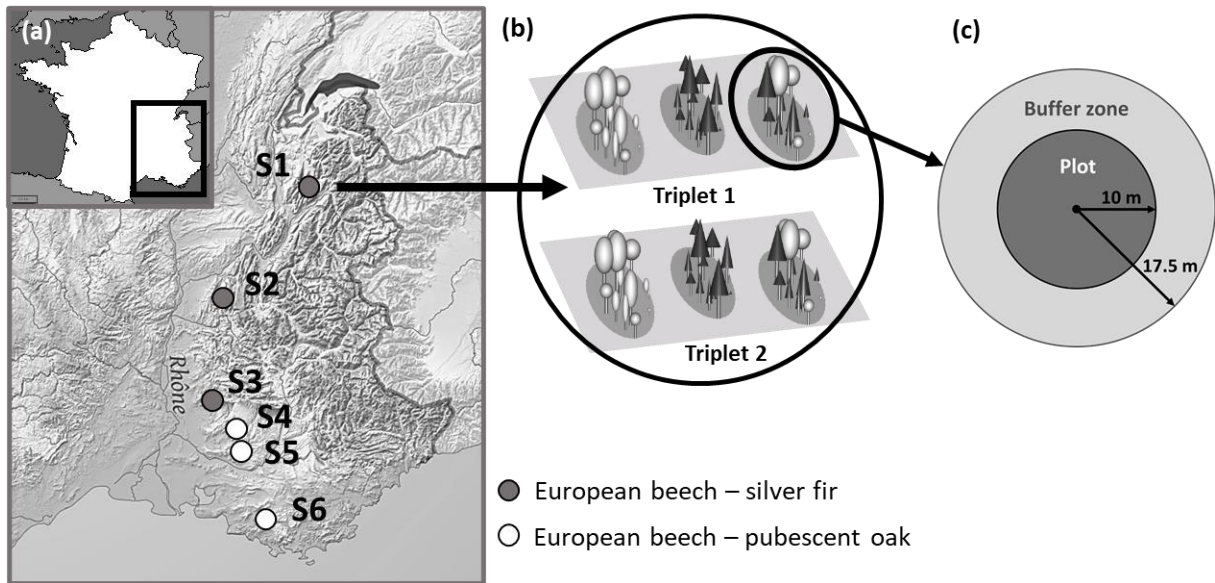
Fig. 1. Field design of the GMAP network (Mediterranean and Alpine Plot Gradient, set up by X. Morin in 2013). **(a)** Study area and location of the six sites in south-eastern France. Dark points (sites S1 to S3) represent northern sites with European beech (*Fagus sylvatica*) and silver fir (*Abies alba*) forests. White points (sites S4 to S6) represent southern sites with European beech and pubescent oak (*Quercus pubescens*) forests. The sites are located in the following mountain ranges of the French pre-Alps: Bauges (S1), Vercors (S2), Ventoux (S3), Lubéron Lagarde (S4), Grand Lubéron (S5), Sainte-Baume (S6). **(b)** Schematic representation of a site, with the two triplets per site. Each triplet is made up of two pure plots in monospecific stands and one mixed plot in a mixed-species stand. **(c)** Representation of an individual plot. Plots are circular with a central plot area (10 m radius) and a buffer zone (7.5 m radius). All the trees in the central plot with a DBH > 7.5 cm were sampled.

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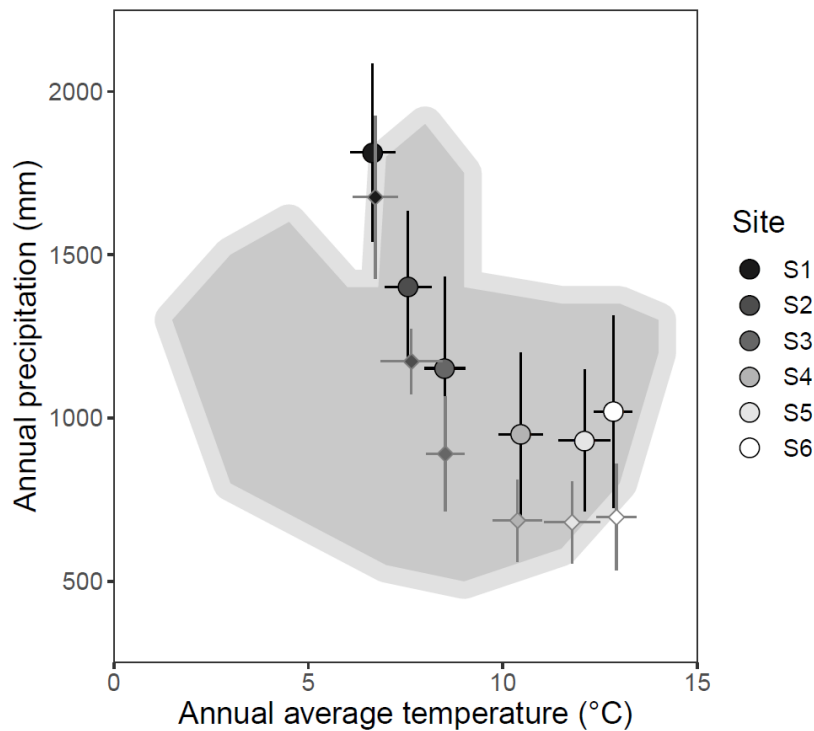
1106

1107 **Fig. 2.** Climate-space diagram of presence for beech in Europe, study site annual average

1108 temperature and annual sum of precipitation 20-year averages. The shaded area represents

1109 observed beech presence in Europe in all national forest inventories in terms of annual climatic

1110 conditions for temperature and precipitation (adapted from the European Atlas of Forest Tree

1111 Species, Houston Durrant et al., 2016). Large circular points represent the 20-year averages

1112 (1994-2013) from the SAFRAN weather data for each of our study sites. Small diamond points

1113 represent the 5-year averages of the selected dry years (2003-2007) for each site. The x-axis is

1114 the annual average of daily average temperatures. The y-axis is the annual sum of daily

1115 precipitation. Error bars represent ± one standard deviation around the mean.

1116
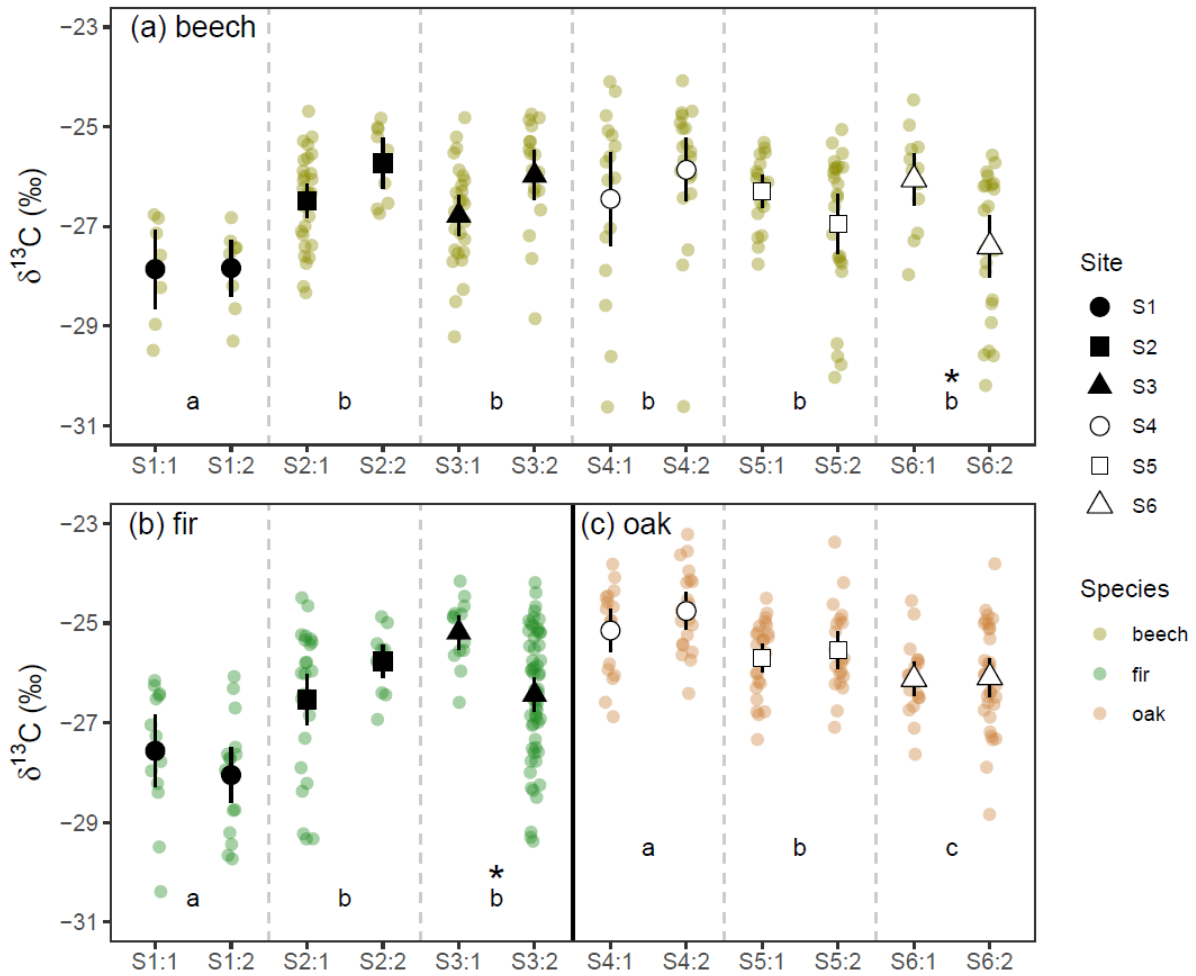
1117

Fig. 3. Tree-ring $\delta^{13}C$ values for all the trees sampled in pure stands, by site and triplet, for **(a)** beech, **(b)** fir, and **(c)** oak. Closed and open symbols with error bars (95% confidence intervals) show mean plot values. Letters at the bottom of each graph indicate results for differences among sites by species (Eq. 5). The pure plots of the two triplets within each site are shown separately (i.e. S1:1 is triplet 1 in site S1). Stars indicate significant differences between the triplets of a site in pure stands of the same species (Eq. 5). Dots in the background represent individual tree data. For individual points, a jitter function is used to add a small amount of random noise on the x-axis to show overlapping points.

Fig. 4. Tree-ring δ^{13}C values for all the trees in the dominant-tree subsample for pure stands, by site and triplet for **(a)** beech, **(b)** fir, and **(c)** oak. Closed and open symbols with error bars (95% confidence intervals) show mean plot values. Letters at the bottom of each graph indicate results for differences among sites by species (Eq. 5). The pure plots of the two triplets within each site are shown separately (i.e. S1:1 is triplet 1 in site S1). Stars indicate significant differences between the two triplets of a site in pure stands of the same species (none here) (Eq. 5). Dots in the background represent individual tree data. For individual points, a jitter function is used to add a small amount of random noise on the x-axis to show overlapping points.
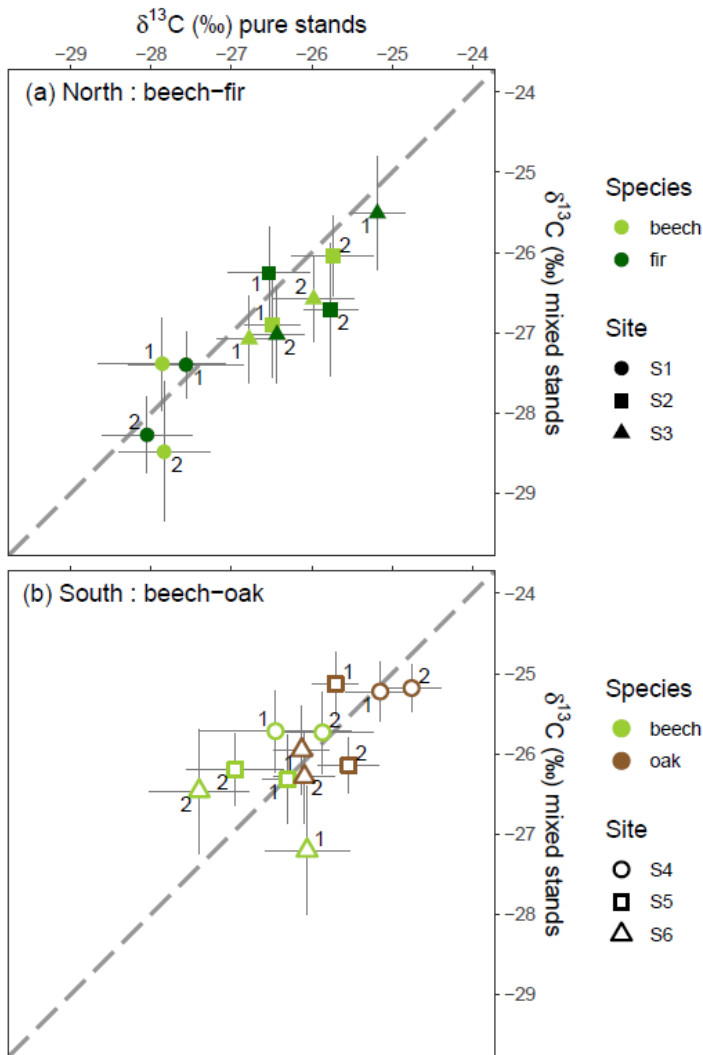
Fig. 5. Mean tree-ring $\delta^{13}C$ values of all the sampled trees in pure versus mixed stands by species, site and triplet for **(a)** beech-fir sites (S1 to S3, closed symbols) and **(b)** beech-oak sites (S4 to S6, open symbols). Symbols with error bars (95% confidence intervals) show mean plot values for pure (x-axis) vs. mixed (y-axis) stands. Numbers 1 and 2 represent the triplet number within the site. The dashed lines are the identity lines of slope 1 and intercept 0.

1143
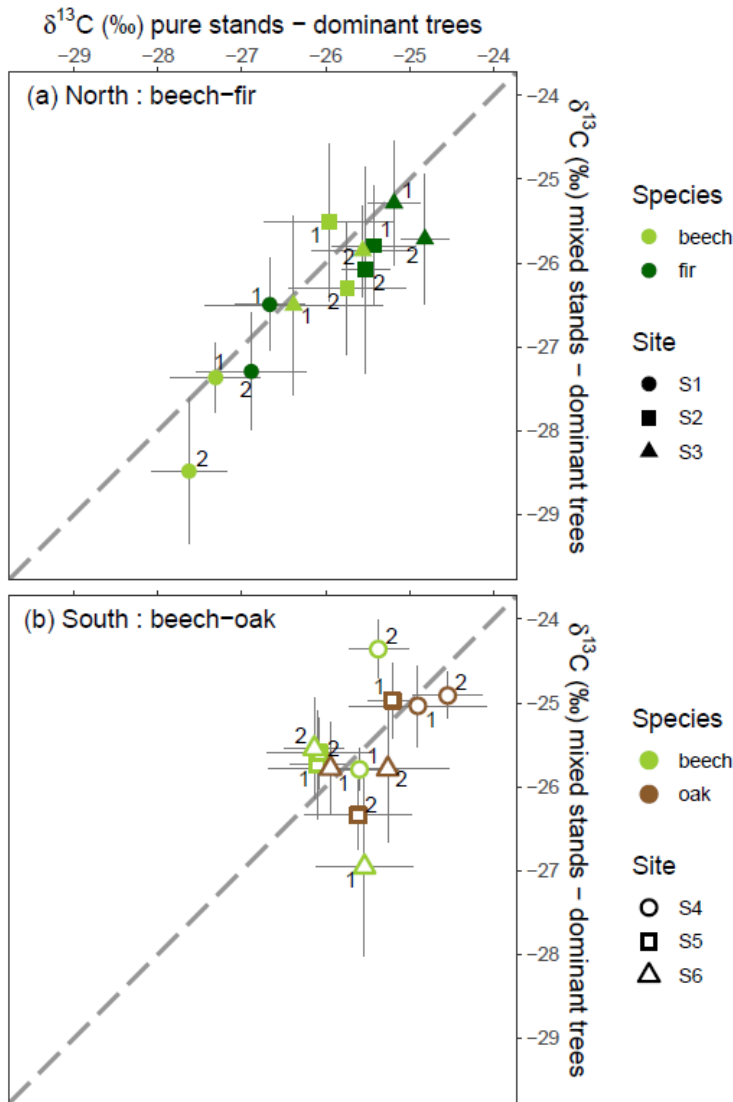
1144

1145   **Fig. 6.** Mean tree-ring $\delta^{13}C$ values of the dominant-tree subsample for pure versus mixed stands

1146   by species, site and triplet for **(a)** beech-fir sites (S1 to S3, closed symbols) and **(b)** beech-oak

1147   sites (S4 to S6, open symbols). Symbols with error bars (95% confidence intervals) show mean

1148   plot values for pure (x-axis) vs. mixed (y-axis) stands. Numbers 1 and 2 represent the triplet

1149   number within the site. The dashed lines are the identity lines of slope 1 and intercept 0.
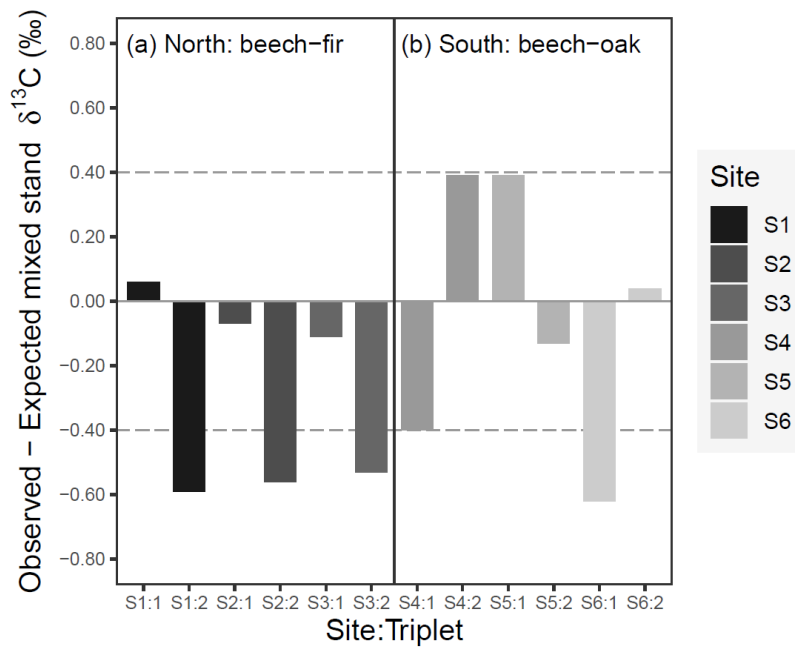
Fig. 7. Net Biodiversity Effect (NBE) for δ^{13}C values at the stand level (see Eq. 9) for the dominant-trees subsample. Differences under 0.4 ‰ are considered as not different from 0.0 ‰. A positive NBE means that the observed mixed stand had a higher (less negative) δ^{13}C value than expected based on data from pure stands. A negative NBE means that the observed mixed stand had a lower (more negative) δ^{13}C value than expected based on data from pure stands.

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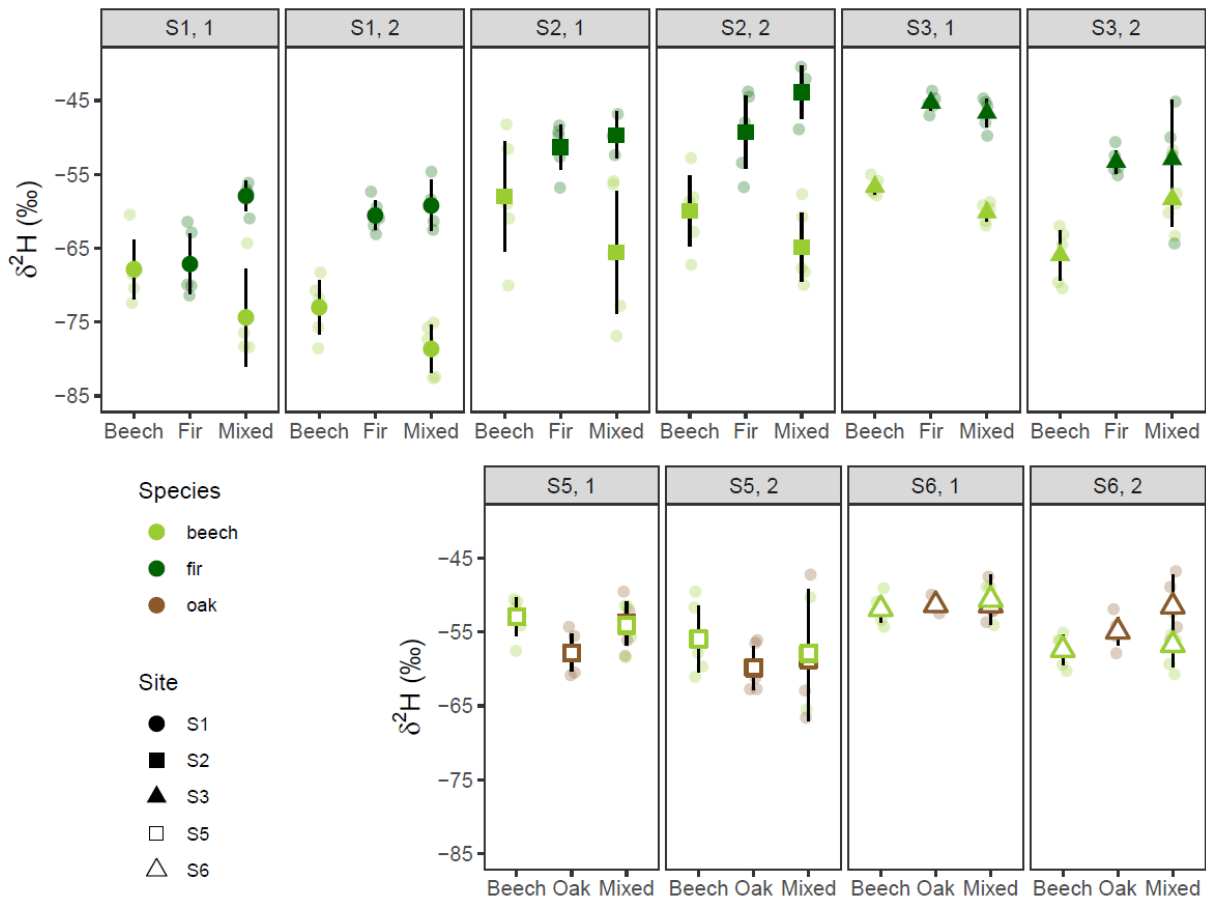
Fig. 8. Xylem-water δ^2H values of the dominant-tree subsample by triplet and species. Each box represents a triplet with values for both species (beech-fir in the top line and beech-oak in the bottom line). The x-axis represents the different plots within a triplet (two pure plots and one mixed plot). Closed and open symbols with error bars (95% confidence intervals) show mean plot values. Dots in the background represent individual tree data. For site S5:1 in the mixed plot, the average for beech and oak overlap. No data could be recorded at site S4.

Appendices

Appendix A : Additional plot characteristics

See Tables A1-A3

Table A1 Environmental characteristics of the triplets.

Site	Triplet	Triplet ID	Lat. (°N)	Lon. (°E)	Mean Elevation (m)	Mean Slope (°)
S1	1	S1:1	45.70548	6.2192	1198	21
S1	2	S1:2	45.71188	6.21625	1025	18
S2	1	S2:1	44.90231	5.3513	1390	20
S2	2	S2:2	44.9411	5.32774	1137	11
S3	1	S3:1	44.17788	5.24168	1354	27
S3	2	S3:2	44.18707	5.25234	1328	27
S4	1	S4:1	43.97376	5.48	1094	13
S4	2	S4:2	43.97661	5.47961	1082	12
S5	1	S5:1	43.8203	5.53343	1005	26
S5	2	S5:2	43.82269	5.53423	889	34
S6	1	S6:1	43.33197	5.77038	736	8
S6	2	S6:2	43.33281	5.77328	746	12

Table A2 Stand structure for each plot, triplet and site. Plot dendrometric variables include: the dominant height of the plot (H_{dom}, in m) and the basal area of all stems by plot and species (in $m^2\ ha^{-1}$).

Site	Triplet	Plot	H_{dom} (m)	Basal area ($m^2\ ha^{-1}$)				
				Beech	Fir	Oak	Other sp.	Total
S1	1	Pure beech	37	80.3	_	_	4.5	84.8
S1	1	Pure fir	30	0.2	81.0	_	8.5	89.7
S1	1	Mixed	28	27.4	29.6	_	4.3	61.4
S1	2	Pure beech	34	46.0	5.5	_	_	51.5
S1	2	Pure fir	34	_	62.5	_	7.4	69.9
S1	2	Mixed	33	32.7	45.5	_	5.1	83.3
S2	1	Pure beech	16	24.9	_	_	3.0	27.9
S2	1	Pure fir	22	_	50.5	_	1.4	51.9
S2	1	Mixed	18	16.0	18.9	_	1.4	36.2
S2	2	Pure beech	33	63.0	2.4	_	_	65.5
S2	2	Pure fir	30	0.6	64.4	_	_	65.0
S2	2	Mixed	26	27.5	19.2	_	_	46.7
S3	1	Pure beech	22	62.0	0.2	_	_	62.2
S3	1	Pure fir	16	0.9	47.5	_	0.8	49.3
S3	1	Mixed	18	17.6	39.4	_	_	57.0
S3	2	Pure beech	20	58.5	1.0	_	_	59.6
S3	2	Pure fir	17	14.1	52.1	_	1.2	67.5
S3	2	Mixed	20	32.0	20.5	_	4.7	57.3
S4	1	Pure beech	20	42.5	_	_	1.3	43.8
S4	1	Pure oak	12	1.3	_	25.3	2.0	28.6
S4	1	Mixed	16	12.5	_	24.2	9.8	46.5
S4	2	Pure beech	19	41.9	_	0.3	_	42.2
S4	2	Pure oak	13	1.0	_	19.0	0.7	20.6
S4	2	Mixed	15	35.5	_	11.9	1.7	49.1
S5	1	Pure beech	13	24.7	_	0.9	11.9	37.5
S5	1	Pure oak	11	_	_	34.4	9.7	44.1
S5	1	Mixed	11	15.2	_	13.0	8.0	36.2
S5	2	Pure beech	16	30.5	_	_	8.0	38.5
S5	2	Pure oak	10	_	_	24.3	4.2	28.5
S5	2	Mixed	12	11.9	_	12.0	5.4	29.3
S6	1	Pure beech	30	54.8	_	_	13.3	68.1
S6	1	Pure oak	19	_	_	61.6	18.2	79.8
S6	1	Mixed	21	23.3	_	24.2	22.5	70.0
S6	2	Pure beech	20	32.5	_	3.0	7.7	43.2
S6	2	Pure oak	16	_	_	33.4	0.5	33.9
S6	2	Mixed	19	22.3	_	21.2	8.1	51.7

Table A3 Average summer rainfall and summer temperature for 4-month summer periods (June to September) at each site calculated over two time periods and mean number of days with water stress (REW < 0.4) over 2003-2007 from BILJOU© model.

Site	Name of region	Summer Rain (mm) 1994-2013	Summer Rain (mm) 2003-2007	Mean summer temp. (°C) 1994-2013	Mean summer temp. (°C) 2003-2007	Mean number of days with water stress 2003-2007
S1	Bauges	619 (±50)	581 (±158)	13.7 (±0.41)	14.3 (±1.34)	1
S2	Vercors	429 (±48)	332 (±46)	14.5 (±0.41)	15.1 (±0.93)	52
S3	Ventoux	293 (±39)	221 (±35)	15.6 (±0.37)	16.2 (±1.01)	94
S4	Lubéron Lagarde	246 (±38)	172 (±36)	17.8 (±0.37)	18.2 (±1.08)	105
S5	Grand Lubéron	227 (±30)	169 (±40)	19.8 (±0.35)	20.2 (±1.16)	107
S6	Sainte-Baume	212 (±39)	158 (±56)	20.1 (±0.37)	20.7 (±0.93)	108

Appendix B : Additional model results

See Tables B1-B2

Table B1 Linear mixed model output for *Species* effect, *log(DBH)* and their interaction on $\delta^{13}C$ values in pure stands by type of mixture (Eq. 6).

	Beech-fir sites				Beech-oak sites			
	χ^2	df	P	post-hoc	χ^2	df	P	post-hoc
All trees		228				238		
log(DBH)	183	1	<.001***		113	1	<.001***	
Species	7	1	<.01**	beech<fir	92	1	<.001***	beech<oak
log(DBH):Species	17	1	<.001***		24	1	<.001***	
R2m (R2c)	*0.30 (0.72)*				*0.40 (0.61)*			
Dominant-tree subsample		54				56		
log(DBH)	0	1	0.61		0	1	0.48	
Species	11	1	<.001***	beech<fir	7	1	<.01**	beech<oak
log(DBH):Species	0	1	0.72		4	1	0.06	
R2m (R2c)	*0.09 (0.63)*				*0.18 (0.28)*			

Note: Stars indicate significant *log(DBH)*, *Species* and interaction effects. *Species* is a two-level factor (beech-fir or beech-oak). For significant *Species* effects, the direction of the estimate is indicated by beech<fir (beech<oak) because fir (oak) trees have a higher $\delta^{13}C$ estimate than beech stands. *χ2*: chi square test results for type "II" ANOVA. *df*: degrees of freedom. *P*: p-value for fixed terms. *Triplet ID* is used as a random factor in all four models. A pseudo R-squared for mixed effect models was used to determine the variance explained by the two fixed terms only (marginal coefficient of determination, R^2_m) and the entire model including the random terms (conditional coefficient of determination, R^2_c).

Table B2 Linear mixed model output for *Species* effect, *Mixture* and their interaction on δ^2H of the dominant-tree subsample by type of mixture (Eq. 10).

	Beech-fir sites				Beech-oak sites		
	χ^2	*df*	*P*	post-hoc	χ^2	*df*	*P*
Dominant-tree subsample		104				65	
Species	149	1	<0.001***	beech<fir	0	1	0.71
Mixture	0	1	0.61		1	1	0.33
Species:Mixture	10	1	0.002**	beech: m<p	2	1	0.16
				fir: m=p			
				pure: beech<fir			
				mixed: beech<fir			
R2m (R2c)	*0.36 (0.75)*				*0.03 (0.32)*		

Note: Stars indicate significant *Species*, *Mixture* and interaction effects. *Species* is a two-level factor (beech-fir or beech-oak); *Mixture* is also a two-level factor (pure vs. mixed). For significant *Species* effects, the direction of the estimate is indicated by beech<fir because fir trees have a higher δ^2H estimate than beech. Post-hoc results for the *Species:Mixture* interaction are similarly indicated. $\chi2$: chi square test results for type "II" ANOVA. *df*: degrees of freedom. *P*: p-value for fixed terms. *Triplet ID* is used as a random factor in both models. A pseudo R-squared for mixed effect models was used to determine the variance explained by the two fixed terms only (marginal coefficient of determination, R^2_m) and the entire model including the random terms (conditional coefficient of determination, R^2_c).