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Application of Loreau & Hector's (2001) partitioning method to complex functional traits

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RUNNING TITLE

Complex traits in B-EF studies

AUTHORSHIP

CG and DB elaborated the adaptation of the method to complex functional traits. DB, AG¹, AG² and MSL designed the experimental study. DB, MP and AG¹ performed the experimental work. CG, DB, AG¹ and AG² analyzed the results. CG and DB wrote the first draft of this manuscript and all authors substantially contributed to revisions.

24 **Key words**

25 Biodiversity effect, BIOTREE, carbon isotope composition, complementarity effect,
26 FunDivEUROPE, method, selection effect, water use efficiency

27

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29

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33

34 **Tweetable abstract:**

35 We applied Loreau and Hector's (2001) method to calculate biodiversity effects of complex
36 functional traits.

Abstract

1- In 2001, Loreau & Hector proposed a method to calculate the effect of biodiversity on ecosystem-level properties that distinguished selection effects from complementarity effects. The approach was designed, and has been widely used, for the study of yield in mixed-species situations taking into account the relative abundance of each species in ecosystem-level yield. However, complex functional traits commonly used to integrate ecosystem-level properties cannot be analyzed like yield data because the weighted contribution of each species is not determined by its relative abundance.

2- We adapted the original method by clearly identifying ecologically meaningful weighting coefficients to represent species specific contributions to ecosystem function.

3- We applied the adapted method of analysis to tree foliar carbon isotope composition in an experimental plantation in order to test the influence of species richness on plot water use efficiency (WUE_{plot}). The appropriate weights for the WUE_{plot} of each species are leaf CO_2 assimilation rate.

4- We observed a large range of WUE_{plot} and biodiversity effects among plots. The absence of a significant selection effect on WUE_{plot} indicated that the overall net biodiversity effect was primarily driven by a complementarity effect. The net biodiversity and complementarity effects were mostly negative, suggesting that interspecific interactions resulted in a decrease in the ratio between carbon acquisition and transpiration at the ecosystem level.

5- The application of the method to complex components of ecosystem functioning provides important new insights for the practical and conceptual aspects of functional biodiversity research.

INTRODUCTION

The loss of biodiversity occurring in most natural environments worldwide has sparked an interest among the scientific community in the relationship between biodiversity and ecosystem functioning (Hooper *et al.* 2012). More than twenty years of ecological studies have led to a consensus that ecosystem performance is highly dependent on species richness and on species functional characteristics (Loreau *et al.* 2001; Hooper *et al.* 2005; Zhang *et al.* 2012). However, the mechanisms underlying biodiversity-function relationships have been hotly debated. Two major groups of mechanisms were initially proposed to explain positive effects of biodiversity: (1) a sampling or selection effect which arises, as species richness increases, from the increasingly probable occurrence of one or several species that strongly contribute to the ecosystem function observed (Aarssen 1997; Huston 1997; Tilman 1997), and (2) a complementarity effect driven either by niche differentiation among species, which tends to increase the efficiency with which coexisting species use the available resources or to facilitation or other mutualistic interactions among species (Tilman *et al.* 1997; Loreau 1998). Recently, the combination of evenness, richness, and life-history variations were also successfully linked to the mechanisms producing positive biodiversity effects (Zhang *et al.* 2012).

To quantitatively evaluate this biodiversity - ecosystem functioning relationship and partition the underlying mechanisms, Loreau & Hector (2001) proposed a convenient method to calculate the influence of species mixture on ecosystem productivity: the net biodiversity effect on the yield (ΔY) of a given mixture can be calculated as the difference between the observed total yield in the mixture (Y_O) and the expected total yield in the mixture (Y_E) under the null hypothesis that intra- and inter-specific interactions are identical. The original method was extended by Fox (2005) to include trait-dependent and trait-independent complementarity effects in addition to the dominance effect (tripartite partitioning).

Loreau & Hector's method has been widely used and so far more than 100 peer-reviewed papers analyzing the relationships between species richness and ecosystem functioning in highly diverse biomes have been published. Even though the method has proven to be very popular, existing studies have focused on a limited number of ecosystem functions, mostly on standing biomass.

One explanation for this limited application could stem from the fact that some ecological functions in mixed species stands cannot be treated at the ecosystem level in the same way as observed yield. For complex functional properties, ecosystem-level values correspond to the mean value of the species present in the community weighted by the contribution of each species to the given function; this weighted contribution can be totally different from the relative abundance of these species in terms of frequency or biomass, which the original method does not imply. These complex functions include, among others, any measurement related to the efficiency of individuals to acquire and use resources (e.g. water use efficiency, photosynthesis efficiency, nutrient use efficiency), whatever the ecosystem (plant or aquatic ecosystems, bacterial communities...). The isotope composition of organic or mineral elements in biological material or the density of any gas flux (e.g. sap flow density, density of CO₂ respiration) are also examples of such complex functional traits.

In this paper, we extend Loreau & Hector's (2001) method to complex functional traits by clearly identifying ecologically meaningful weighting coefficients which represent species specific contributions to ecosystem functioning. We illustrate the usefulness of the adapted equations for leaf carbon isotope composition. More precisely, we analyze the effect of species richness on foliar carbon isotope composition ($\delta^{13}C$) at the ecosystem level in a temperate mixed-species tree plantation. Foliar $\delta^{13}C$ is a convenient proxy for time-integrated intrinsic water use efficiency (WUE_{int}) (Farquhar *et al.* 1982) and reflects the trade-off between CO₂ acquisition and stomatal regulation of transpiration at the leaf level. Foliar $\delta^{13}C$

and WUE_{int} can only be obtained at the individual level and ecosystem-level $\delta^{13}C$ cannot be calculated by simply taking into account the summed contribution of the individuals in terms of biomass or frequency. Instead, the proportional amount of CO_2 assimilated by each species in the plot needs to be considered to correctly weight each species' contribution to ecosystem-level functioning.

APPLICATION OF LOREAU & HECTOR'S (2001) METHOD TO COMPLEX FUNCTIONAL TRAITS

According to Loreau & Hector (2001), the net biodiversity effect on the yield (ΔY) of a given mixture of species is the difference between the observed total yield in the mixture (Y_O) and the expected total yield in the mixture (Y_E) calculated as the sum of the products between the yield of the different species in their corresponding monocultures and the proportion of the species in the mixture (defined in terms of individual frequency or biomass):

$$\Delta Y = Y_O - Y_E \quad \text{Eqn 1}$$

where N is the number of species in the mixture, Y_{Os} and Y_{Es} denote the observed and expected yield of species s in the mixture, M_s is yield of species s in the monoculture, RY_{Os} is the observed relative yield of species s in the mixture, and RY_{Es} is the expected relative yield of species s in the mixture. RY_{Os} is calculated as the ratio of the observed yield of species s in the mixture and the yield of species s in the monoculture, whereas RY_{Es} is simply the proportion of species s seeded or planted in the mixture.

For complex functional properties where the contribution of each species to a given ecosystem-level function is not simply proportionate to the frequency of these species or their

proportion in biomass, we introduce a weighting coefficient (W_{Os}) to calculate the contribution of species s to the complex function (F) in the mixed plots. W_{Os} is normalized to one and therefore represents a proportional contribution. Thus, the net biodiversity effect on a complex function (ΔF) is written as:

$$\Delta F = F_{O_i} - F_{E_i} \quad \text{Eqn 2}$$

where F_{O_i} and F_{E_i} denote the observed and expected value of the function of species i in the mixture. This equation is a generalisation of the equation proposed by Loreau & Hector (2001). If one considers that F_{O_i} is the observed biomass of species i in the mixed plot and W_{O_i} is the proportion of species i in the mixed plot in terms of the number of seeded individuals or in terms of biomass, then $F_{O_i} \times W_{O_i}$ equals Y_{O_i} . Similarly, if one considers that F_{E_i} is the observed biomass of species i in the monoculture and W_{O_i} is the proportion of species i in the mixed plot in terms of the number of seeded individuals or in terms of biomass, then $F_{E_i} \times W_{O_i}$ equals Y_{E_i} .

The weighting coefficients are specific to each studied complex property and must take into account the underlying biological and ecological mechanisms to correctly estimate the contribution of each species to ecosystem-level functioning. In Table 1, we have listed some weighting factors that could be used in plant ecological studies. Let us illustrate this point with sapflow density. Sapflow density ($L_{\text{sap}} \text{ dm}^{-2} \text{ sapwood h}^{-1}$) represents the density of the flow of raw sap circulating in the xylem vessels of trees and can be directly measured with sapflow sensors at the single tree level. However, sapflow density cannot simply be added among the trees to calculate total plot sapflow density and to estimate the influence of biodiversity on this ecosystem-level property, because the proportion in biomass or tree frequency among

species in the mixture does not give the proportional contribution of each species to total plot sapflow density. Rather, sapwood area (the cross-sectional, water conducting area in the trunk) of each tree is the correct weighting coefficient and should be used as the quantity W_{oi} in equation 2.

The goal of Loreau & Hector's (2001) method is to partition ΔY into two effects generated by species interactions in mixtures: selection effects (SE) and complementarity effects (CE). SE arises from interspecific competition leading to the dominance of a given species with particular functional traits. CE reflects the degree to which niche differences and facilitation outweigh interference competition and other negative species interactions (Loreau *et al.* 2012). As when calculating ΔY , partitioning the effects of complex functions into SE and CE must also take W_{oi} into account. The equation to calculate CE can be rewritten as:

, Eqn 3

and the equation for SE is:

. Eqn 4

As our approach is intended to be a generalisation of the original method and can be applied to complex functions which are not directly related to yield, changes in species contribution with time are not taken into account in equation 4. The selection effect thus here stresses the dominance of a one or more species for the considered complex ecosystem function at a given time.

APPLICATION OF THE MODIFIED EQUATIONS TO ECOSYSTEM-LEVEL CARBON ISOTOPE COMPOSITION

A positive effect of species mixture on forest ecosystem productivity (Paquette & Messier 2011; Zhang *et al.* 2012) and transpiration (Forrester *et al.* 2010; Kunert *et al.* 2012) has previously been reported. Complementarity effects (*CE*) and selection effects (*SE*) for these ecosystem-level parameters were highlighted with Loreau & Hector's (2001) method, thus making it possible to explain differences in ecosystem functioning among species richness levels. Promoting a mixture of tree species to enhance the ratio of ecosystem-level productivity to transpiration (i.e. high water use efficiency) has been advocated for sustainable forest management (McCarthy *et al.* 2011), of particular importance in a context of climate change. To further investigate this relationship, we applied the widely used carbon isotope approach (Farquhar *et al.* 1982) to study the impact of tree species mixtures on ecosystem-level water use efficiency (WUE_{plot}) in a temperate mixed plantation.

At leaf level, intrinsic water use efficiency (WUE_{int}) represents the ratio between photosynthetic assimilation of CO_2 by the leaf (A) and stomatal conductance for water vapor (g_s) and depends on the molar fraction of CO_2 in the air (C_a) and in the leaf intercellular spaces (C_i) following this equation:

Eqn 5

During photosynthetic assimilation of CO_2 , plants discriminate against molecules of CO_2 containing ^{13}C because $^{13}CO_2$ diffuses more slowly from the atmosphere to the site of carboxylation (stomatal diffusion) than does $^{12}CO_2$. $^{13}CO_2$ also reacts less with the primary

carboxylating enzyme (Rubisco) (Fig. 1). Farquhar *et al.* (1982) showed that foliar carbon isotope composition ($\delta^{13}\text{C}$, ‰) is strongly negatively correlated with WUE_{int} following this simplified equation:

$$\delta^{13}\text{C} = a + b \cdot WUE_{int} \quad \text{Eqn 6}$$

where $\delta^{13}\text{C}_{air}$ is the carbon isotope composition of the air and a and b are factors characterising the discrimination against $^{13}\text{CO}_2$ during stomatal diffusion and carboxylation, respectively. Therefore, $\delta^{13}\text{C}$ provides a convenient time-integrated estimate of WUE_{int} . Values for $\delta^{13}\text{C}$ are obtained at the individual tree level by sampling representative subsets of leaves or needles, but individual values cannot simply be added to represent ecosystem-level carbon isotope composition ($\delta^{13}\text{C}_{plot}$). Instead, the contribution of a single tree to the carbon isotope composition of the whole population depends on tree-specific CO_2 assimilation rates that control carbon isotope fractionation during photosynthesis (Lloyd & Farquhar 1994). Consequently, when $\delta^{13}\text{C}$ values are scaled up from tree or species level to ecosystem level, tree or species $\delta^{13}\text{C}$ values should be weighted by these assimilation rates (Fig. 2). Since direct measurements of CO_2 assimilation rates cannot easily be made for each tree in the field, we used a convenient proxy for canopy-level, species CO_2 assimilation. In a given environment, the quantum yield for reduction of end electron acceptors at the PSI acceptor side is strongly positively correlated with time-integrated leaf CO_2 exchanges (Genty *et al.* 1989). Thus, the proportions of the measured quantum yield of species i can therefore be used as the weighting coefficient (W_{oi}) for $\delta^{13}\text{C}$ values.

Materials

We conducted our study at the BIOTREE tree biodiversity experimental site in Germany (Kaltenborn site, Scherer-Lorenzen *et al.* 2007), which was planted in winter 2003/2004. This plantation is located on acidic sandy soils and includes four species: *Fagus sylvatica* (L.), *Quercus petraea* (Matt.), *Picea abies* (L.) Karst. and *Pseudotsuga menziesii* (Mirb.) Franco. The plantation was designed to assure maximum above- and below-ground interactions among species at the adult stage. Therefore, in the mixed plots of 120 x 48m, each species was planted in monospecific rectangular patches of 8 x 8m, arranged in a regular pattern in order to reduce out-competition of slow growing species at an early stage and to maximize interspecific interactions along borders and corners (Scherer-Lorenzen *et al.* 2007). In summer 2011, we sampled leaves and needles from four trees per species and per plot in monocultures (n = four plots), two-species mixtures (n = six), three-species mixtures (n = four) and four-species mixtures (n = one). In each plot, we only took samples from trees at the corners of the patches. The samples were oven-dried at 60°C for 48 hours, then finely ground. $\delta^{13}\text{C}$ analysis was carried out at the Stable Isotope Facility of UC Davis, USA. The $\delta^{13}\text{C}$ (‰) values are expressed relative to the Vienna Pee Dee Belemnite standard. The quantum yield for reduction of end electron acceptors at the PSI acceptor side was measured with a HandyPea fluorimeter (Hansatech Instruments, Pentney-Norfolk, UK) on leaves or needles in close vicinity to the ones harvested for elemental and isotope analyses following the procedure described by Strasser *et al.* (2010). This value was then used as a weighting coefficient (W_{oi}) when calculating biodiversity effects on $\delta^{13}\text{C}_{plot}$. We used the non-parametric Wilcoxon test to check for complementarity, selection and net effects among mixture levels and t-tests to evaluate whether all the indices differed significantly from zero (SAS 9.3, SAS Institute, Cary, USA).

Results and Discussion

254 We found large differences in ΔF , CE , and SE among plots, with either positive or negative
255 values (Fig. 3, Table 2). Positive or negative values confirmed that in this plantation,
256 interactions among species drive $\delta^{13}C_{plot}$, and thus WUE_{plot} . When considering individual
257 species richness levels, we found that both the net and complementarity effects were
258 significantly different from zero ($P < 0.05$) for the two-species mixtures; however, we found
259 no selection effect ($P = 0.27$). Furthermore, the net and complementarity effects were mostly
260 negative, suggesting lower observed $\delta^{13}C_{plot}$ than what would have been expected based on the
261 monoculture values. Since $\delta^{13}C$ and water use efficiency are positively related (Farquhar *et al.*
262 1982), our findings point toward lower WUE_{plot} when several different species coexist. This
263 result contrasts with previous patterns of enhanced water use efficiency found in species
264 mixtures (Forrester *et al.* 2010; Kunert *et al.* 2012).

265 The absence of a significant selection effect on WUE_{plot} indicates that the overall negative net
266 biodiversity effect observed in the 2-species mixtures was primarily driven by a
267 complementarity effect. Our interpretation is that the species coexisting in the mixed plots are
268 in direct competition for the same resources because they still share the same ecological niche
269 at the early establishment stage (7 years after planting, at the time of our measurements). This
270 competition most likely caused a decrease in the ratio between carbon acquisition and
271 transpiration at the ecosystem level in the two-species mixtures. As no overshading was
272 observed, the competition among species is presumably occurring belowground. This
273 assumption is consistent with the strong competition among fine roots observed by Lei *et al.*
274 (2012) in this plantation.

275 Furthermore, we did not observe any significant effect of richness level for any of the
276 biodiversity effects ($P > 0.05$). This indicates that the number of species competing for
277 resources does not significantly affect the difference in $\delta^{13}C_{plot}$ between observed and expected

values. The $\delta^{13}C$ value for a given species in the two-, three- and four-species mixtures did not greatly change.

CONCLUSION

Applying the version of Loreau & Hector's (2001) method to complex components of ecosystem functioning will provide important new practical applications as well as conceptual insights in functional biodiversity research. We have shown here that, with the appropriate weighting factors for specific, complex functional properties, the method can be applied to a broad range of functional properties, rather than to yield alone. In our case, we used the quantum yield for reduction of end electron acceptors at the PSI acceptor side as the weighting factor for ecosystem-level carbon isotope composition. This combination of weights and functions provides an estimate of intrinsic water use efficiency in mixed species plots. For other complex traits, the selection of the most pertinent weighting factor should make it possible to determine the contribution of each species to the studied ecosystem property. Some of the weighting factors may be difficult to measure precisely with currently available equipment, as in the case of CO₂ assimilation rates in our study. Nevertheless, if appropriate measurements are not easily obtainable, proxies could be found that provide the same proportional contributions; modelling approaches may be of help in this case. In the young mixed temperate plantation in our study, complementarity rather than selection effects were the substantial drivers of plot water use efficiency. As the plantation ages and taller trees with broader root systems begin to compete for light and soil resources, the complementarity effects might increase and selection effects might arise due to more frequent interspecific interactions. It will thus be interesting to follow the changes in the relative importance of

302 these two components of net biodiversity effects for a multitude of ecological processes and
303 functions.

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315

316 **REFERENCES**

- 317 Aarssen, L.W. (1997) High productivity in grassland ecosystems: effects by species diversity
318 or productive species? *Oikos*, **80**, 183-184.
- 319 Bonal, D., Barigah, T.S., Granier, A. & Guehl, J. (2000) Late stage canopy tree species with
320 extremely low $\delta^{13}\text{C}$ and high stomatal sensitivity to seasonal soil drought in the tropical
321 rainforest of French Guiana. *Plant, Cell and Environment*, **23**, 445-459.
- 322 Cascio, C., M. Schaub, K. Novak, R. Desotgiu, F. Bussotti, & R. J. Strasser. (2010) Foliar
323 responses to ozone of *fagus sylvatica* L. seedlings grown in shaded and in full sunlight
324 conditions. *Environmental and Experimental Botany*, **68**, 188-197.
- 325 Farquhar, G.D., O'Leary, M.H., & Berry, J.A. (1982) On the relationship between carbon
326 isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian*
327 *Journal of Plant Physiology*, **9**, 121-137.
- 328 Forrester, D.I., Theiveyanathan, S., Collopy, J.J. & Marcar, N.E. (2010) Enhanced water use
329 efficiency in a mixed *Eucalyptus globulus* and *Acacia mearnsii* plantation. *Forest Ecology*
330 *and Management*, **259**, 1761-1770.
- 331 Fox, J.W. (2005) Interpreting the selection effect of biodiversity on ecosystem function.
332 *Ecology letters*, **8**, 846-856.
- 333 Genty B, Briantais J-M, Baker N.R. (1989) The relationship between the quantum yield of
334 photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et*
335 *Biophysica Acta (BBA) - General Subjects*, **990**, 87-92.
- 336 Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H.,
337 Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. &
338 Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current
339 knowledge. *Ecological Monographs*, **75**, 3-35.

340 Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L.,
 341 Gonzalez, A., Duffy, J.E., Gamfeldt, L. & O'Connor, M.I. (2012) A global synthesis reveals
 342 biodiversity loss as a major driver of ecosystem change. *Nature*, **486**, 105-108.
 343 Huston, M.A. (1997) Hidden treatments in ecological experiments: re-evaluating the
 344 ecosystem function of biodiversity. *Oecologia*, **110**, 449-460.
 345 Kunert, N., Schwendenmann, L., Potvin, C. & Hölscher, D. (2012) Tree diversity enhances
 346 tree transpiration in a Panamanian forest plantation. *Journal of applied Ecology*, **49**, 135-144.
 347 Lei, P., Scherer-Lorenzen, M. & Bauhus, J. (2012) Belowground facilitation and competition
 348 in young tree species mixtures. *Forest Ecology and Management*, **265**, 191-200.
 349 Lloyd, J. & Farquhar, G.D. (1994) ¹³C discrimination during CO₂ assimilation by the
 350 terrestrial biosphere. *Oecologia*, **99**, 201-215.
 351 Loreau, M. (1998) Separating sampling and other effects in biodiversity experiments. *Oikos*,
 352 **82**, 600-602.
 353 Loreau, M. & Hector, A. (2001) Partitioning selection and complementarity in biodiversity
 354 experiments. *Nature*, **412**, 72-76.
 355 Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U.,
 356 Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. (2001) Biodiversity and
 357 ecosystem functioning: current knowledge and future challenges. *Science*, **294**, 804-808.
 358 Loreau, M., Sapijanskas, J., Isbell, F. I. & Hector, A. (2012) Niche and fitness differences
 359 relate the maintenance of diversity to ecosystem function: comment. *Ecology*, **96**, 1482-1487.
 360 McCarthy, N., Bentsen, N.S., Willoughby, I. & Balandier, P. (2011) The state of forest
 361 vegetation management in Europe in the 21st century. *European Journal of Forest Research*,
 362 **130**, 7-16.

363 Paquette, A. & Messier, C. (2011) The effect of biodiversity on tree productivity: from
 364 temperate to boreal forests. *Global Ecology and Biogeography*, **20**, 170-180.

365 Scherer-Lorenzen, M., Schulze, E.-D., Don, A., Schumacher, J. & Weller, E. (2007) Exploring
 366 the functional significance of forest diversity: A new long-term experiment with temperate
 367 tree species (BIOTREE). *Perspectives in Plant Ecology, Evolution and Systematics*, **9**, 53-70.

368 Strasser RJ, Tsimilli-Michael M, Qiang S, Goltsev V. (2010) Simultaneous in vivo recording
 369 of prompt and delayed fluorescence and 820-nm reflection changes during drying and after
 370 rehydration of the resurrection plant *Haberlea rhodopensis*. *Biochimica et Biophysica Acta*
 371 (*BBA*) – *Bioenergetics* **1797**, 1313-1326.

372 Tilman, D. (1997) Distinguishing between the effects of species diversity and species
 373 composition. *Oikos*, **80**, 185.

374 Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. & Siemann, E. (1997) The influence
 375 of functional diversity and composition on ecosystem processes. *Science*, **277**, 1300-1302.

376 Zhang, Y., Chen, H.Y.H. & Reich, P.B. (2012) Forest productivity increases with evenness,
 377 species richness and trait variation: a global meta-analysis. *Journal of Ecology*, **100**, 742-749.

378

Table 1. Examples of complex functional properties used in plant ecological studies, with units and suitable corresponding weighting coefficients.

Properties	Units	Weighting coefficient
Sap flow density	$\text{L dm}^{-2} \text{ h}^{-1}$	Sapwood area
Bark CO_2 efflux	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	Trunk surface
Photosynthesis	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	Leaf area
Leaf water use efficiency	$\mu\text{mol mol}^{-1}$	Leaf CO_2 exchange
Carbon isotope composition	‰	Leaf CO_2 exchange
Plant water use efficiency	Kg L^{-1}	Leaf area
Nutrient uptake rates	$\mu\text{mol g}^{-1} \text{ h}^{-1}$	Root surface area

Table 2. Mean species foliar carbon isotope composition measured in the mixed plots (F_{Oi} , ‰), expected mean species foliar carbon isotope composition measured in the monoculture plots (F_{Ei} , ‰), and proportional weighting coefficient (W_{Oi}) of species i , in each studied plot for the four studied species: *Fagus sylvatica* (Fs), *Pseudotsuga menziesii* (Pm), *Quercus petraea* (Qp) and *Picea abies* (Pa). Species richness, net biodiversity effect (ΔF), complementarity effect (CE), selection effect (SE) and calculated plot carbon isotope composition (F_o) are shown for each plot.

Plot	Richness level	Species	F_{Oi}	F_{Ei}	W_{Oi}	ΔF	CE	SE	F_o
3	2	Fs	-28.46	-28.82	0.46	0.26	0.26	3.11×10^{-3}	-26.99
		Pm	-25.74	-25.91	0.54				
6	2	Qp	-27.43	-26.60	0.50	-0.48	-0.47	-5.91×10^{-3}	-26.64
		Pa	-25.84	-25.72	0.50				
7	2	Pm	-26.36	-25.91	0.51	-0.52	-0.52	-7.38×10^{-4}	-26.77
		Qp	-27.19	-26.60	0.49				
10	2	Pm	-26.13	-25.91	0.58	-0.30	-0.30	2.14×10^{-4}	-26.13
		Pa	-26.15	-25.72	0.42				
12	2	Fs	-28.99	-28.82	0.41	-0.52	-0.53	1.59×10^{-2}	-28.02
		Qp	-27.36	-26.60	0.59				
16	2	Fs	-28.58	-28.82	0.36	-0.26	-0.28	2.54×10^{-2}	-27.11
		Pa	-26.26	-25.72	0.64				
4	3	Fs	-28.60	-28.82	0.25	-0.22	-0.24	1.61×10^{-2}	-27.06
		Qp	-26.73	-26.60	0.39				
		Pa	-26.35	-25.72	0.36				
5	3	Fs	-28.57	-28.82	0.29	0.15	0.15	-5.72×10^{-4}	-26.84
		Pm	-25.57	-25.91	0.36				
		Qp	-26.75	-26.60	0.35				
9	3	Fs	-28.82	-28.82	0.27	-0.07	-0.07	3.58×10^{-3}	-26.70
		Pm	-25.70	-25.91	0.34				
		Pa	-26.09	-25.72	0.39				

15	3	Pm	-26.25	-25.91	0.38				
		Qp	-27.20	-26.60	0.30	-0.47	-0.47	2.54×10^{-2}	-27.11
		Pa	-26.23	-25.72	0.31				
8	4	Fs	-28.92	-28.82	0.18				
		Pm	-25.24	-25.91	0.25				
		Qp	-26.95	-26.60	0.30	-0.03	-0.02	-3.15×10^{-3}	-26.61
		Pa	-26.00	-25.72	0.27				

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Figure legends:

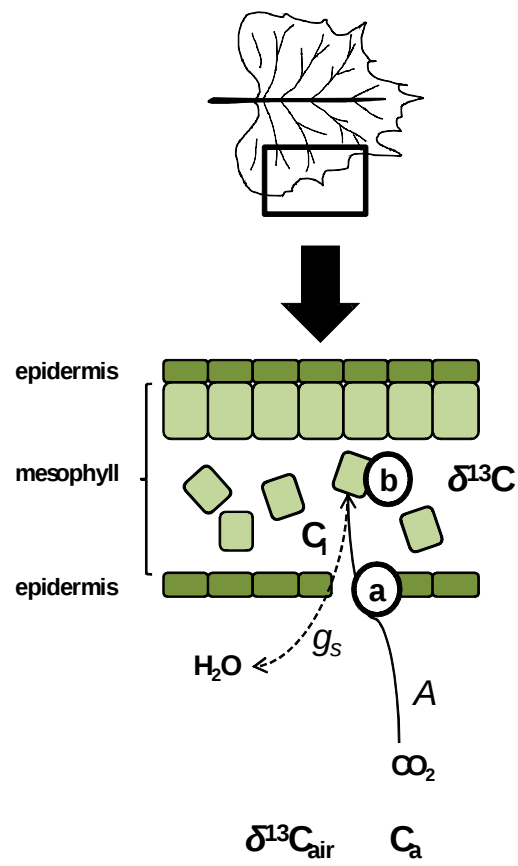
Figure 1: Schematic representation of the processes involved in leaf carbon isotope discrimination during photosynthesis. The carbon isotope composition ($\delta^{13}\text{C}$) of total leaf organic matter is determined by the carbon isotope composition of CO_2 in the air ($\delta^{13}\text{C}_{\text{air}}$) and the CO_2 concentration in the air (C_a) and in the leaf intercellular spaces (C_i). Discrimination processes against $^{13}\text{CO}_2$ occur during photosynthetic CO_2 assimilation (A) when CO_2 passes through the stomata (g_s) from the outside air (fractionation factor a) and during the carboxylation process by the Rubisco enzyme inside the chloroplasts (fractionation factor b). The plain arrow represents A and the dotted arrow represents the transpiration flux.

Figure 2: Comparison of the factors taken into account to calculate the observed trait at plot level for yield (Y_o) and carbon isotope composition (F_o). For yield, the observed biomass of each species i in the mixture (B_{oi}) and the proportion of each species in the mixture (p_i) are taken into account to calculate Y_o . For carbon isotope composition, F_o is dependent on the observed carbon isotope composition of species i in the mixture ($\delta^{13}\text{C}_{oi}$, i.e. F_{oi}) and the corresponding CO_2 assimilation rate during photosynthesis (A_i) representing the species-specific contribution to plot level carbon isotope composition. Letters on the trees denote different species.

Figure 3: Application of the adapted method to plot carbon isotope composition ($\delta^{13}\text{C}_{\text{plot}}$). Net, complementarity and selection effects calculated for $\delta^{13}\text{C}_{\text{plot}}$ for the different richness levels. Asterisks denote significant differences from zero for each effect (t-test, *, $P < 0.05$).

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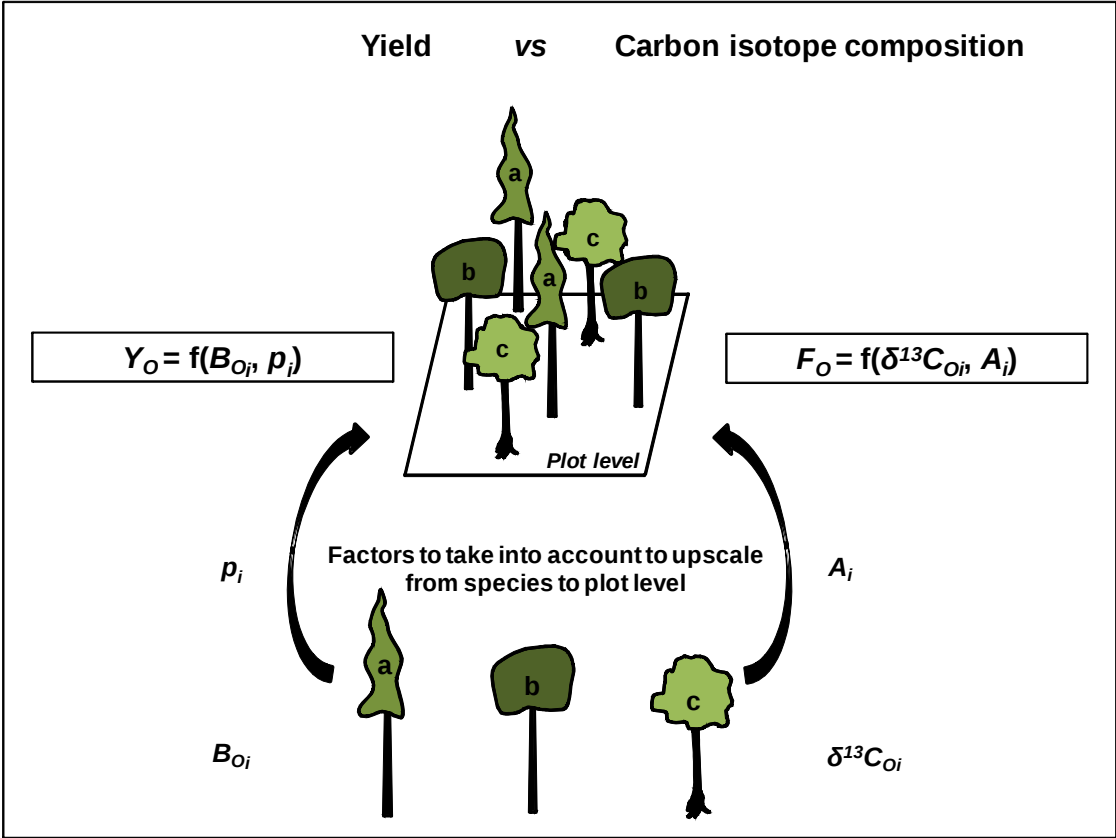
Figure 1



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Figure 2

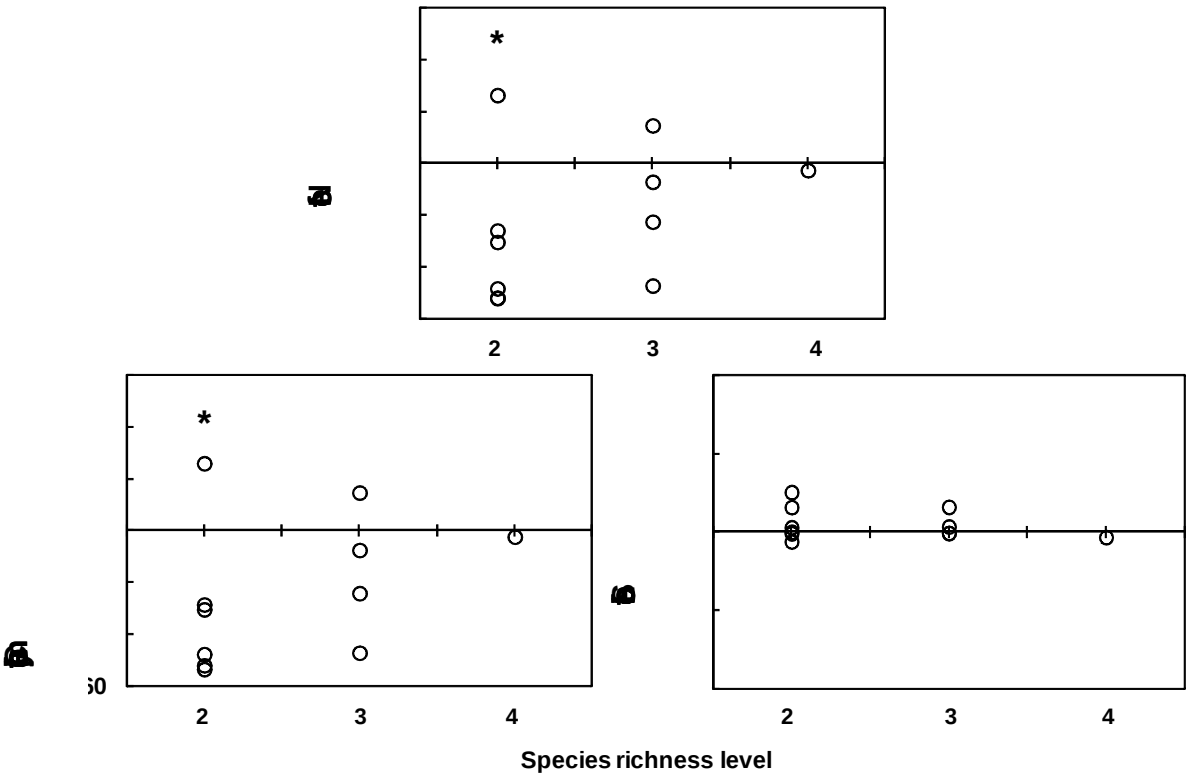


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Figure 3



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