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To cite this version:

Xin Jing, Christel Baum, Bastien Castagneyrol, Nico Eisenhauer, Olga Ferlian, et al.. Leaf isotopes reveal tree diversity effects on the functional responses to the pan-European 2018 summer drought. New Phytologist, 2024, pp.1-17. 10.1111/nph.19931 . hal-04642132

HAL Id: hal-04642132
https://hal.inrae.fr/hal-04642132v1

Submitted on 9 Jul 2024

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New Phytologist

Leaf isotopes reveal tree diversity effects on the functional responses to the pan-European 2018 summer drought

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Received: *19 February 2024*
Accepted: *5 June 2024*

New Phytologist (2024)
doi: 10.1111/nph.19931

Key words: biodiversity, climate change, drought resistance, ecosystem functioning, species richness, stable isotope, TreeDivNet, tree plantations.

Summary

- Recent droughts have strongly impacted forest ecosystems and are projected to increase in frequency, intensity, and duration in the future together with continued warming. While evidence suggests that tree diversity can regulate drought impacts in natural forests, few studies examine whether mixed tree plantations are more resistant to the impacts of severe droughts.
- Using natural variations in leaf carbon (C) and nitrogen (N) isotopic ratios, that is δ^{13}C and δ^{15}N, as proxies for drought response, we analyzed the effects of tree species richness on the functional responses of tree plantations to the pan-European 2018 summer drought in seven European tree diversity experiments.
- We found that leaf δ^{13}C decreased with increasing tree species richness, indicating less drought stress. This effect was not related to drought intensity, nor desiccation tolerance of the tree species. Leaf δ^{15}N increased with drought intensity, indicating a shift toward more open N cycling as water availability diminishes. Additionally, drought intensity was observed to alter the influence of tree species richness on leaf δ^{15}N from weakly negative under low drought intensity to weakly positive under high drought intensity.
- Overall, our findings suggest that dual leaf isotope analysis helps understand the interaction between drought, nutrients, and species richness.

Introduction

Drought occurrences have seen an upward trend alongside climate change and are anticipated to further amplify in terms of frequency, intensity, and duration in the future (IPCC, 2021). Europe has experienced multiple severe drought events since the start of the twenty-first century (Stahl *et al.*, 2016), and warm-season droughts with prolonged soil water scarcity are recognized as the dominant drought type in Europe (Markonis *et al.*, 2021). During the summer of 2018, large parts of the European continent faced a record-breaking severe drought and heat wave (Schuldt *et al.*, 2020). Importantly, during the 2018 summer drought, the air temperature was 3.3°C warmer than the long-term average between 1961 and 1990 (Schuldt *et al.*, 2020). Due to the likelihood that drought and heat wave will occur more frequently as human-caused climate change progresses, there is an urgent need to assess how ecosystems respond to warm-season droughts, which continues to be a primary goal of ecological research (Allen *et al.*, 2010; Doblas-Miranda *et al.*, 2015; Keenan, 2015; Markonis *et al.*, 2021).

Drought impacts on natural forests have been well documented, such as early leaf senescence, canopy dieback, growth reduction, and tree mortality (Schuldt *et al.*, 2020; Schnabel *et al.*, 2022). To better understand how to mitigate drought impacts, and to help adapt management practices to the novel drought conditions, a better understanding of the processes involved is

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required. For example, research has theoretically and experimentally demonstrated that biodiversity not only promotes tree productivity (Scherer-Lorenzen, 2014; Ammer, 2019; van der Plas, 2019) but can also enhance the stability of natural forests in response to droughts (Grossiord, 2020; Schnabel *et al.*, 2021; Hatton *et al.*, 2024). Two nonmutually exclusive processes potentially contribute to the mitigating effects of tree diversity on the functional responses to droughts (Kunert & Mercado, 2015; Grossiord, 2020; Trogisch *et al.*, 2021): selection and complementarity effects. Selection effects can arise from: a stochastic component, suggesting that as species richness increases, there is a greater probability of desiccation-tolerant species being incorporated into the community (Huston, 1997); while the functional component of selection effects suggests that desiccation-tolerant species can become dominant, thereby increasing drought resistance of the community. Conversely, complementarity effects may stem from: niche differentiation, leading to not only reduced resource competition for water in space and time but also more efficient use of resources that are often not evenly distributed across the vertical stratification of roots and canopy; as well as from acilitation, wherein one species experiences positive impacts from the presence of other species, for example, due to hydraulic redistribution (Wright *et al.*, 2017; Grossiord, 2020). Although these processes theoretically suggest positive effects of tree species diversity on forest drought responses, empirical evidence is not conclusive, and only 42% of the empirical studies in a recent review showed positive effects of tree species diversity in mediating drought impacts on forests (Grossiord, 2020). Compared with monocultures, mixed forests are typically more productive and require more water, which could cause the water supply in the soil to run out sooner and leave them more susceptible to severe droughts (Grossiord *et al.*, 2014b; Forrester, 2015). These research findings hold considerable significance, given the growing recognition of forest planting as a pivotal nature-based solution to counteract climate change, rehabilitate degraded land, and sustain biodiversity and ecosystem functioning (Messier *et al.*, 2021).

Nonetheless, it is important to acknowledge that transferring research insights, derived from natural forests, directly into tree plantations might not be straightforward. Plantations typically comprise stands of uniform age, exhibiting reduced structural and genetic diversity. Over time, the impact on biodiversity may evolve and strengthen (Meyer *et al.*, 2016; Huang *et al.*, 2018; Wagg *et al.*, 2022), while susceptibility to drought can also vary with plantation age (Socha *et al.*, 2023). Moreover, differences in drought vulnerability between naturally regenerated and planted forests have been documented (Zadworny *et al.*, 2014). Hence, there is still debate over whether mixed tree plantations are more drought-resistant than monocultures in forest plantations (Kunert & Mercado, 2015; Hajek *et al.*, 2022). Two mechanisms are crucial to define drought resistance, that is desiccation delay and desiccation tolerance (Tyree *et al.*, 2003). The former is associated with plant traits such as deep rooting depth and early stomatal closure, which can improve access to water while decreasing water loss. Desiccation tolerance is associated with plant traits such as leaf relative water content and water potentials, which enable plants to endure desiccation. However, prior research has rarely elucidated the mechanisms behind the drought resistance of tree plantations. Therefore, a better mechanistic understanding of the drought resistance in tree plantations is required to improve the design and management of forest ecosystems in response to droughts (Messier *et al.*, 2021).

Across a broad spectrum of species and environmental conditions, the carbon (C) isotopic ratio ($\delta^{13}C$) in the aboveground tissues is recognized as a reliable indicator to assess drought impacts on plants, because $\delta^{13}C$ is associated with plant water availability (Grossiord *et al.*, 2014b; Bonal *et al.*, 2017; Jucker *et al.*, 2017). For instance, under drought conditions, C_3 plants may close their stomata to prevent desiccation (i.e. desiccation delay). Consequently, the intercellular concentrations of CO_2 will decrease (Grossiord *et al.*, 2014b; Jucker *et al.*, 2017), which results in a greater amount of ^{13}C being fixed, thereby increasing $\delta^{13}C$ in plant tissues (Farquhar *et al.*, 1982, 1989; Arndt & Wanek, 2002; Peuke *et al.*, 2006). With tree diversity mitigating drought impacts due to complementarity or selection effects, increases in $\delta^{13}C$ will be lower in species-rich forests than those in monospecific stands. As a result, a negative relationship between tree species richness and $\delta^{13}C$ should be observed during droughts (Grossiord *et al.*, 2014b; Forrester *et al.*, 2016; Jucker *et al.*, 2017). However, earlier work in mature European forests on plant $\delta^{13}C$ using an approach of dendrochronological analysis has demonstrated inconsistent results, including positive, neutral, or negative effects of tree diversity on desiccation tolerance (Grossiord *et al.*, 2014b; Forrester *et al.*, 2016; Jucker *et al.*, 2017). These studies were performed in natural forest ecosystems, where confounding factors may override potential tree diversity effects. Within the present study, we propose to use observations from tree diversity experiments across a wide range of climatic and environmental conditions using the natural C isotopic ratio. Moreover, such controlled tree diversity experiments provide a unique opportunity to investigate the mechanisms underlying tree diversity effects on the functional response of tree plantations to droughts.

The interaction of droughts with nutrient availability and uptake is important for understanding drought impacts on trees (Gessler *et al.*, 2017). However, patterns of nitrogen (N) isotopic ratio ($\delta^{15}N$) in leaves are often highly variable, since it is affected by many factors, such as changes in N availability, soil microbial activities, distribution of N assimilation between roots and shoots, and N metabolism within the plants (Robinson, 2001). Unlike $\delta^{13}C$ in plant aboveground tissues, the response of leaf $\delta^{15}N$ to droughts is not well understood. Previous literature has shown that plant $\delta^{15}N$ increases with a decline in mean annual precipitation (MAP) (Handley *et al.*, 1999; Scherer-Lorenzen *et al.*, 2003; Craine *et al.*, 2009; Ma *et al.*, 2012). This implies that N cycling in water-limited systems is more open and typically results in higher ^{14}N losses, but a higher ^{15}N retention (Ma *et al.*, 2012). Consequently, there is an enrichment of the soil $\delta^{15}N$ pool, which results in a subsequent increase in plant $\delta^{15}N$ under droughts (but see Peuke *et al.*, 2006; Dudney *et al.*, 2023). These studies, however, did not investigate the effects of tree

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species richness on leaf $\delta^{15}N$, because most of them are centered on individual tree species. Earlier work in grasslands showed that leaf $\delta^{15}N$ is negatively correlated with plant species richness (Spehn *et al*., 2002; Temperton *et al*., 2007; Gubsch *et al*., 2011; Kleinebecker *et al*., 2014). One reason is that these studies often include N-fixing species. It is well recognized that symbiotically fixed N is ^{15}N depleted (Spehn *et al*., 2002; Temperton *et al*., 2007) and that the rate of N-fixation increases with increasing plant diversity due to the selection effect (Huston, 1997). This leads to the depletion of soil $\delta^{15}N$ as well as a decline in leaf $\delta^{15}N$ in highly diverse grassland communities. Furthermore, an increase in plant productivity and N utilization, accompanied by lower levels of soil N losses, may be achieved through increasing plant diversity (Scherer-Lorenzen *et al*., 2003; Zak *et al*., 2003; Lang *et al*., 2013; Chen *et al*., 2020). N lost through denitrification and leaching of inorganic N forms is depleted in ^{15}N, enriching the remaining soil N pool with ^{15}N (Gubsch *et al*., 2011). We would, therefore, expect a negative relationship between plant diversity and leaf $\delta^{15}N$, also in the absence of N-fixing plants. However, since there are no studies that have investigated the simultaneous impacts of species diversity and drought on leaf $\delta^{15}N$, it is unclear how plant diversity mediates drought impacts on leaf $\delta^{15}N$.

Here, we investigated the effects of tree species richness on responses to an extreme drought event with the combined use of leaf C and N isotopic ratios. We took advantage of the pan-European extreme drought between April and October 2018 (Schuldt *et al*., 2020). We followed a climatological definition of extreme droughts, independent of their biological impact (Zwiers *et al*., 2013; Bailey & van de Pol, 2016). We collected leaf samples using seven tree diversity experiments of the TreeDivNet platform (Verheyen *et al*., 2016; Paquette *et al*., 2018) across three European countries (Belgium, France, and Germany; Table 1). The use of the European tree diversity experiments allows attributing effects to differences in research site (between experiments) and differences in tree diversity and composition (within experiments; Materials and Methods section) across a wide range of drought intensities. We measured leaf $\delta^{13}C$ and $\delta^{15}N$ for 28 typical European tree species across the diversity gradients of seven tree diversity experiments. We evaluated the following main hypotheses (Fig. 1):

H1: Tree diversity lowers the need to close the stomata and reduces drought-related increases in leaf $\delta^{13}C$, leading to a negative relationship between tree species richness and $\delta^{13}C$ during droughts (H1a). Both complementarity and selection effects may potentially underlie diversity effects. However, given that all tree species have an equal chance of presence across diversity gradients and isotope composition is measured at the leaf level, the observed diversity effects can be attributed to complementarity effects alone (see Materials and Methods section). Furthermore, since positive species interactions (e.g. facilitation) are commonly observed in conditions of high abiotic stress (Forrester & Bauhus, 2016), we expect that the higher the drought intensity, the stronger the effects of tree species richness on leaf $\delta^{13}C$ become (H1b; Fig. 1b).

H2: In the absence of N-fixing tree species in communities, patterns in leaf $\delta^{15}N$ are likely related to differences in N losses between diversity levels. Because tree diversity mitigates N loss and thus results in lower soil $\delta^{15}N$ and leaf $\delta^{15}N$ values, we expect a negative relationship between tree species richness and leaf $\delta^{15}N$ (H2a; Fig. 1c). Droughts are expected to increase leaf $\delta^{15}N$, but there are no *a priori* expectations on differences in this increase between diversity levels (H2b; Fig. 1d).

Finally, we explored whether species-specific responses are driven by their desiccation tolerance and mycorrhizal association type, as they are expected to mediate interspecific leaf $\delta^{13}C$ and $\delta^{15}N$ patterns (Evans, 2001; Phillips *et al*., 2016; Isaac-Renton *et al*., 2018; Liese *et al*., 2018). We anticipated a positive correlation between desiccation tolerance and the species-specific relationships between species richness and $\delta^{13}C$ in the presence of complementarity effects, or no correlation in their absence (See Supporting Information Fig. S1). Yet, whether arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) associations confer superior drought resistance to their host trees and thus lower $\delta^{13}C$ remains uncertain owing to contrasting results (Mohan *et al*., 2014; Liese *et al*., 2018).

Materials and Methods

Study design

This study uses seven tree diversity experiments of TreeDivNet (https://treedivnet.ugent.be/index.html), a global network of tree diversity experiments focusing on tree species diversity and ecosystem functioning relationships in major forest types around the world (Verheyen *et al*., 2016; Paquette *et al*., 2018). The seven tree diversity experiments are ECOLINK-Salix in Rostock, Germany (Baum *et al*., 2018), MyDiv in Bad Lauchstädt, Germany (Ferlian *et al*., 2018), FORBIO in Hechtel-Eksel, Zedelgem, and Gedinne, Belgium (Verheyen *et al*., 2013), IDENT in Freiburg, Germany (Wein *et al*., 2016), and ORPHEE in Pierroton, France (Castagneyrol *et al*., 2013; see Table 1 for more details). The set of experiments spans more than nine degrees in latitude within the temperate forest biome in Europe. Mean annual temperature (MAT) ranges from 8.3°C at Gedinne to 13.8°C at Pierroton, and MAP ranges from 484 mm at Bad Lauchstädt to 1336 mm at Gedinne. The former land-use types represent typical land-use history including agriculture, heathland converted to pine plantations, forest plantations, and grasslands. Soil types are diverse but are mostly restricted to dry sandy soils (Table 1).

The seven tree diversity experiments were established between 2008 and 2015 (Table 1). The basic experimental design followed a classical synthetic community approach with a replacement series design. In other words, tree species are selected from a common local species pool and are all planted as monocultures and together as polycultures. Plant density is kept constant in both monocultures and polycultures. The levels of tree diversity were replicated using a randomized block design (Table 1). Specifically, the ECOLINK-Salix experiment aims to study the effects of genotype identity and diversity in willow short-rotation coppice on various

Table 1 Site characteristics, experiment design, and sampling information of the seven experiments of the tree diversity network (TreeDivNet).

	ECOLINK	MyDiv	FORBIO	FORBIO	FORBIO	IDENT	ORPHEE
Site characteristics							
Location	Rostock	Bad Lauchstädt	Hechtel-Eksel	Zedelgem	Gedinne	Freiburg	Pierroton
Country	Germany	Germany	Belgium	Belgium	Belgium	Germany	France
Latitude/ Longitude	54°N, 12°E	51°N, 12°E	51°N, 5°E	51°N, 3°E	50°N, 5°E	48°N, 8°E	45°N, −1°E
Elevation (m asl)	13	118	55–56	10–16	367–376, 421–426	278	60
Mean annual temperature (°C)	8.4	8.8	10.2	10.9	8.3	10.4	13.8
Mean annual precipitation (mm)	591	484	887	850	1336	887	944
Former land use	Agriculture	Agriculture	Heathland converted to pine plantation in 1908	Agriculture: crops and grass	Forest: broadleaved until 1920, spruce plantations until 2005	Military training area and barracks until 1945, then grassland with sheep grazing	Forest: *Pinus pinaster* plantation
Soil type	Stagnic cambisol	Haplic Chernozem developed from Loess	Dry sandy soil with gravel substrate	Relatively dry sandy soil to moderately wet loamy sand soil	Moderately dry stony loam soils	Cambisol, loam	Sandy podzol on sandstone bedrock
Experiment information							
Planting year	2014	2015	2012	2009, 2010	2010	2013	2008
Experiment design	Randomized block	Randomized block	Randomized block	Randomized block	Randomized block	Randomized block	Randomized block
Number of blocks	3	2	2	2	2	4	8
Number of plots	9	80	41	42	44	415	256
Plot size (m^2)	92	121	1296	1764	1575, 1764	13	400
Number of trees planted	1296	11 200	23 040	32 810	33 404	19 992	25 600
Level of genetic diversity	1, 2	–	–	–	–	–	–
Level of species richness	–	1, 2, 4	1, 2, 3, 4	1, 2, 3, 4	1, 2, 3, 4	1, 2, 4, 6	1, 2, 3, 4, 5
Size of species pool	2	10	5	5	5	12	5

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Table 1 (Continued)

	ECOLINK	MyDiv	FORBIO	FORBIO	FORBIO	IDENT	ORPHEE
Species pool	*Salix* varieties: *Salix dasyclados* var. 'Loden', *Salix schwerinii* × *Salix viminalis* var. 'Tora'	*Acer pseudoplatanus*, *Aesculus hippocastanum*, *Fraxinus excelsior*, *Prunus avium*, *Sorbus aucuparia*, *Betula pendula*, *Carpinus betulus*, *Fagus sylvatica*, *Quercus petraea*, *Tilia platyphyllos*	*Betula pendula*, *Fagus sylvatica*, *Pinus Sylvestris*, *Quercus robur*, *Tilia cordata*	*Betula pendula*, *Larix kaempferi*, *Pinus Sylvestris*, *Pseudotsuga menziesii*, *Quercus petraea*	*Acer pseudoplatanus*, *Fagus sylvatica*, *Larix eurolepis*, *Pseudotsuga menziesii*, *Quercus petraea*	*Acer platanoides*, *Acer saccharum*, *Betula papyrifera*, *Betula pendula*, *Larix decidua*, *Larix laricina*, *Picea abies*, *Picea pungens* var. *glauca*, *Pinus strobus*, *Pinus sylvestris*, *Quercus robur*, *Quercus rubra*	*Betula pendula*, *Pinus pinaster*, ***Quercus pyrenaica***, *Quercus robur*, ***Quercus ilex***
Sampling information							
Sampling year/month	2018/06	2018/10	2018/08	2018/10	2018/10	2018/08	2018/10
Number of blocks sampled	3	2	2	2	2	4	3
Number of plots sampled	9	79	40	42	43	193	24
Number of samples	12	197	99	102	99	470	44
Mean δ^{13}C ± SE (‰)	−27.1 ± 0.12de	−27.6 ± 0.08e	−28.2 ± 0.07 cd	−30.5 ± 0.09a	−28.2 ± 0.12 cd	−29.1 ± 0.08b	−28.5 ± 0.14c
Mean δ^{15}N ± SE (‰)	−0.55 ± 0.26d	0.22 ± 0.10d	−5.77 ± 0.14a	0.89 ± 0.15e	−4.86 ± 0.13b	1.52 ± 0.06f	−2.48 ± 0.07c

Bold indicates that leaf samples of the tree species were not collected. Different letters indicate significant differences in leaf C and N isotopic ratios among research locations at $P < 0.05$ (the Tukey Honest Significant Differences method was used). The seven experiments are ECOLINK in Rostock, MyDiv in Bad Lauchstädt, FORBIO in Hechtel-Eksel, FORBIO in Zedelgem, FORBIO in Gedinne, IDENT in Freiburg, and ORPHEE in Pierroton (see more details in Materials and Methods section). The research location is sorted from north to south.

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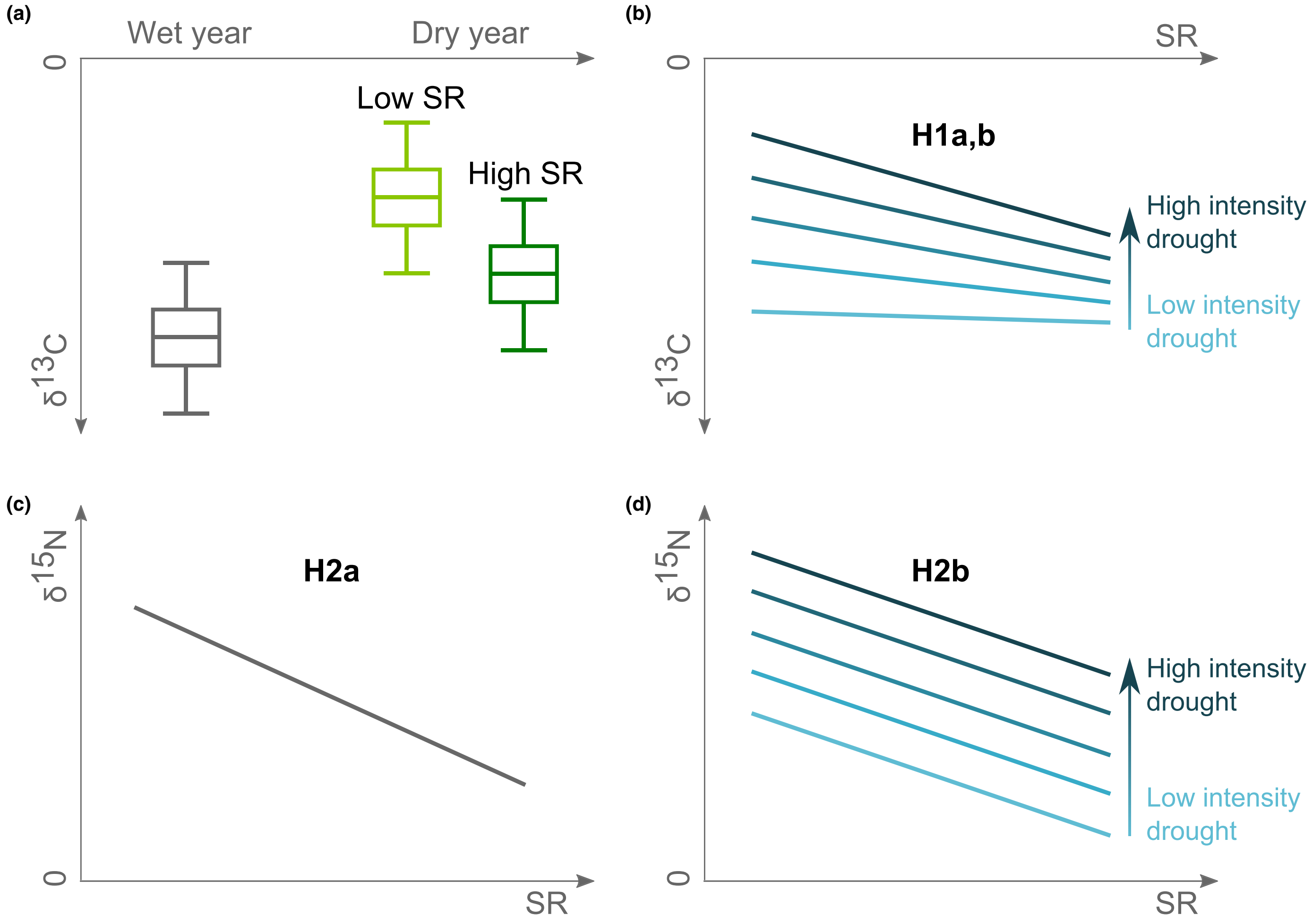

Fig. 1 Conceptual framework illustrating how the relationships between tree species richness (SR) and leaf C and N isotopic ratios are expected to change with climatic conditions. (a) In dry years, drought increases $\delta^{13}C$, while species-rich plantations (High SR) are expected to have lower water stress and lower ^{13}C being fixed, and thus have lower ^{13}C than species-poor (Low SR) plantations. Box plots showcase the distribution of $\delta^{13}C$ in wet years (grey) and in dry years (light green represents Low SR and dark green represents High SR). (b) We expect a negative relationship between tree species richness and $\delta^{13}C$ in dry years (H1a), and this negative relationship might become stronger under more intense drought conditions (H1b). Dark blue lines represent high-intensity droughts and light blue lines represent low-intensity droughts. (c) We expect that species-rich plantations may have lower $\delta^{15}N$ (H2a). (d) Droughts are expected to increase leaf $\delta^{15}N$, but there are no *a priori* expectations on differences in this increase between diversity levels (H2b).

ecosystem functions. At the Rostock site, two Salix varieties (*Salix dasyclados* var. 'Loden' and *S. schwerinii* × *S. viminalis* var. 'Tora') were planted alone or in a mixture in nine plots (each 92 m^2 in size) in three replicate blocks. The MyDiv experiment investigates the significance of mycorrhizas in tree diversity–ecosystem functioning relationships. Ten tree species (five AM species and five ECM species) were planted as monocultures, two- and four-species polycultures in 80 plots (each 121 m^2 in size) distributed over two blocks. The FORBIO experiment examines the effects of tree species diversity on ecosystem functioning at three sites in Hechtel-Eksel, Zedelgem, and Gedinne, Belgium, with contrasting environmental conditions. Five tree species were planted in 127 plots (41, 42, and 44 plots per site respectively, between 1296 and 1764 m^2 in size) with one up to four tree species per plot. The experiment IDENT Freiburg is part of the International Diversity Experiment Network with Trees (IDENT), to study tree diversity effects at early stages after planting by using high-density planting (Wein *et al.*, 2016). Twelve tree species were planted in 415 plots (each 13 m^2 in size) in four blocks with monocultures, two-species, four-species, and six-species polycultures, from which the nonfertilized ones were used here (total of 193 plots). Finally, the ORPHEE experiment in the southwest of France is established to study the effects of climate change on tree diversity and ecosystem functioning relationships. Five tree species were planted in 256 plots (each 400 m^2 in size) in eight blocks with monocultures, two- and five-species polycultures.

Leaf sampling and C and N stable isotope measurement

Leaves were collected between June and October 2018 from each tree species per plot following the protocol of Perez-Harguindeguy *et al.* (2013). In total, 28 different European tree species were sampled (Tables 1, S1). Since initial signs of stress recovery in trees are typically detectable through the regeneration of leaves (Dobbertin, 2005), newly formed leaves (i.e. deciduous and

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evergreen species) and needles (i.e. coniferous species) during the drought were collected to detect the signal of drought. That is, the collected leaves and needles were the youngest ones, unless it was clear that they were formed after the drought. Specifically, leaves were collected using the following criteria: (1) leaves were fully expanded sun leaves or as close to full sun leaves as possible, that is between 2/3 and the top of the living crown; (2) leaves were collected from each of three trees per species in each plot; and (3) a minimum of five leaves and 4 g of fresh leaf/needle materials were collected and pooled into one sample per species per plot. Leaf samples were stored in paper envelopes and oven-dried at 60°C for 48 h in the laboratory to constant mass immediately after collecting and then stored under dry conditions until stable isotope composition analysis.

To detect the effects of tree species richness on tree responses to extreme drought, we measured leaf C and N stable isotopes during the 2018 summer European drought. We focused on leaf isotope composition, rather than plant growth for three reasons. First, isotope composition does not directly connect to plant growth (i.e. C sequestration and allocation to wood) in response to drought (Jucker *et al*., 2017). Second, leaf isotope composition, that is $\delta^{13}C$, is defined by only few and specific leaf-level physiological changes, in particular stomatal conductance, while growth is determined by many more factors, in particular sink limitation during growth (no growth due to hydraulic constraints). This gives then raise to different information contained in the two variables, that is leaf isotope composition and plant growth, with their relationship extremely variable (Timofeeva *et al*., 2017). Third, we wanted to shed more light on the mechanisms driving species diversity effects, and leaf isotope composition gives us more information on what happens in the plant. Leaf samples were first grounded with a ball mill to a fine powder. Next, leaf $\delta^{13}C$ and $\delta^{15}N$ were analyzed at the Stable Isotope Facility of KU Leuven, Leuven, Belgium (except for leaf samples from IDENT Freiburg, which were measured at the Swiss Federal Institute for Forest, Snow and Landscape Research, Forest Dynamics, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland). All samples were combusted in an elemental analyzer at 1020°C and analyzed using a continuous-flow isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). Leaf $\delta^{13}C$ and $\delta^{15}N$ were calculated using the following equations:

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{\text{leaves}}}{\left(\frac{^{13}C}{^{12}C}\right)_{\text{VPDB}}} - 1 \right) \times 1000 \qquad \text{Eqn 1}$$

where $\delta^{13}C$ was expressed as the C isotopic ratio ($^{13}C : {}^{12}C$) of the leaf samples and of the Vienna Pee Dee Belemnite standard (VPDB, ‰).

$$\delta^{15}N = \left(\frac{\left(\frac{^{15}N}{^{14}N}\right)_{\text{leaves}}}{\left(\frac{^{15}N}{^{14}N}\right)_{\text{AIR}}} - 1 \right) \times 1000 \qquad \text{Eqn 2}$$

where $\delta^{15}N$ was expressed as the N isotopic ratio ($^{15}N : {}^{14}N$) of leaf samples and of the International Atomic Energy Agency standard air (AIR, ‰). The precision for the repeated analysis of standard materials was 0.1‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

Tree trait data

To better understand how tree species richness affects leaf C and N isotopic ratios under drought conditions, we compiled data on desiccation tolerance and mycorrhizal type for each tree species. Desiccation tolerance data were compiled from the study of Niinemets & Valladares (2006) based on the rankings of site characteristics and plant physiological potentials for conifers, deciduous and evergreen broad-leaf species. Specifically, the duration of the dry period, the ratio of precipitation to potential evapotranspiration (PET), and the total annual precipitation were among the site characteristics. The physiological potential of plants was the lowest amount of soil water that may be sustained over an extended period without causing more than 50% of leaf damage or plant dieback. Desiccation tolerance was categorized into five categories: (1) very intolerant; (2) intolerant; (3) moderately tolerant; (4) tolerant; and (5) very tolerant. Of the 28 tree species that were examined, four species (*Larix eurolepis* A., *Pinus pinaster* Ait., *S. dasyclados* var. 'Loden', and *S. schwerinii* × *S. viminalis* var. 'Tora') lack desiccation tolerance data and were subsequently excluded from this analysis (Table S2). We classified the 28 tree species into two groups of mycorrhizal types based on the literature (Harley & Harley, 1987). These mycorrhizal types were AM and ECM. Since the roots of Salix varieties may be colonized by AM and ECM fungi, as well as dark-septate endophytes (Baum *et al*., 2018; Huang *et al*., 2018), we subsequently excluded *S. dasyclados* var. 'Loden' and *S. schwerinii* × *S. viminalis* var. 'Tora' from this analysis (Table S3).

Climate data

To test whether climate mediates the effects of tree species richness on leaf C and N isotopic ratios, we compiled climate data for each of the seven study sites. These climate data included the Standardized Precipitation Evapotranspiration Index (SPEI; unitless), MAT (°C), MAP (mm yr^{-1}), and PET (mm). SPEI data were compiled from the SPEI Global Drought Monitor database (Vicente-Serrano *et al*., 2010). The SPEI data were derived based on monthly precipitation and PET at a spatial resolution of 0.5 degrees between 1950 and 2019. We only used the SPEI data throughout the sampling month for statistical analysis, while the SPEI data between 1950 and 2018 were used to visualize and inspect the temporal trends of SPEI (See Figs S2–S4 for more details). MAT, MAP, and PET data were compiled from the gridded agro-meteorological database in Europe, that is AGRI4CAST (http://mars.jrc.ec.europa.eu/mars). The database contains interpolated climate data derived from meteorological observations, remote sensing imagery, and agro-meteorological modeling at a 25 km × 25 km spatial resolution in the form of a daily basis between 1979 and 2020. After an inspection of the correlations between climate variables, we selected MAP and PET to calculate a MAP : PET ratio that was used to define long-term water

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availability in a region as in Jucker *et al.* (2016) for further data analysis.

Soil characteristics data

To test whether soil characteristics mediate the effects of tree species richness on leaf C and N isotopic ratios, we compiled soil characteristics data of each research site from the Land Use and Cover Area frame Statistical Survey (LUCAS) database (Ballabio *et al.*, 2016). These soil characteristics data included soil available water capacity (vol %), bulk density ($\mathrm{T\,m^{-3}}$), topsoil sand proportion (%), topsoil clay proportion (%), and topsoil silt proportion (%) (see Table S4 for details). The LUCAS database was derived using the model of multivariate additive regression splines based on *c.* 20 000 topsoil samples in Europe at 500 m × 500 m spatial resolution. After an inspection of the correlations between these soil properties, we selected soil sand content that was used to estimate soil nutrient and water retention potential as in Ratcliffe *et al.* (2017) for further data analysis.

Statistical analyses

In this study, we developed a two-step analytical approach. First, we examined the general relationship between the isotope composition of leaves across a diversity gradient within specific sites. During severe drought conditions, leaf isotope composition changes because stomata close to avoid desiccation, with the rate of closure increasing with greater exposure to drought. Given consistent climatic and soil conditions within each site, this set-up allows us to identify tree diversity effects on the need for desiccation avoidance and stomatal closure. Two key mechanisms potentially underlie the diversity effects: complementarity effects, driven by niche complementarity and facilitation, mitigate drought stress experienced by individual trees, alongside selection effects that promote the presence and dominance of drought-resistant species as tree diversity increases. However, given that all tree species have an equal chance of presence across diversity gradients, and we sampled leaves from all species present in each plot, our analyses cannot identify selection effects, and the observed diversity effects can be attributed to complementarity effects alone. In a subsequent analysis, we examined whether the species-specific impacts of tree diversity on leaf isotope composition were influenced by their desiccation tolerance (Niinemets & Valladares, 2006). *Pinus sylvestris* L. was an extreme outlier in this analysis, inducing a marginally significant effect (Fig. S5). We therefore report the results excluding this species. Given the acknowledged influence of mycorrhizas on plant drought resistance, we also investigated the correlation between species-specific effects and the mycorrhizal type of the studied trees.

To examine the hypothesized effects of tree species richness on leaf C and N isotopic ratios in the summer drought of 2018, and how these tree diversity effects rely on drought conditions, we separately fitted the linear mixed-effects models for leaf δ^{13}C and δ^{15}N. Fixed effects included tree species richness ($\log_2$ transformed), drought intensity (i.e. SPEI) throughout the sampling month, long-term water availability (MAP : PET ratio) of a site, and soil sand content. To evaluate whether drought intensity influences the effects of tree species richness on leaf isotopes, an interaction term between tree species richness and drought intensity was included. Fixed factors were centered to allow the main effects (tree species richness and drought condition variables) to be biologically explainable (Schielzeth, 2010). Random effects included in the linear mixed-effects models were a block factor nested within a site factor (locality), a species identity factor, and an interaction factor between species identity and species richness. A visual inspection of residual plots was conducted to identify any deviations from a linear form. For the estimated coefficients of fixed factors, we used the bootstrap sample to calculate the 95% confidence intervals. We conducted 500 repetitions of bootstrap samplings for each linear mixed-effects model. A pseudo-R^2 was estimated to determine the marginal coefficient of determination (variance explained by the fixed factors) and the conditional coefficient of determination (variance explained by both the fixed and random factors; Nakagawa *et al.*, 2017). We conducted a sensitivity analysis of linear mixed-effects models by removing the Zedelgem site owing to the lower values of the leaf δ^{13}C, specifically -30.5 ± 0.09‰ (Table S5).

To determine species-specific effects of tree species richness on leaf C and N isotopic ratios in the summer drought of 2018, we conducted general linear models following Hector *et al.* (1999). For each of the 28 tree species (Table S1), leaf δ^{13}C and δ^{15}N were separately regressed on tree species richness ($\log_2$ transformed). If the estimated coefficients (slopes) were positive, the leaf δ^{13}C and δ^{15}N values were higher in polycultures. Instead, if the estimated coefficients (slopes) were negative, the leaf δ^{13}C and δ^{15}N values were higher in monocultures. Experimental sites (i.e. locality) and blocks were also included before the tree species richness term was entered into the general linear models. We, therefore, calculated the total sums of squares explained by tree species richness by accounting for the influences of experimental sites and blocks.

To determine whether tree traits mediate species-specific effects of tree species richness on leaf C and N isotopic ratios, we assessed the associations between the estimated coefficients of general linear models (i.e. the effects of tree species richness on leaf isotopes) and desiccation tolerance and mycorrhizal type, respectively. We computed Pearson correlation coefficients to investigate the relationships between estimated coefficients and desiccation tolerance. A permutation test on correlation was further used to assess the significance of correlation coefficients. We conducted general linear models to assess the effects of mycorrhizal type on the estimated coefficients. A permutation *t*-test was further used to assess the significance of mycorrhizal type effects (Figs S6, S7). All permutation tests were conducted with 9999 simulations.

All statistical analyses were performed in R (v.4.0.3) (R Development Core Team, 2019). We used LME4 package (Bates *et al.*, 2015) for linear mixed-effects models and the TIDYVERSE package (Wickham *et al.*, 2019) for data preprocessing and visualization.

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Results

Effects of tree species richness on leaf δ^{13}C and δ^{15}N

A total of 1023 leaf samples were analyzed from the seven European tree diversity experiments (Table 1). We found that leaf δ^{13}C decreased significantly with tree species richness (Fig. 2a); the estimated coefficient/slope was −0.19 with 95% confidence interval [−0.33, −0.05] (Table 2). The predicted mean leaf δ^{13}C ranged from −28.1‰ (95% confidence interval [−29.2‰, −27.1‰]) in monocultures to −28.6‰ (95% confidence interval [−29.7‰, −27.6‰]) in mixtures of six tree species (Fig. 2a). The effects of tree species richness on leaf δ^{13}C were independent of SPEI, MAP : PET, and soil sand content (all interaction terms were not significant in the linear mixed-effects model, Table 2). Among the random effects assessed, the variability in leaf δ^{13}C was mainly explained by site (38.0%) and species identity (31.5%; Table 2).

Leaf δ^{15}N and tree species richness relationships varied with drought intensity, as represented by the interaction between tree species richness and SPEI in the sampling months (the estimated effects of interaction = −0.22, 95% confidence interval [−0.32, −0.12]; Table 2). Specifically, the predicted mean of leaf δ^{15}N increased with tree species richness under high drought intensity, while it decreased with tree species richness under low drought intensity (Fig. 2b). However, the main effects of tree species richness were negligible (the estimated effects of tree species richness = 0.02, 95% confidence interval [−0.08, 0.13] and the estimated main effects of SPEI = −2.68, 95% confidence interval [−8.68, 3.31]; Table 2). Among the random effects, the variability in leaf δ^{15}N was mainly explained by the site (84.4%), followed by species identity (7.7%; Table 2).

Species-specific effects of tree species richness on leaf δ^{13}C and δ^{15}N

The effects of tree species richness on leaf δ^{13}C varied among species (Fig. 3a,b). After adjusting for the influences of research sites and experimental blocks, nine tree species (32%) out of 28 showed significantly negative effects of tree species richness on leaf δ^{13}C (i.e. no overlap between zero and the 95% confidence interval; Fig. 3a; Table S6). *Quercus robur* L. had the largest negative effect of tree species richness, followed by three coniferous tree species *Pinus strobus* L., *Larix laricina* (Du Roi) K., and *P. sylvestris*. *L. eurolepis* was the only species with significantly positive effects of tree species richness (Fig. 3a). Variance in leaf δ^{13}C explained by tree species richness varied between 0 and 30.1% (Fig. 3b; Table S6).

The effects of tree species richness on leaf δ^{15}N varied considerably among species (Fig. 3c,d). After adjusting for differences in research sites and experimental blocks, *Fagus sylvatica* L. and *Pseudotsuga menziesii* (Mirb.) F. were the only two species, comprising only 7% of the total 28 species evaluated, which exhibited significantly negative effects of tree species richness, while *Picea abies*, *P. strobus*, and *Q. robur* had significantly positive effects of tree species richness, comprising 11% of the species studied (Fig. 3c,d; Table S7).

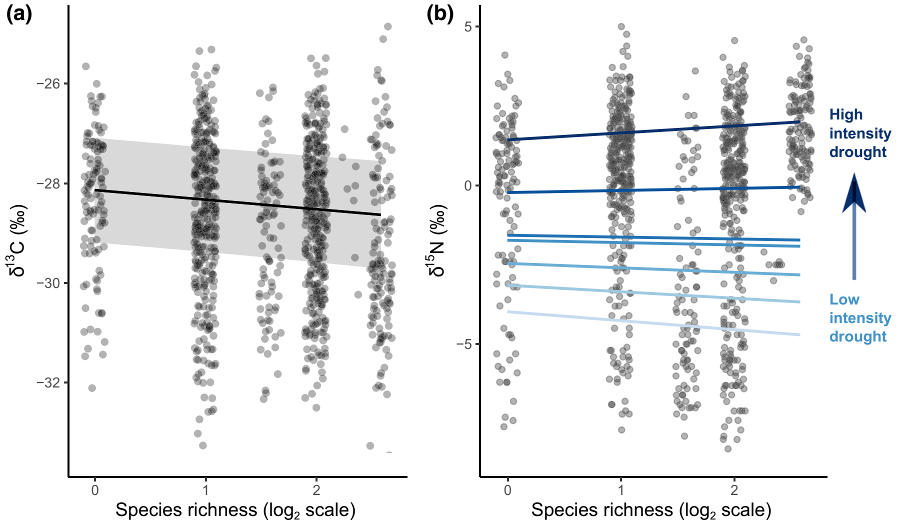

Fig. 2 Effects of tree species richness on leaf δ^{13}C (a) and δ^{15}N (b) in the summer drought of 2018 across the seven European tree diversity experiments. (a) Tree species richness significantly decreases leaf δ^{13}C, while the effect does not vary with drought intensity (i.e. no interaction). The line represents the estimated coefficient, and the band represents the 95% confidence intervals of the linear mixed-effects models. (b) The effect of tree species richness on leaf δ^{15}N varies with drought intensity. The line represents the estimated coefficients. Dark lines represent high-intensity droughts and light lines represent low-intensity droughts. The points in (a, b) are jittered for visualization and represent the predicted values of leaf δ^{13}C and δ^{15}N using the linear mixed-effects models. Drought intensity is represented by the Standardized Precipitation Evapotranspiration Index (SPEI) which is derived from the Global SPEI database for the given sampling month at each tree diversity experiment site (Materials and Methods section). See Table 2 for detailed test statistics and supporting Information Figs S2–S4 for the trend of SPEI at each tree diversity experiment site.

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Table 2 Effects of tree species richness on leaf carbon (C) and nitrogen (N) isotopic ratios in the summer drought of 2018.

	$\delta^{13}C$	$\delta^{15}N$
Fixed effects		
Intercept	**−28.4 [−30.1, −26.8]**[†]	−0.65 [−5.06, 3.76]
Species richness (log$_2$ scale)	**−0.19 [−0.33, −0.05]**	0.02 [−0.08, 0.13]
SPEI	0.14 [−2.14, 2.43]	−2.68 [−8.68, 3.31]
PPET	−1.65 [−11.5, 8.24]	−11.4 [−36.9, 14.2]
Sand	−0.01 [−0.07, 0.05]	−0.01 [−0.17, 0.14]
Richness × SPEI	0.06 [−0.05, 0.17]	**−0.22 [−0.32, −0.12]**
Variance components (VC%)		
Site	1.15 (38.0)	8.99 (84.4)
Block	0.02 (0.6)	0.02 (0.2)
Species identity (Identity)	0.96 (31.5)	0.81 (7.7)
Richness × Identity	0.07 (2.4)	0.02 (0.2)
Residuals	0.84 (27.5)	0.80 (7.5)
Pseudo-R^2[‡]		
Marginal	0.07	0.21
Conditional	0.74	0.94

Fixed effects, variance components of random factors, and pseudo-R^2 are shown for the linear mixed-effects models of leaf $\delta^{13}C$ and $\delta^{15}N$, respectively. Significant fixed terms are shown in bold. Insignificant interaction terms are excluded from the linear mixed-effects models. SPEI denotes the Standardized Precipitation Evapotranspiration Index, PPET denotes MAP : PET ratio, and sand denotes sand content. MAP, mean annual precipitation; PET, potential evapotranspiration.

[†] Estimated coefficients of the linear mixed-effects models with 95% confidence interval.

[‡] Marginal R^2 represents model variation explained by the fixed factors, and conditional R^2 represents model variation explained by the fixed and random factors.

Associations between species-specific effects of tree species richness on leaf isotopes and desiccation tolerance and mycorrhizal type

We found that desiccation tolerance did not mediate the species-specific effects of tree species richness on leaf $\delta^{13}C$ (Fig. 4a; Pearson correlation coefficient $r = -0.28$, P-value = 0.211, P-value of the permutation test = 0.208). Similarly, species-specific effects of tree species richness on leaf $\delta^{15}N$ were not significantly associated with desiccation tolerance (Fig. 4b; $r = -0.38$, P-value = 0.078, P-value of the permutation test = 0.078). Species-specific effects of tree species richness on leaf $\delta^{13}C$ and $\delta^{15}N$ were not mediated by mycorrhizal type (Figs 4c,d, S6, S7).

Discussion

The seven research sites of tree diversity experiments experienced an extreme natural drought event in 2018. This occurrence offers a rare chance to evaluate whether and how the impacts of extreme drought on leaf C and N isotopic ratios can be mediated by tree species richness. We show a generally negative relationship between tree species richness and leaf $\delta^{13}C$ across the seven tree diversity experiments (Fig. 2). We further show that the effects of tree species richness on leaf $\delta^{13}C$ vary depending on the tree species examined but found no evidence that they are modulated by their desiccation tolerance (Figs 3, 4). Although an overall relationship between tree species richness and leaf $\delta^{15}N$ did not become evident, our work demonstrates that this relationship is modulated by drought intensity. The relationship varies from weakly negative under low-intensity droughts to weakly positive under high-intensity droughts (Fig. 2).

Effects of tree species richness on leaf C isotopic ratio under drought conditions

Increases in C isotope values are frequently observed during droughts (Sarris *et al.*, 2013; Jucker *et al.*, 2017; Siegwolf *et al.*, 2023). Our work shows a significantly negative relationship between tree species richness and leaf $\delta^{13}C$ under drought conditions (Fig. 2), indicating that the drought-induced increase in leaf $\delta^{13}C$ is smaller in species-rich tree plantations than in species-poor tree plantations. This result is in line with our hypothesis H1a and is supported by previous research that often found negative effects of tree species richness on C isotopic ratio using stable C isotope values from tree rings rather than leaves (Grossiord *et al.*, 2014b; Forrester *et al.*, 2016). As species-rich forests and tree plantations usually show better resource partitioning and facilitation (e.g. hydraulic lift of deeper soil water by tree species with deeper rooting depth) than species-poor ones (Grossiord, 2020), this reduces water stress and, consequently, lowers the rise in $\delta^{13}C$ values during drought events (but see the exceptions in Grossiord *et al.*, 2014b; Forrester *et al.*, 2016). Importantly, we found that tree species richness decreased leaf $\delta^{13}C$ by 0.5‰ from monocultures to six tree species plots. Grossiord *et al.* (2014b) reported that, on average across a wide range of European forests, droughts increased tree ring $\delta^{13}C$ from 0.55‰ to 1.11‰ (i.e. the differences in tree ring $\delta^{13}C$ between a dry year and a wet year). Our result, therefore, suggests that tree species richness may completely or partially mitigate the impacts of drought on $\delta^{13}C$. These findings highlight that biodiversity is an important ecosystem property that can promote forest drought resistance (Sousa-Silva *et al.*, 2018; Grossiord, 2020).

When delving into the species-specific relationships, we found considerable variation in the slopes between tree diversity and $\delta^{13}C$. Only nine of the 28 tree species (*c.* 32%) have significantly lower leaf $\delta^{13}C$ values in species-rich tree plantations than in species-poor ones (Fig. 3). We even found a positive slope for *L. eurolepis*, indicating that this species is more water-stressed in species-rich tree plantations than in species-poor ones. *L. eurolepis* is among the fastest-growing tree species in the species pool. Its higher observed drought stress when mixed with other tree species could be related to the corresponding reduced belowground competition due to facilitation and resource partitioning among species (Grossiord, 2020), which in turn might have resulted in a proportionally lower investment in the root system. However, *L. eurolepis* has only been planted in one experiment (FORBIO-Gedinne in Belgium; Table S1), so results must be interpreted with caution. Contrary to our expectations under the presence of complementarity effects, the species-specific effects of tree species richness on leaf $\delta^{13}C$ did not tend to change with species desiccation tolerance (Fig. 4). Although our

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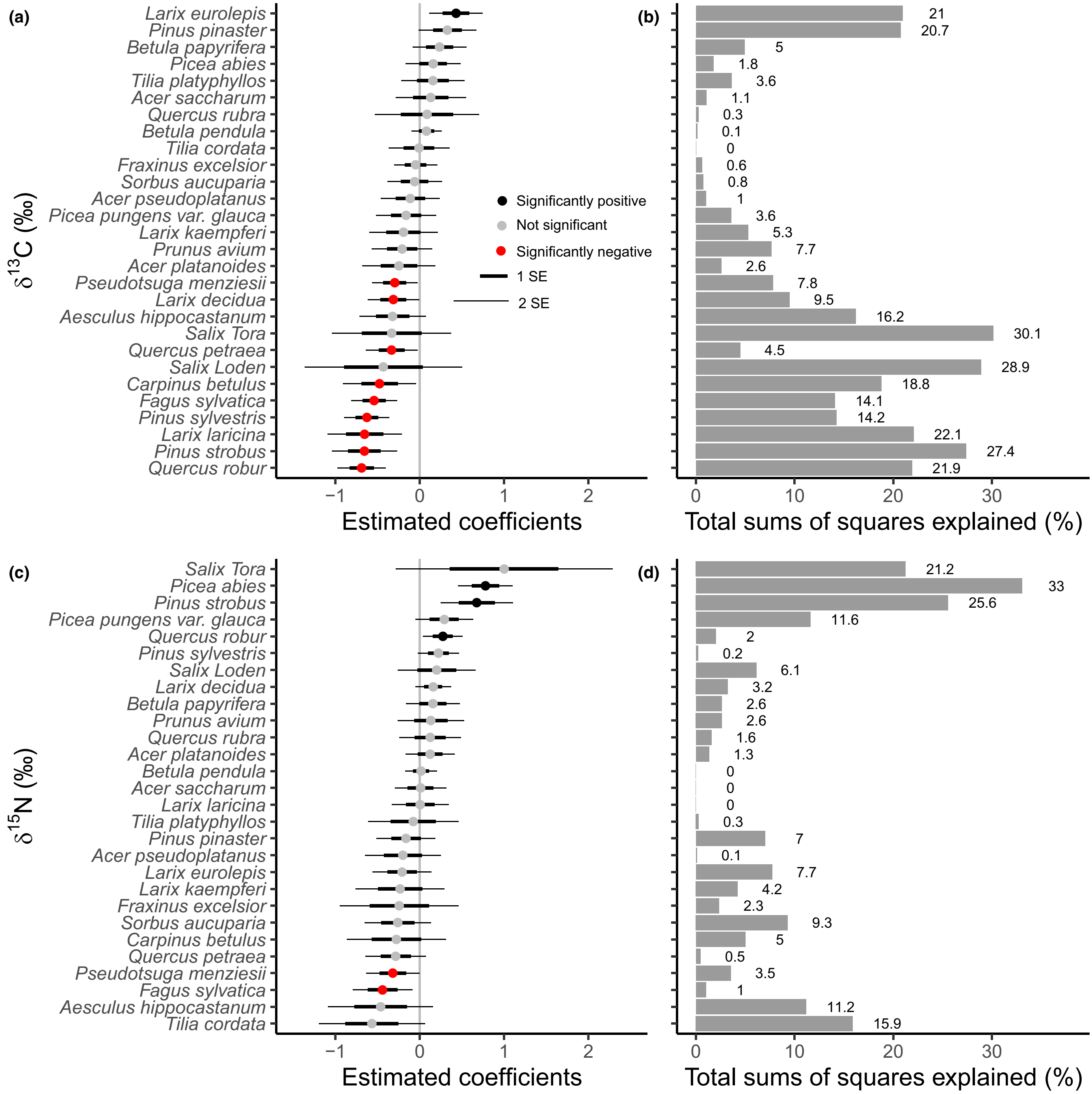

Fig. 3 Species-specific effects of tree species richness on leaf δ^{13}C (a, b) and δ^{15}N (c, d) in the summer drought of 2018 across the seven European tree diversity experiments. Species-specific effects of tree species richness are indicated by the estimated coefficients/slopes of general linear models. Tree species are ranked by the estimated coefficients/slopes of leaf δ^{13}C (a) or δ^{15}N (c) that show the magnitude of species-specific effects of tree species richness on leaf δ^{13}C and δ^{15}N. Black points represent significantly positive coefficients, red points, significantly negative coefficients, and grey points, nonsignificant coefficients. Thick error bars represent the estimated coefficients ±1 SE, and thin error bars represent the estimated coefficients ±2 SE (i.e. 95% CI). The thin bar does not overlap zero which is considered a significant coefficient. The bars and numbers in (b, d) represent the total variance (sums of squares) explained by tree species richness after adjusting for differences in experimental sites and blocks. See Supporting Information Tables S6 and S7 for detailed test statistics.

methodology may not have been capable of detecting selection effects, this outcome implies that the presence of such selection effects associated with drought resistance is improbable. This might be driven by the maladaptation of the studied forest plantations to drought events as rare and extreme as the 2018 pan-European drought.

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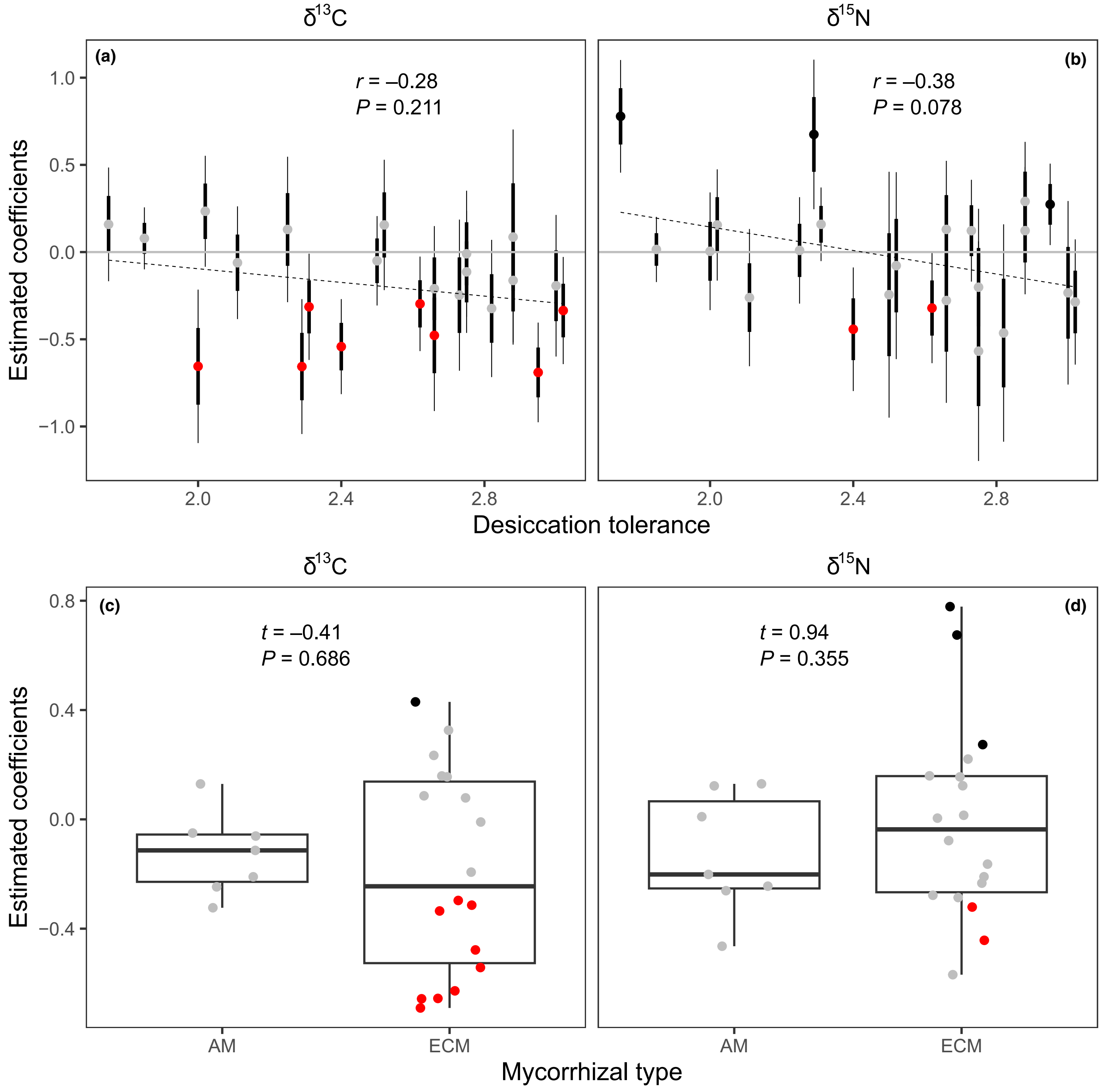

Fig. 4 Species-specific effects of tree species richness on leaf $\delta^{13}C$ (a, c) and $\delta^{15}N$ (b, d) as a function of desiccation tolerance (a, b) and mycorrhizal type (c, d). Species-specific effects of tree species richness are indicated by the estimated coefficients/slopes of general linear models. (a, b) Associations between species-specific effects of tree species richness and desiccation tolerance. Pearson correlation coefficients and the associated *P*-values are shown. Dashed fitted line represents a nonsignificant association. Thick error bars represent the estimated coefficients ±1 SE, and thin error bars represent the estimated coefficients ±2 SE. (c, d) Associations between species-specific effects of tree species richness and mycorrhizal type. The *t*-values of general linear models and the associated *P*-values are shown. AM represents arbuscular mycorrhiza and ECM represents ectomycorrhiza. Black points represent significantly positive coefficients, red points significantly negative coefficients, and grey points nonsignificant coefficients.

Environmental conditions, especially climatic conditions, are known to modulate biodiversity and ecosystem functioning relationships (Jucker *et al.*, 2016; Ratcliffe *et al.*, 2017; Fei *et al.*, 2018; Eisenhauer *et al.*, 2019; Jing *et al.*, 2022). Indeed, context dependency in tree diversity effects on $\delta^{13}C$ is regularly observed in the literature. For example, Grossiord *et al.* (2014b) observed a negative relationship between tree species diversity and tree ring $\delta^{13}C$ in two major European forest types (i.e.

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temperate beech and thermophilous deciduous), a positive one in boreal forests (Grossiord *et al.*, 2014a), while three other forest types (hemi-boreal, mountainous beech, and Mediterranean) did not show significant tree species diversity effects. Similarly, Forrester *et al.* (2016) observed two negative, one positive, and three neutral effects of tree diversity on tree ring $\delta^{13}C$. However, unlike these previous studies, our work focuses on leaf $\delta^{13}C$, while the others studied $\delta^{13}C$ in woody tissues (Arndt & Wanek, 2002; Grossiord *et al.*, 2014b; Forrester *et al.*, 2016). Leaf $\delta^{13}C$ is highly associated with the processes of stomatal regulation of stable C isotope discrimination during C fixation (Farquhar *et al.*, 1982, 1989) and can be immediately affected by droughts (Arndt & Wanek, 2002; Siegwolf *et al.*, 2023). On the contrary, it may take time to incorporate photosynthetically fixed $\delta^{13}C$ into woody tissues with multiple C allocation processes involved (Arndt & Wanek, 2002). Additionally, most tree ring growth occurs during the spring, and therefore, the subsequent tree ring stable C isotope signal may not accurately represent a period of dry and hot summer climate with very little tree ring growth (Sarris *et al.*, 2013; Jucker *et al.*, 2017; Siegwolf *et al.*, 2023). Our work shows that the negative effects of tree species richness on leaf $\delta^{13}C$ are present across the seven tree diversity experiments, irrespective of the variation in drought intensity during the sampling months across sites, as well as the long-term water availability and soil water retention capacity at the study sites (Table 2). These results do not support our hypothesis that higher drought intensity increases the effects of tree species richness on leaf $\delta^{13}C$ (i.e. stress gradient hypothesis; H1b). This underscores the likelihood that the mechanisms driving these effects may differ significantly between sites (Fig. S8; Tables S8, S9; Grossiord *et al.*, 2014b). Future research could aim to more precisely discern the relative significance of resource partitioning and facilitation, among other mechanisms not addressed in this study, including competition, species dominance, neighbor identity, and environmental filtering (Grossiord, 2020).

Effects of tree species richness on leaf N isotopic ratio under drought conditions

The overall effect of tree species richness on leaf $\delta^{15}N$ under the 2018 summer drought is not significant. This result does not support our hypothesis that there is a negative relationship between tree species richness and leaf $\delta^{15}N$ (H2a), driven by lower soil N losses in high-diversity plots (Zak *et al.*, 2003; Lang *et al.*, 2013; Chen *et al.*, 2020). Zooming in on each of the 28 tree species examined, we found no tree species richness effects on leaf $\delta^{15}N$ for 82% of tree species (Fig. 3). Species-specific results were not found to be mediated by the type of mycorrhizal association (Fig. 4).

We found that experimental sites accounted for 84.2% of the variability in leaf $\delta^{15}N$ (Table 2), implying that the relationship between tree species richness and leaf $\delta^{15}N$ is highly context-dependent. Such strong context-dependency effects on biodiversity–ecosystem functioning relationships have been reported in forest ecosystems before (Jucker *et al.*, 2016; Ratcliffe *et al.*, 2017; Fei *et al.*, 2018; Mori, 2018). Moreover, although the main effect of tree species richness on leaf $\delta^{15}N$ was not significant, we highlight the role of drought intensity in shaping context dependency. Specifically, we observed a significantly negative interaction between species richness and drought intensity. The marginal effects plot, incorporating both main and interaction effects (Fig. 2b), illustrates that leaf $\delta^{15}N$ increases with drought intensity, partially aligning with our hypothesis H2b that droughts elevate leaf $\delta^{15}N$ and indicating a transition toward more open N cycling as water availability declines. Furthermore, we demonstrate that severe droughts may counterbalance the adverse effects of tree species richness, resulting in a weakened negative association between tree species richness and leaf $\delta^{15}N$ under heightened drought intensity. We found a slightly negative relationship between tree species richness and leaf $\delta^{15}N$ under low drought intensity, while under higher drought intensity the relationship becomes slightly positive. Hence, not only the magnitude of tree species richness effects on leaf $\delta^{15}N$ changes with drought intensity, but also the direction of tree diversity effects. Future research is necessary to further reveal the mechanisms driving the interaction between drought intensity and species richness, offering insights into N cycling under extreme droughts.

Conclusion

Considering the increasing frequency of droughts due to climate change (IPCC, 2021), and the need to design resilient tree plantations capable of providing diverse ecosystem services even during extreme drought conditions (Messier *et al.*, 2021), understanding how tree diversity influences ecosystem responses to severe droughts could enable mitigation of their impacts on tree plantations. In our study, we present clear evidence that drought directly contributes to an increase in leaf $\delta^{13}C$, while tree species richness decreases leaf $\delta^{13}C$. The negative relationship between tree species richness and leaf $\delta^{13}C$ is likely driven by complementarity effects. Furthermore, we found that the species-specific effects of tree species richness on leaf $\delta^{13}C$ did not vary with species desiccation tolerance, suggesting that the presence of drought-related selection effects in the studied forest plantations is improbable. Unlike leaf C isotopic ratio, the effect of tree species richness on leaf $\delta^{15}N$ depends on drought intensity, with an increasingly positive effect observed under high drought intensity. Thus, our findings suggest that dual leaf isotope analysis aids in comprehending the relationship among species richness, nutrients, and drought. Our work holds significant relevance for the management of tree plantations, with more diverse plantations being more resilient – and thus economically more attractive – under ongoing climate change with more frequent droughts. However, given the considerable variation in the observed impact of tree species richness on leaf $\delta^{13}C$ across different sites, further research is required to clarify the relative significance of resource partitioning and facilitation, among other mechanisms not addressed in this study. It would also be of interest for future research to broaden the framework for better understanding how tree species interact with each other in response to extreme climate events across varying environmental gradients.

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Acknowledgements

The authors thank site managers, field technicians, and student helpers for establishing and managing the TreeDivNet experiments and for collecting the leaf samples. We thank Marijn Bauters and Pascal Boeckx for the discussion about the interpretation of the results of the nitrogen isotope. We thank Margaux Casier for the help in compiling data on desiccation tolerance. We thank the team of the Stable Isotope Research Centre (SIRC) at WSL Birmensdorf for support in the laboratory. The research was supported by the Internal Funds of the KU Leuven (XJ). CB acknowledges funding from the German Research Foundation (DFG, project no.: BA 1494/9-1). NE and OF acknowledge the support from the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig funded by the German Research Foundation (DFG– FZT 118, 202548816) and funding by the DFG (Ei 862/29-1 and Ei 862/31-1). LR, PH, CAN, and MSL acknowledge funding by the German Research Foundation (DFG 384026712 to LR; DFG 316733524 to CAN) and by the University of Freiburg (Innovationsfonds Forschung to MSL). The maintenance of the FORBIO-Gedinne site is partly supported by the 5-yr research program 'Accord-cadre de recherches et de vulgarisation forestières' funded by the Walloon Forest Service (SPW-DNF). The research presented here contributes to the Global Network of Tree Diversity Experiments (TreeDivNet; https://treedivnet.ugent.be/).

Competing interests

None declared.

Author contributions

KVM, BM, and KV conceived of the study. XJ and KVM led the analyses and writing of the manuscript. All the authors contributed substantially to the result interpretation and manuscript revision.

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Data availability

The data for replication of the results in this work are openly available in Tables S2, S3, S6, S7, and S10.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Hypothesis on the relationship between desiccation tolerance of tree species and their species-specific relationship between species richness and leaf δ^{13}C.

Fig. S2 Pattern of the Standardized Precipitation Evapotranspiration Index (SPEI) at the tree diversity experimental sites between 1950 and 2019.

Fig. S3 Changes in the Standardized Precipitation Evapotranspiration Index (SPEI) in 2018 across the studied regions.

Fig. S4 Trends of the Standardized Precipitation Evapotranspiration Index (SPEI) in 2008 across the tree diversity experiments.

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Fig. S5 A sensitivity analysis on the species-specific effects of tree species richness on leaf $\delta^{13}C$ and leaf $\delta^{15}N$ as a function of desiccation tolerance when including *Pinus sylvestris*.

Fig. S6 Permutation *t*-test for the associations of species-specific effects of tree species richness on leaf $\delta^{13}C$ as a function of mycorrhizal type.

Fig. S7 Permutation *t*-test for the associations of species-specific effects of tree species richness on leaf $\delta^{15}N$ as a function of mycorrhizal type.

Fig. S8 The effects of tree species richness on leaf $\delta^{13}C$ vary with sites.

Table S1 Number of leaf samples measured in the laboratory per species per location.

Table S2 Desiccation tolerance of the tree species examined in this study.

Table S3 Mycorrhizal types of the tree species examined in this study.

Table S4 Soil characteristics of the research site.

Table S5 Sensitivity analysis for the effects of tree species richness on leaf C and N isotopic ratios in the summer drought of 2018 by removing the Zedelgem site.

Table S6 Species-specific relationships between tree species richness and leaf $\delta^{13}C$ in the summer drought of 2018.

Table S7 Species-specific relationships between tree species richness and lead $\delta^{15}N$ in the summer drought of 2018.

Table S8 Effects of tree species richness on leaf $\delta^{13}C$ across sites.

Table S9 Effects of tree species richness on leaf $\delta^{13}C$ across sites and species.

Table S10 Leaf isotope data for replication of the results in this work.

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