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Research article

Ectomycorrhizal fungi and root water uptake respond independently to water availability

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Temperate forests on their warm and dry distribution limits are expected to be most vulnerable to reductions in water availability. This prediction is mostly based on studies assessing single forest functions, mainly growth. Water and nutrient cycling are functions that rely on tree roots and their symbiotic association with ectomycorrhizal (ECM) fungi. Trees can compensate for seasonal reductions in water availability by shifting root water-uptake (RWU) towards deeper soil layers, but ECM fungi mostly dwell in the topsoil, thus suffering from desiccation and compromising nutrient uptake. We hypothesised that drier sites should depict larger seasonal shifts in RWU, but at the expense of lower ECM fungal diversity and colonization of fine roots by ECM fungi. We selected three beech *Fagus sylvatica* forests in their warm distribution limit with contrasting geographic locations and mean annual precipitation: northern Atlantic (2500 mm), intermediate transitional (1150 mm) and southern Mediterranean (780 mm). We collected soil, stem and root samples in spring (wet) and summer (dry) to: 1) quantify fine-root density and colonization by ECM fungi, 2) infer RWU from isotopic composition of plant and soil water and 3) characterize ECM communities through DNA-metabarcoding. Generalized and linear mixed models revealed that high topsoil moisture benefited ECM diversity, but higher diversity and ECM colonization did not imply larger contributions of the topsoil to RWU. The prevailing climate and abiotic conditions determined how ECM communities were structured, more than seasonal climatic variability. Across sites, communities differed in their functional diversity: ECM fungi with long hyphae, more vulnerable to water scarcity, dominated at the southernmost site, where water availability was the highest. Our results suggest that, in a climate change scenario, increasing drought might not

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compromise RWU, but it would still be detrimental for ECM communities, compromising key ecosystem services such as nutrient cycling and productivity.

Keywords: community composition, drought, *Fagus sylvatica*, root, water isotopes

Introduction

Climate change is increasing the frequency and severity of drought episodes worldwide (Dai 2013). Drought risk is now extending to regions not considered water limited, including European temperate forests. In the past two decades, European forests have suffered multiple severe droughts, with detrimental impacts for forest health and productivity (Ciais et al. 2005, Camarero et al. 2015, Senf et al. 2018, Schuldt et al. 2020, Treydte et al. 2023). European beech *Fagus sylvatica* forests, which are widely distributed over the continent, constitute an iconic example of this drought-related decline (Geßler et al. 2007, Leuschner 2020). Numerous studies have shown that drought has detrimental effects on beech performance, mainly based on decreased radial growth, but also on canopy vigour or on ecophysiological performance (Zimmermann et al. 2015, Serra-Maluquer et al. 2019, Martinez del Castillo et al. 2022, Mazza et al. 2024). This drought sensitivity suggests that, under climate change, the distribution range of beech could contract along its southern and low elevation limits. Evidence for such range contraction and lower performance along the drier and warmer limits has been found for various beech forests (Peñuelas and Boada 2003, Rozas et al. 2015, Martinez del Castillo et al. 2022). In contrast, similar studies have shown that marginal beech populations or those inhabiting their rear-edge are not necessarily more drought sensitive (Weber et al. 2013, Vilà-Cabrera and Jump 2019, Bert et al. 2022). Most of these studies based their predictions on a single aboveground forest function, radial growth. Yet, drought and climate change impact all forest functions, including not only those aboveground, but also belowground.

Ectomycorrhizae, symbiotic associations between tree roots and fungi, are crucial belowground interactions for ecosystem functioning (Van der Heijden et al. 2015, Tedersoo et al. 2020). In this association, the ectomycorrhizal (ECM) fungi (mainly from the phyla Basidiomycota and Ascomycota) form a hyphal mantle around the tree roots, enhancing nutrient and water uptake and providing pathogen resistance for their host in exchange for carbohydrates (Smith and Read 2008, Rasmann et al. 2017). ECM fungal hyphae improve nutrient uptake by exudating enzymes, organic acids and other compounds that mobilize N and P locked in the soil organic matter or that help weather mineral surfaces (Finlay 2008). ECM fungi should also enhance water uptake by increasing the soil volume explored through the extension of their hyphal mantle. However, the importance of ECM fungi in plant water uptake depends on their functional traits, particularly exploration type (Navarro-Fernández et al. 2016, Prieto et al. 2016, Johnson 2018). The pattern and extent of the hyphal network (short contact versus long exploration types) is a morphological trait that is also likely useful to determine the soil exploration capacity (Agerer 2001), and hence it would have crucial implications for the ECM fungal community structure and functioning (Koide et al. 2014). At the same time, factors like climate, past land use, soil pH or nutrient levels strongly influence the structure and composition of the ECM fungal community (Querejeta et al. 2009b, Erlandson et al. 2016, Reis et al. 2018, Van der Linde et al. 2018, Correia et al. 2021, Rodríguez-Uña et al. 2024). Water availability, in particular, is a key factor determining ECM fungal survival and abundance, as ECM communities that experience recurrent droughts tend to converge with a few closely related fungal taxa becoming dominant (Gehring et al. 2020). The hyphae of ECM fungi are particularly sensitive to water availability because they dwell mainly in the topsoil (Neville et al. 2002), they lack regulatory mechanisms to control for water loss and suffer indirectly from the effects of drought on their host (Brunner et al. 2015, Gehring et al. 2017).

At the onset of drought, plants deploy various mechanisms to preserve the integrity of their hydraulic system (Magnani et al. 2002, Brunner et al. 2015). For example, closing stomata or taking advantage of fine roots as hydraulic fuses that decouple the plant from the drying soil (Jackson et al. 2000). To activate this latter mechanism, lacunae (air gaps) form in the cortical cells of fine roots, triggering loss of conductivity (Cuneo et al. 2016), but this inevitably affects their associated ECM fungi (Gehring et al. 2017). Drought also impairs photosynthesis and phloem transport, and hence reduces the amount and type of carbohydrates trees export to their fungal partners (Shi et al. 2002, Ruehr et al. 2009, Köhler et al. 2018). Overall, drought decreases the abundance, diversity, biomass and enzymatic activity of ECM fungi (Gehring et al. 2017, Nickel et al. 2018), inducing shifts in community composition, mainly by increasing the relative abundance of drought-tolerant species (Shi et al. 2002, Gehring et al. 2014). For example, drought may drive a shift in the ECM fungal community towards a higher portion of ECM associations with fungal partners with a lower C-demand, i.e. contact- and short-exploration ECM fungal types (Fernandez et al. 2017, Castaño et al. 2018). In contrast, several studies have reported that ECM symbiosis enhances drought tolerance by increasing water absorbing surface area; hence, under drought, ECM networks formed by fungi with long emanating tissues (i.e. medium- and long-exploration ECM fungal types) should be favoured, although these are generally more C-demanding (Allen and Kitajima 2014, Brunner et al. 2015, Köhler et al. 2018, Nickel et al. 2018, Leuschner 2020). It is thus unclear which ECM functional

types would be most resistant and beneficial for tree survival and growth under low water availability, as well as the extent of the contribution of ECM fungi to root water uptake.

Inferring tree root water uptake is challenging mainly due to the uncertainty of the distribution of live fine roots along the depth profile. Furthermore, traditional methods using root biomass distributions may not even accurately represent water uptake patterns (Mazzacavallo and Kulmatiski 2015). Concurrent analyses of the isotopic composition of tree water and its potential sources, mainly the different soil layers, is a powerful method to infer root water uptake, while causing minimal disturbances to both the trees and the soil (Ceperley et al. 2024). The rationale behind this approach is that the soil is the main source of water for vegetation uptake and water stored in different soil layers can be distinguished based on differences in their isotopic composition, that is the ratios of the rarer and heavier isotopes to the lighter and more abundant ones: $^{2}H/^{1}H$ and $^{18}O/^{16}O$, usually reported using the δ notation. Differences in water isotopic composition among soil layers are caused by evaporation enrichment, which affects mainly the upper soil layers, and by the seasonal differences in incoming precipitation, as deeper soil layers usually carry the isotopic signature of the lighter winter and autumn precipitation (Sprenger et al. 2016). During root water uptake, typically, no isotopic fractionation occurs, therefore the isotopic composition of the water that flows through the xylem vessels should reflect that of its sources (Dawson and Ehleringer 1991). Apparent isotopic fractionation resulting from methodological artefacts can bias inference on water uptake from isotopic analyses (Barbeta et al. 2019, 2022, Chen et al. 2020), but these apparent biases are consistent within species and can be corrected (Duvert et al. 2022). In temperate forests, including beech forests, analyses of water isotopes have shown that trees would increase the proportion of water taken up from deeper soil layers as topsoil water decreases (Brinkmann et al. 2019, Gessler et al. 2022). These findings suggest that beech water uptake could exhibit certain plasticity in response to drought. However, although this plasticity could contribute to resist transient drought periods, in the mid- to long-term, reduced topsoil moisture and water uptake from this layer could compromise nutrient uptake (Querejeta et al. 2021a). Nutrients concentrate in the topsoil (Jobbágy and Jackson 2001) as do ECM fungi (Neville et al. 2002). Thus, although reductions in topsoil water could be compensated with shifts in water uptake towards deep soil layers, reduced water uptake from the topsoil might impact negatively on nutrient uptake and on the activity and functionality of the ECM fungal community.

Here, we assess how seasonal and geographic variations in water uptake are connected to changes in the ECM fungal community, along a geographic gradient at the southernmost distribution limit of *F. sylvatica*, in the Iberian Peninsula. We aimed to answer three questions: 1) how do seasonal water uptake patterns vary across a geographic gradient? We expected a greater shift in relative water uptake from upper to deeper soil layers, from spring to summer at sites with lower water availability. 2) How do seasonal and geographical variability affect ECM fungal communities? We hypothesized that at sites with lower water availability, ECM fungal colonization rates and diversity would decrease more from spring to summer. 3) Are ECM fungal diversity and community structure associated with water uptake patterns? We hypothesized that the seasonal shift of water uptake towards deeper soil layers will result in less abundant and diverse ECM fungal communities.

Material and methods

Study sites

We conducted our study in the north of Spain, where European beech reaches its southern distribution limit (except for some small isolated populations in Sicily, Italy, and central and coastal Spain, Leuschner 2020). In this area, we selected three mature monospecific beech forests along a geographic gradient (Table 1). The climate in the three selected sites varied markedly (Table 1, Supporting information). The northernmost and rainiest site, Artikutza (Navarra, Spain, hereafter 'Northern site'), was the closest to the coast and experienced a temperate oceanic climate with a mean annual precipitation of 2488.1 ± 62.1 mm and a mean annual temperature of 15.5 ± 1.2°C (mean ± SE, 1970–2015, according to the closest meteorological station of the Regional Basque Meteorological Agency, Euskalmet). The second site, Iturrieta (Álava, Spain, hereafter 'Intermediate site'), experienced a predominantly Atlantic climate with a mean annual precipitation of 1151.4 ± 64.4 mm and mean annual temperature of 13.3 ± 1.5°C (2001–2015, Euskalmet). The southernmost and most continental site, Diustes (Soria, Spain, hereafter 'Southern site') experienced a Mediterranean climate with a mean annual precipitation of 779.4 ± 20.5 mm and mean annual temperature of 14.1

Table 1. Location, mean (± SE) age and size (diameter at 1.3 m height, DBH) of sampled trees and mean climatic values of the three study sites in northern Spain: Northern, (Artikutza, Navarra), Intermediate (Iturrieta, Álava) and Southern (Diustes, Soria). Climatic values are: mean annual precipitation (MAP) and temperature (MAT), mean monthly precipitation of the driest (P_{dry}) and wettest (P_{wet}) months, mean monthly temperature of the coldest (T_{cold}) and hottest (T_{hot}) months. Climatic data provided by the Basque Regional Meteorological Agency (Euskalmet, for the Northern and Intermediate sites) and by the Spanish Meteorological Agency (AEMET, for the Southern site).

Site	Northern	Intermediate	Southern
Latitude (N)	43°12'50.4"	42°47'20.8"	42°06'12.9"
Longitude (W)	1°47'47.5"	2°19'34.0"	2°24'15.2"
Elevation (m a.s.l.)	590	1000	1290
Tree age (years)	119 ± 9	144 ± 7	124 ± 9
DBH (cm)	81.8 ± 5.7	89.0 ± 1.9	88.2 ± 3.1
MAP (mm year^{-1})	2488.1 ± 62.1	1151.4 ± 64.4	779.4 ± 20.5
MAT (°C)	12.0 ± 0.1	8.9 ± 0.2	9.8 ± 0.1
P_{dry} (mm month^{-1})	122.8 ± 9.3	28.7 ± 6.6	37.6 ± 4.5
P_{wet} (mm month^{-1})	293.8 ± 27.4	160.0 ± 17.4	87.7 ± 6.2
T_{cold} (°C)	6.2 ± 0.2	1.7 ± 0.5	2.9 ± 0.4
T_{hot} (°C)	18.4 ± 0.2	16.7 ± 0.5	17.9 ± 0.3

± 1.6°C (1967–2017, Spanish Bureau of Meteorology). To assess the impact of regional and intra-annual climatic variability on the ECM fungal community, besides climate, we prioritised management history and homogeneity in soil properties as our selection criteria, because these are crucial drivers of the ECM fungal community composition and structure (Rodriguez-Uña et al. 2019, 2024, Correia et al. 2021). Our selected sites had been free of major impacts for ~ 90 years (except for some minor and occasional wood extraction), had a non-calcareous bedrock and were located on acidic soils (pH < 5.5, Table 1). Time since the end of major activities was checked with dendrochronological analyses of wood cores extracted from all selected trees. Wood cores were extracted in August 2020 using a Pressler increment borer. The wood cores were air-dried, glued onto wooden mounts, polished until tree rings were visible and then visually cross-dated (Stokes and Smiley 1968). Soil geology was identified on maps of the Spanish Geographic Institute and pH was measured on a set of soil samples from various depths, collected at each site.

Experimental design and sample collection

At each study site, we chose eight mature (> 90 years) dominant individuals of *F. sylvatica* (3 sites × 8 trees/site = 24 trees in total). We performed two sampling campaigns, one in June 2020 (spring campaign), when we did not expect water availability to be limiting, and a second one in August 2020 (summer campaign), when water availability was expected to limit the physiological activity of both the vegetation and the associated fungal community. The same selected trees were sampled in both campaigns.

For analyses of ECM fungal diversity and to determine colonization of fine roots by ECM fungi, on each campaign and at each tree, we selected four points on the forest floor located 1.0–1.5 m from the base of the tree. In spring/June, these points were located in north, south, west and east directions and in summer/August, we selected four similar points in northwest, northeast, southeast and southwest direction. At each one of these sampling points, we dug a shallow (0–15 cm) hole (~ 15 cm in diameter) and collected fine root segments. Fine root segments were transferred to Ziploc bags with ~ 200 g of accompanying soil. These bags were transported in a cool-box to the laboratory, where they were stored at 4°C until they were processed, within the following 48 h. Roots were carefully washed before randomly selecting three fine root segments (< 2 mm in diameter and 5 cm in length each). In total, we collected 192 samples of fine roots (3 sites × 8 trees/site × 4 samples/tree × 2 campaigns = 192 samples). In addition, at one of the four locations per tree and season, we also collected an intact 5–10 cm root segment of diameter > 2 mm in each campaign, to determine fine root density per unit of soil volume.

Samples for analyses of water isotopic composition and volumetric water content (VWC), the amount of water per unit of soil volume, a proxy of water availability and hence an indicator of drought stress, were collected from five out of the eight selected trees at each study site and on each campaign. The same five trees per site were sampled in both campaigns. We collected one lignified branch segment (diameter: 1–2 cm, length: ~ 10 cm) per tree and for those trees where branches were not accessible, we collected a small wood core (~ 10 cm) extracted at 1.3 m height. Bark, phloem and cambium were removed before placing the branch samples or small wood cores into Exetainers glass vials, sealed with parafilm. Soil samples for analyses of soil VWC and water isotopic composition were collected from a location 1.5–2.0 m away from the base of each tree and at least 1 m away from any of the sampling points selected for fine root collection (to avoid sampling some disturbed topsoil that might have undergone some evaporative enrichment). At each selected location, we dug a hole down to 50 cm and collected soil samples at three depth intervals: 0–5, 20–25 and 40–50 cm. All samples were collected into screw-cap glass vials and sealed with parafilm. All samples for isotopic analyses were placed in a cool-box and once in the laboratory, they were stored at −20°C. In total, we collected 30 branches or cores (3 sites × 5 trees/site × 2 campaigns = 30 samples), 90 soil samples (3 sites × 5 trees/site × 3 depths × 2 campaigns = 90 samples) and 6 water samples (3 sites × 2 campaigns = 6 samples) for analyses of water isotopic composition.

Characterization of soil physical and chemical properties

We estimated VWC from gravimetric water content (W_b), obtained from the difference between wet and dry masses of the soil samples used for isotopic analyses (below) using bulk density pedotransfer functions, including organic carbon and texture (Martin et al. 2017). We acknowledge that soil water available for water uptake is best characterized by means of measurements of soil water potential, not soil VWC. This is because soils that differ in their texture will have contrasting water availability for the same VWC. Here we found some differences in soil texture among sites (below), yet we opted to report VWC instead of water potential because we found that further estimating soil water potential from VWC using standard pedotransfer functions (Campbell and Norman 2000) rendered estimates of soil water potential that masked the seasonal variability in water availability within sites, as well as differences among sites during the spring sampling (data not shown). Soil chemical and physical (texture) properties were measured from additional soil samples collected in November 2020. For this latter sample collection, we randomly selected three locations at each site among the sampled trees. At each location, we dug a 50 cm hole and collected soil samples at the same depth intervals as for water isotopic analyses (0–5, 20–25 and 40–45 cm), i.e. we collected a total of 27 samples (3 sites × 3 locations/site × 3 depths/location = 27 samples). These soil samples were sieved (2 mm) and oven dried (48 h at 70°C) for texture, pH and nutrient content analyses. Texture analyses involved estimating percentages of sand, silt and clay by laser diffraction. Nutrient analyses consisted of the determination of total C, total N, total P, pH and conductivity (Supporting information).

Cryogenic water extraction and analyses of water isotopic composition

Water extraction from branches or cores and soil samples was performed by cryogenic vacuum distillation using the design and methodology proposed by Orlowski et al. (2013), following the methodology detailed in Jones et al. (2017). Briefly, the cryogenic extraction line consisted of six branches each with four vacuum extraction lines connected on one end to the glass vials containing the samples and on the other end to U-shaped tubes. At the onset of the extraction, samples in the glass vials were quickly frozen by immersing them into liquid N. The extraction line was then evacuated down to an atmospheric (static) pressure < 1 Pa, and then the U-shape tubes were immersed in liquid nitrogen to create a cold trap. At the same time, samples were immersed in a water bath at ambient temperature, the water bath was gradually heated up to 80°C for 1 h and samples remained in the heated bath at 80°C for 2 h, i.e. total extraction time: 3 h. Pressure in the extraction line was continuously monitored with sub-atmospheric pressure sensors (APG100 Active Pirani Vacuum Gauges, Edwards) to check that the lines remained leak-tight throughout the entire extraction and that the water extraction had ended. Samples were weighed before and after the extraction and before and after being oven-dried for 24 h at 105°C to assess the water extraction efficiency and to calculate sample relative water content. The extraction efficiency was always above 98%, ensuring that the isotopic composition of extracted water had not been affected by incomplete Rayleigh distillation processes (Araguas-Araguas et al. 1995, Ceperley et al. 2024).

We measured the isotopic composition (δ^2H and $\delta^{18}O$) of the extracted water samples with an off-axis integrated cavity optical spectrometer (TIWA-45EP, Los Gatos Research) coupled to a liquid auto-sampler and vaporiser (LC-xt, PAL systems). All measured values were calibrated using two internal standards and expressed on the VSMOW-SLAP scale (Jones et al. 2017). When analysing water samples extracted from plant tissues with laser-based instruments (Schultz et al. 2011, Leen et al. 2012), the presence of organic compounds (ethanol and methanol mainly) can lead to large isotopic biases (Martín-Gómez et al. 2015). Therefore, we developed a post-correction algorithm, specific for our instrument, to correct for the presence of organic compounds based on the narrowband (for methanol) and broadband (for ethanol) metrics of the absorption spectra (Leen et al. 2012).

Recent studies have shown that cryogenically extracted water from plant samples does not always reflect the isotopic composition of its sources, particularly for δ^2H (Zhao et al. 2016, Barbeta et al. 2020, Chen et al. 2020, de la Casa et al. 2021). Barbeta et al. (2022) demonstrated that this discrepancy would be caused by xylem sap water, the water transported through the xylem conduits, being more enriched than cryogenically extracted bulk xylem water. This is because bulk xylem water consists of a mix of xylem sap water and storage water and only the former reflects source water, whereas the latter is isotopically depleted with respect to the sources (Barbeta et al. 2022). Here, we estimated the isotopic composition of xylem sap water (δ_{sap}, the one that reflects the source) from bulk xylem water ($\delta_{tree\text{-}bulk}$, cryogenically extracted) by subtracting to our measurements of $\delta_{tree\text{-}bulk}$ the mean difference between $\delta_{tree\text{-}bulk}$ and δ_{sap}. We calculated this mean difference from a dataset obtained from a separate set of samples of the same species (*F. sylvatica*). This set of samples consisted of 35 branch samples collected on seven dates in 2019 from five adult beech trees, similar in age and height to ours, at the Ciron Valley (France; see Barbeta et al. 2019 for the site description). Each branch sample was divided in two sub-samples, one used for extraction of δ_{sap}, using the centrifugation method (Barbeta et al. 2022), and a second used for extraction of $\delta_{tree\text{-}bulk}$, using the same cryogenic distillation line described above. Mean (± sd) differences in isotopic composition between sap and bulk water ($\delta_{tree\text{-}bulk} - \delta_{sap}$) were: −13.8 ± 0.83 and −0.65 ± 0.19‰, for δ^2H and $\delta^{18}O$, respectively (n = 35), and there were no significant differences either among trees, or among dates (Martín-Gómez unpubl.). In addition, for those sites with high clay content (Northern and Southern), we also performed a correction to estimate the isotopic composition of unbound soil water from bulk soil water (Supporting information).

We used measurements of water isotopic composition to determine the relative contribution to corrected tree xylem sap water ($\delta_{tree\text{-}sap}$) of each one of the potential water sources using Bayesian mixing models (Parnell et al. 2010). These models assume that all considered water sources can be accessed simultaneously by the plants. To produce plausible simulations of the most likely relative contribution of each source the models apply Markov chain Monte Carlo (MCMC) methods (Stock et al. 2018). Our model inputs were mean and standard deviation of water isotopic compositions (δ^2H and $\delta^{18}O$) of soil water. We used estimated unbound soil water ($\delta_{soil\text{-}u}$) at the Northern and Southern sites, with very clayey soils, and bulk soil water ($\delta_{soil\text{-}bulk}$) at the Intermediate site. Mean and standard deviation of isotopic compositions were calculated for each source per study site and sampling campaign. Soil water from intermediate (20–25 cm) and deep (40–45 cm) soil layers were pooled together because their isotopic compositions did not differ significantly (Supporting information). The target values were individual isotopic compositions of each tree and sampling campaign. Trophic enrichment factor was set to 0, as we assumed that no fractionation occurred during root water uptake. We also assumed no concentration dependency, i.e. no differences in the plausible proportions before considering the isotope data (Muñoz-Villers et al. 2018). We ran 400 000 iterations and discarded the first 200 000. Convergence was checked according to the Gelman and Geweke diagnostics. All models were run in R ver. 4.1.3 (www.r-project.org; R Core Team 2021) using the 'MixSIAR' package (Stock and Semmens 2016).

Fine root density and ECM fungal colonization

Root colonization by ECM fungi was assessed by counting the number of live ECM root tips in each segment under the stereomicroscope, always by the same two observers. ECM

fungal colonization per centimeter of fine root at each location was estimated as the average of the three segments.

The additional larger root segment (diameter > 2 mm, one segment collected per tree and sampling campaign) was rinsed and scanned with WinRHIZO (Regent Instruments). From this analysis, we used the total length of fine roots per unit of soil volume (in cm m^{-3}) to calculate a standardized rate of ECM fungal colonization, expressed as the number of live ECM tips per cubic meter of soil, and hereafter referred as 'density of live ECM tips'.

Molecular identification of ECM fungi present in topsoil (0–15 cm)

All live ECM tips counted from the three randomly selected segments from each sample were collected into Eppendorf tubes, i.e. we generated one sample (one Eppendorf tube) per sampled location. This produced four samples per tree for each sampling campaign. DNA was extracted from these mycorrhizal root tips using the DNeasy PowerSoil Pro Kit (Qiagen) following manufacturer's instructions. Extraction negative controls were also created to ensure lack of contamination during DNA extraction. The following steps of molecular analysis and bioinformatics were carried out at a commercial sequencing facility (www.allgenetics.eu). First, a fragment of the internal transcribed spacer (ITS) genomic region of the fungal rRNA (ca 400 bp) was amplified using the primers ITS1-F (5′ CTT GGT CAT TTA GAG GAA GTA A 3′) (Gardes and Bruns 1993) and ITS2 (5′GCT GCG TTC TTC ATC GAT GC 3′) (White et al. 1990). PCRs were carried out in a final volume of 12.5 µl, containing 1.25 µl of template DNA, 0.5 µM of the primers, 3.25 µl of Supreme NZYTaq 2× Green Master Mix (NZYTech) and ultrapure water up to 12.5 µl. The reaction mixture was incubated as follows: an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 47.7°C for 45 s, 72°C for 45 s and a final extension step at 72°C for 7 min. The libraries were run on 2% agarose gels stained with GreenSafe (NZYTech) and imaged under UV light to verify the library size. A second PCR round (with identical conditions but only 5 cycles and 60°C as the annealing temperature) was carried out to attach the oligonucleotide indices required for multiplexing different libraries in the same sequencing pool. Subsequent library purification was performed using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek). Then, libraries were pooled in equimolar amounts according to the quantification data provided by the Qubit dsDNA HSAssay (Thermo Fisher Scientific). The pool was sequenced on an Illumina MiSeq platform using PE300 v3 kits.

The obtained ITS1 amplicon reads were processed using DADA2 (Callahan et al. 2016), implemented in QIIME 2 (rel. 2021.2) (Bolyen et al. 2019). PCR primers were removed, reads were denoised, merged and filtered according to their quality, removing the chimaeric reads and clustering the resulting sequences into Amplicon Sequence Variants (ASVs).

Taxonomy was assigned to the obtained 2273 ASVs with a pre-trained classifier of the UNITE reference database (Abarenkov et al. 2020, updated in February 2020), using the feature-classifier classify-sklearn approach implemented in QIIME2 (Bokulich et al. 2018). We discarded 534 ASVs (23%) that corresponded to 1) singletons (i.e. ASVs containing only one-member sequence in the whole dataset), 2) ASVs occurring at a frequency below 0.01% in each sample or 3) unidentified sequences and sequences assigned only at the kingdom level ('Fungi'). Based on the alpha rarefaction curves created with the number of ASVs obtained for each sample, six samples were discarded because they did not reach the plateau in the number of ASVs observed. ECM fungal communities were characterized with the 675 ASVs (40%, from a total of 1688 ASVs) identified as ECM fungi at the genus or species level, also including 78 ASVs corresponding to families only composed of ECM fungi (i.e. Boletaceae, Clavulinaceae, Elaphomycetaceae, Russulaceae and Thelephoraceae). We manually matched the 183 ASVs that reached the genus level with the UNITE reference database to improve its taxonomy assignment, reaching a total of 209 and 466 ASVs identified at the genus and species level, respectively (based on a threshold similarity > 97%). For simplicity, hereafter we refer to ASVs as 'species', although we acknowledge that ASVs do not necessarily correspond to actual species as defined by the biological species concept.

Diversity and richness indexes, community composition and functional diversity for ECM fungi

ECM fungal species abundances were calculated for each tree and campaign based on the presence of a given species measured in each one of the four samples per tree and campaign. We discarded the use of the number of reads, given the lack of consensus on the level to which the DNA sequences produced actually correspond to the original species abundances in a community (Lamb et al. 2019), due to well-known biological and methodological biases (e.g. different eDNA extraction efficiency or biases during PCR amplification of marker loci; Skelton et al. 2023). We calculated species richness and diversity, i.e. Shannon–Wiener index (H') for each individual tree from the four samples collected per tree and during each campaign (see the Supporting information for details on the formulas).

Community composition for each tree and campaign was characterized with the identity and relative abundance of each species. As a proxy for functional diversity of the ECM fungal community, we classified species according to their capacity to explore the surrounding soil based on their hyphal development, with two categories: long-exploration (including long- and medium-exploration types) versus short-exploration (including short-exploration and contact mycelium, Agerer 2001). Each species was assigned to one of these two categories following Agerer (2006), Ostonen et al. (2017) and Defrenne et al. (2019). We found 9 species out of a total of 151 for which the exploration type was unknown, and therefore these were excluded for further calculations. We then calculated the proportion of species of each functional type (long versus short) for each of the four samples per

tree and sampling campaign (see the Supporting information for details on the formula).

Statistical analyses

All calculations and analyses were conducted in R ver. 4.1.3 (www.r-project.org; R Core Team 2021). Differences in soil physical and chemical properties among study sites (Northern, Intermediate and Southern) and soil depths (0–5, 25–30 and 45–50 cm) were assessed using analysis of variance (ANOVA). For the rest of our variables, we assessed differences among study sites, between spring/June and summer/August sampling campaigns, among sampling depths (for soil VWC only) and their interactions, using linear mixed models (LMMs, for soil VWC, ECM fungal colonization, total fine root length per unit of soil volume, density of live ECM tips and Shannon–Wiener diversity index) or generalized linear mixed models (GLMMs, with a Poisson error distribution for species richness and with a binomial error distribution for proportion of ECM functional group, long- versus short-exploration type), including tree as a random factor. The GLMM with the Poisson error distribution was replaced by a negative binomial to overcome overdispersion. Differences in tree and soil water isotopic composition between sampling campaigns and among depths (for the soil) were also assessed with LMMs, including tree as random factor, but not study site as a fixed factor. Instead, we ran separate LMMs for each study site because we were not interested in differences across study sites due to geographic variability in isotopic composition of precipitation water (Bowen et al. 2005). In addition, we tested for the potential effects of topsoil (0–5 cm) VWC (measured at individual trees on each study site and on each sampling campaign) on ECM fungal species richness and diversity using LMMs. All LMMs and GLMMs were fitted using packages 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017) to estimate p-values. For models with significant study site effects, post hoc tests were performed using 'emmeans' (Lenth et al. 2020). Graphs to visualize our results were generated with 'ggplot2' (Wickham et al. 2016).

We assessed differences among study sites and sampling campaigns on ECM fungal community composition with a permutational multivariate analysis of variance (PERMANOVA), using the *adonis* function with 9999 permutations. The analysis was based on Bray–Curtis because it enables the estimation of community composition dissimilarities considering relative abundances of species. To visualize differences in ECM fungal community composition among study sites and sampling campaigns, we used an ordination analysis of the communities with a non-metric multidimensional scaling (NMDS), based on the Bray–Curtis similarity index, using function *metaMDS* (all functions from R package 'vegan', Oksanen et al. 2019).

Results

Soil volumetric water content, chemical and physical properties

Soil physical and chemical properties were generally homogenous within sites, although there were some differences for certain properties (Supporting information). Notably, soils were sandier at the Intermediate site (Iturrieta); richer in terms of total C, N and P content, at the Northern site (Artikutza); and less acidic at the Southern site (Diustes, Supporting information). Soil volumetric water content (VWC) was higher in spring/June than in summer/August and the topsoil (0–5 cm) was consistently wetter than deeper soil layers (20–25 and 40–45 cm) across all sites (Fig. 1). There were also some significant differences in VWC among study sites: the Intermediate site (Iturrieta) had lower mean VWC than the Northern (Artikutza) and Southern (Diustes) sites (Fig. 1, Table 2). Although the Northern site (Artikutza)

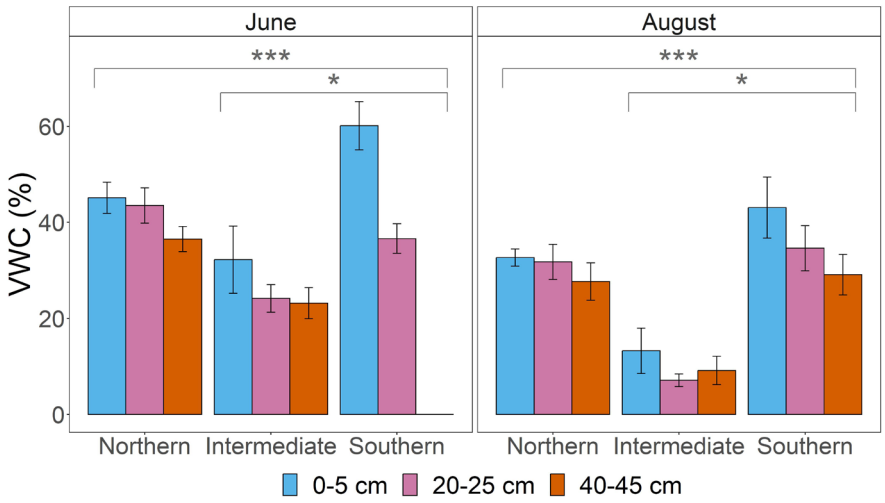

Figure 1. Mean (± SE, n = 5) soil volumetric water content (VWC) different soil depths across the three study sites (Northern, Intermediate and Southern) and during the two sampling campaigns (spring/June and summer/August; no soil samples were collected at 40–45 cm depth at the Southern site in spring/June). Asterisks indicate significant differences between sites.

Table 2. Results of the linear or generalised mixed models for differences among sites (Northern, Intermediate and Southern), campaigns (spring and summer), depths (0–5 cm and 20–50 cm) and their interactions in: VWC; fine root density; and ECM fungal colonization, density of live tips, richness, diversity and fraction of short exploration type. Marginal (R^2_m) and conditional (R^2_c) coefficients indicate variance explained by fixed (R^2_m) or fixed and random (R^2_c) factors.

Soil volumetric water content (VWC)					
Source	df	F	p	R^2_m	R^2_c
Site	2	27.22	**< 0.001**	0.66	0.69
Campaign	1	34.4	**< 0.001**		
Soil depth	2	10.61	**< 0.001**		
Site × Campaign	2	1.36	0.265		
Site × Depth	4	2.23	*0.077*		
Campaign × Depth	2	1.22	0.304		
Fine root density (log-transformed)					
Source	df	F	p	R^2_m	R^2_c
Site	2	2.58	*0.099*	0.17	0.24
Campaign	1	3.83	*0.064*		
Site × Campaign	2	0.49	0.617		
Colonization (log-transformed)					
Source	df	F	p	R^2_m	$\boldsymbol{R}^2_c$
Site	2	9.04	**0.001**	0.26	0.27
Campaign	1	44.92	**< 0.001**		
Site × Campaign	2	0.40	0.674		
Density of live ECM tips (log-transformed)					
Source	df	F	p	R^2_m	R^2_c
Site	2	7.57	**< 0.001**	0.49	0.58
Campaign	1	32.54	**< 0.001**		
Site × Campaign	2	0.12	0.124		
Species richness (negative binomial distribution)					
Source	df	χ^2	p	R^2_m	R^2_c
Site	2	3.95	0.139	0.03	0.04
Campaign	1	0.04	0.846		
Site × Campaign	2	1.49	0.474		
Species diversity (Shannon Index)					
Source	df	F	p	R^2_m	R^2_c
Site	2	3.07	*0.067*	0.13	0.13
Campaign	1	0.26	0.613		
Site × Campaign	2	0.29	0.752		
Short exploration type (Binomial distribution)					
Source	df	χ^2	p	R^2_m	R^2_c
Site	2	9.57	**0.008**	0.08	0.08
Campaign	1	0.92	0.337		
Site × Campaign	2	0.48	0.787		

had the highest mean annual precipitation, the Southern site (Diustes) exhibited the highest VWC.

Water isotopic composition of plant water and its sources

We found that there were some isotopic offsets between plant water and its potential sources (Supporting information). When we applied our corrections and estimated xylem sap water (δ_{sap}) and unbound soil water ($\delta_{soil\text{-}u}$, for the clayey sites, Northern and Southern) these offsets were minimised. Following these corrections, the estimated δ_{sap} values overlapped with the isotopic composition of the potential sources in the dual isotopic space (Supporting information). At the sites with high clay content, this overlap became more evident when we incorporated the correction for estimated unbound soil water ($\delta_{soil\text{-}u}$, Supporting information).

The isotopic compositions of soil water from 40–50 and 20–25 cm depths were not statistically different, neither for $\delta^{18}O$ nor for δ^2H, regardless of the study site or sampling campaign (Supporting information). Thus, values from 40–45 and 20–25 cm depths were pooled together for subsequent analyses. The $\delta^{18}O$ of the topsoil (0–5 cm) was more enriched than that of the deeper soil layers (20–25 and 40–45 cm, Table 3), suggesting isotopic enrichment of the topsoil. Also, the interaction term (campaign × depth) was significant at the Northern and Intermediate sites (Table 3). This

Table 3. Results of the linear mixed models to test for differences in soil and tree water isotope composition (δ^2H and $\delta^{18}O$) between sampling campaigns (spring/June and summer/August), sampling depths (0–5 cm and 20–45 cm, for soil water only) and their interactions, at each one of the sites (Northern, Intermediate and Southern). Tree water δ^2H and $\delta^{18}O$ are those of estimated sap water and soil water δ^2H and $\delta^{18}O$ are those of estimated available soil water: unbound soil water for the clayey sites (Northern and Southern) and bulk soil water for the sandier site (Intermediate). Marginal (R^2_m) and conditional (R^2_c) coefficients indicate the variance explained by fixed only and by fixed and random factors, respectively. df: degrees of freedom. Statistically significant p-values are shown in bold.

		Northern					Intermediate					Southern				
Variable	Source	df	F	p	R^2_m	R^2_c	df	F	p	R^2_m	R^2_c	df	F	p	R^2_m	R^2_c
Soil δ^2H	Campaign	1	34.74	**< 0.001**	0.44	0.68	1	8.72	**0.008**	0.29	0.48	1	20.71	**< 0.001**	0.32	0.62
	Soil depth	1	0.16	0.707			1	3.04	*0.097*			1	0.4	0.534		
	Campaign × Depth	1	4.92	**0.037**			1	2.57	0.125			1	0.02	0.894		
Soil $\delta^{18}O$	Campaign	1	84.74	**< 0.001**	0.69	0.83	1	37.71	**< 0.001**	0.56	0.71	1	17.96	**< 0.001**	0.42	0.63
	Soil depth	1	17.63	**< 0.001**			1	5.19	**0.035**			1	10.44	**0.005**		
	Campaign × Depth	1	18.66	**< 0.001**			1	6.46	**0.02**			1	0.27	0.609		
Tree δ^2H	Campaign	1	< 0.01	0.975	< 0.01	< 0.01	1	0.15	0.722	0.02	0.03	1	3.17	0.149	0.24	0.26
Tree $\delta^{18}O$	Campaign	1	3.22	0.147	0.26	0.26	1	0.54	0.504	0.06	0.06	1	0.8	0.422	0.07	0.07

implies that the isotopic differentiation along the soil profile was stronger in summer, when evaporative enrichment of the topsoil would have been greater, than in spring. In contrast, δ^2H of the topsoil water was not significantly enriched with respect to that of deeper soil layers, likely because δ^2H is less affected by evaporative enrichment than $\delta^{18}O$ due to the lower atomic mass of H. The $\delta^{18}O$ and δ^2H of the soil showed seasonal variation between campaigns across all sites (Table 3, Fig. 2). Meanwhile, $\delta^{18}O$ and δ^2H of estimated tree sap water did not differ between campaigns, in any of the sites (Table 3, Fig. 2).

According to the results of the Bayesian mixed model from the MixSIAR framework, there was a seasonal shift in the relative contributions of topsoil and deeper soil water pools at the Northern and Intermediate site, but not at the Southern site (Fig. 3). At the Southern site, the estimated relative contribution of the topsoil (0–5 cm) was low (Fig. 3), despite high water availability in this soil layer (Fig. 1). At the Southern site, water uptake relied ~ 90% on deep soil, both in spring (89 ± 9%, estimated ± SD) and summer (92 ± 13%, Fig. 3). In contrast, at the other two sites, the estimated relative water uptake (RWU) varied markedly between seasons. In spring. RWU was more evenly distributed between topsoil and deep soil (66 ± 19 and 34 ± 19%, for topsoil and deep soil, and 45 ± 23 and 55 ± 23% at the Northern and Intermediate sites, respectively), whereas in summer the estimated contribution of the topsoil decreased to 14 ± 11% and 19 ± 11%, at the Northern and Intermediate sites, respectively (Fig. 3).

Fine root density and ECM fungal colonization

Mean (± SE) fine root density (total length of fine roots per unit of soil volume) was marginally larger (Table 2) in spring/June (561 ± 73 cm m^{-3}) than in summer/August (399 ± 39 cm m^{-3}). Among sites, fine root density was marginally larger at the Southern site (593 ± 96 cm m^{-3}) than at the Northern site (382 ± 58 cm m^{-3}), whereas density of fine roots at the Intermediate site (464 ± 56 cm m^{-3}) was not different from either site. Colonization of fine roots by ECM fungi (number of live tips per cm of fine root) was higher in spring/June than in summer/August, at all sites (Fig. 4A, Table 2). In addition, we found more colonization of fine roots by ECM fungi at the Southern site than at the Northern and Intermediate sites (Fig. 4A, Table 2). Furthermore, the density of ECM live tips dropped significantly from spring (1519 ± 230 tips m^{-3}) to summer (541 ± 71 tips m^{-3}) and was higher at the Southern site (1568 ± 323 tips m^{-3}) than at the Northern (634 ± 147 tips m^{-3}) and Intermediate sites (887 ± 157 tips m^{-3}, Table 2).

ECM fungal communities

We identified 675 ECM fungal species, of which 578 (85.6%) belonged to the phylum Basidiomycota, 93 (13.8%) to Ascomycota and 4 (0.6%) to Mucoromycota. Among these, we found 38 genera, with *Tomentella*, *Russula* and *Lactarius* being the most abundant. The number of unique species detected was 51, distributed evenly across sites (Supporting information). The mean (± SE) species richness per tree were 11.16 ± 0.69, 10.13 ± 0.86 and 12.47 ± 1.17 in spring and 10.19 ± 0.83, 11.47 ± 0.98 and 12.56 ± 1.07 in summer at the Northern, Intermediate and Southern sites, respectively (Fig. 4B). The species diversity per tree was 3.51 ± 0.09, 3.33 ± 0.12 and 3.63 ± 0.08 in spring and 3.48 ± 0.06, 3.46 ± 0.14 and 3.66 ± 0.11 in summer at the Northern, Intermediate and Southern sites, respectively (Fig. 4C). We found no differences in ECM fungal species richness among study sites or between sampling campaigns (Fig. 4B; Table 2), but a marginally higher species diversity at the Southern site than at the Intermediate site (Fig. 4C, Table 2). In addition, we found that there was greater variability for both species richness and diversity indices at the Southern site (Fig. 4B–C). The PERMANOVA model showed an effect of study site on ECM fungal community composition (Pseudo-F = 4.88, $p < 0.001$), but not of sampling campaign (Pseudo-F = 0.69, $p = 0.97$; Supporting information). NMDS visualization was consistent with this effect of the study site (Fig. 5; stress value = 0.24).

There were differences in the relative proportion of short- and long-exploration types of ECM fungi between the

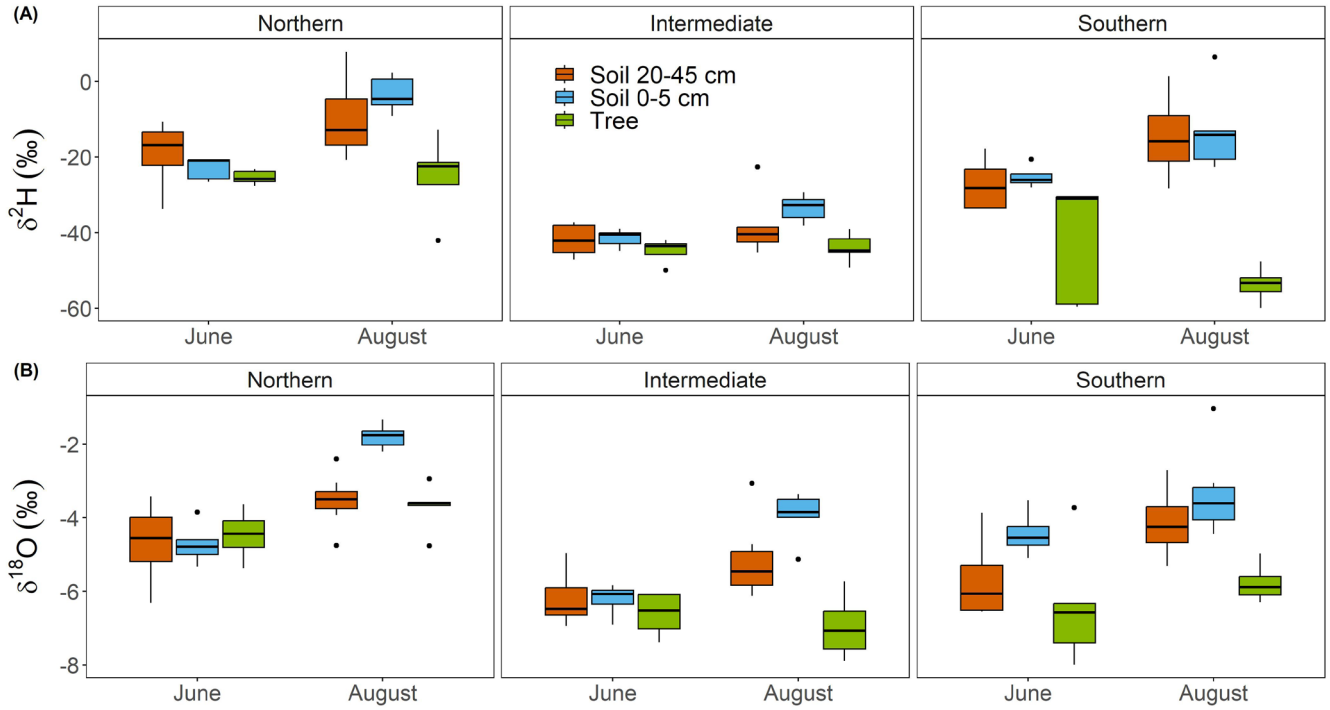

Figure 2. Boxplots of the hydrogen (δ^2H, A) and oxygen ($\delta^{18}O$, B) stable isotope composition for estimated tree sap water (green boxes) and of estimated available soil water for 0–5 cm (blue boxes) and 20–45 cm (orange boxes), for the three sites (Northern, Intermediate and Southern) and the two campaigns (spring/June and summer/August). Estimated δ^2H and $\delta^{18}O$ of soil water are that of unbound soil water for the clayey sites (Northern and Southern) and that of bulk soil water for the sandy site (Intermediate). Within campaigns, the $\delta^{18}O$ of soil water from 0 to 5 cm was significantly more enriched than that from 20 to 45 cm, at the three sites.

Intermediate and the Southern sites, with the former presenting the highest proportion of species with short-exploration types (and the subsequent lowest proportion of long-exploration types) (Fig. 6, Table 2). Finally, we found that soil volumetric water content (VWC) had a significant and positive effect on species diversity, irrespective of the season ($F = 4.48$, $p = 0.044$), but not on species richness ($F = 0.77$, $p = 0.387$; Supporting information).

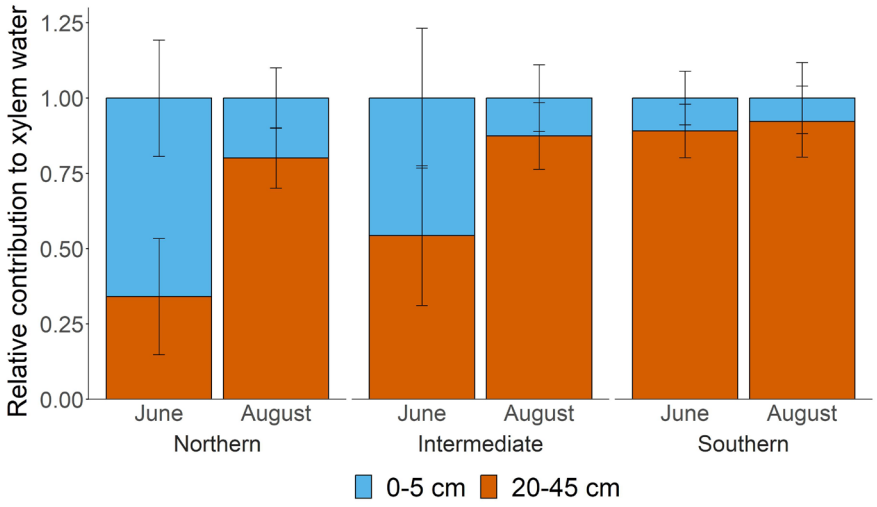

Figure 3. Relative contribution to tree xylem sap water of topsoil (0–5 cm) and deep (20–45 cm) soil water, for the three study sites (Northern, Intermediate and Southern) and the two sampling campaigns (spring/June and summer/August). Bars are the estimated (± SD) relative contribution according to Bayesian mixing models.

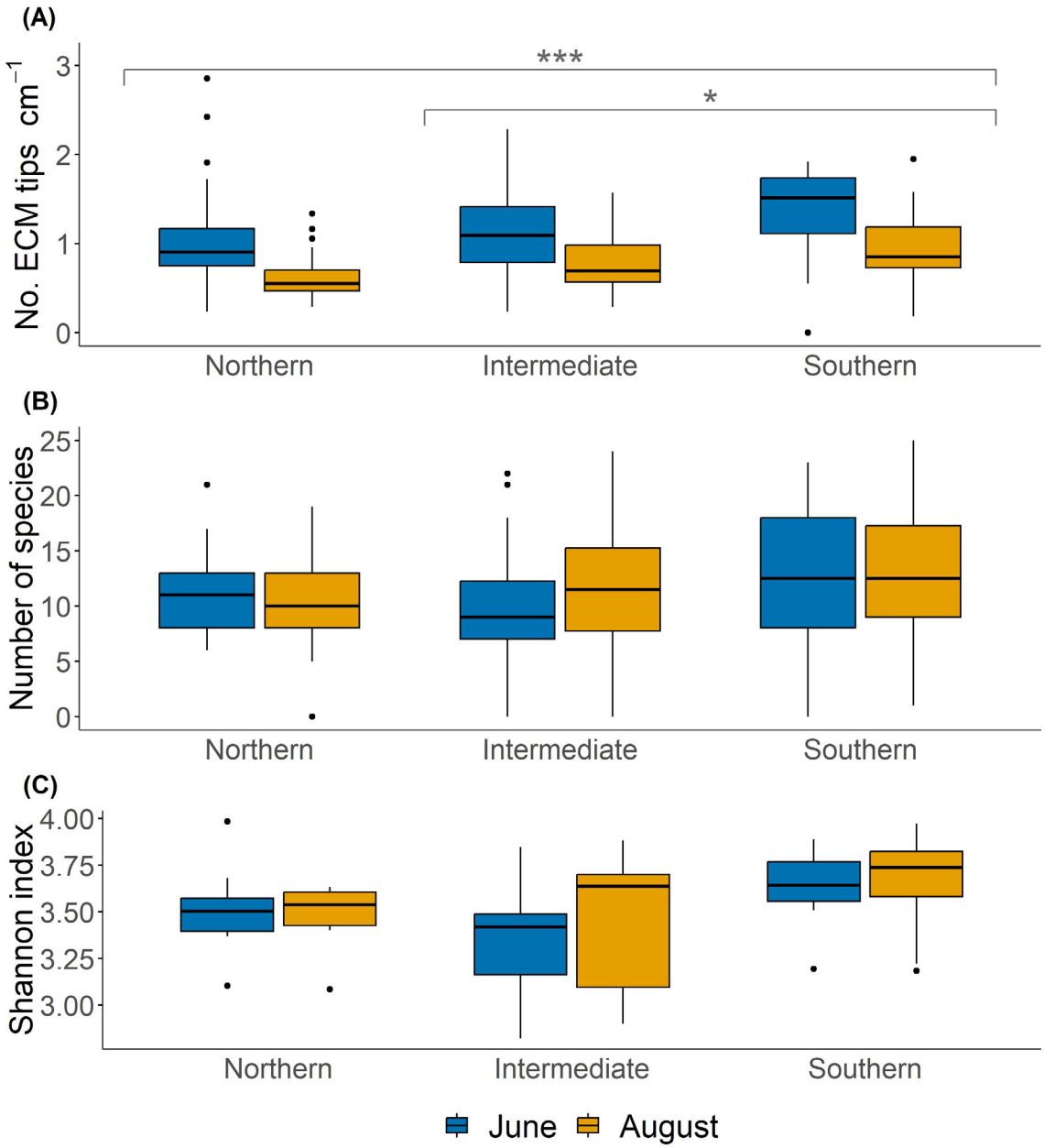

Figure 4. Boxplots of the ectomycorrhizal (ECM) fungal (A) colonization rate, (B) species richness and (C) species diversity for the three study sites (Northern, Intermediate and Southern) and the two sampling campaigns (spring/June and summer/August). Asterisks indicate significant differences (*$p < 0.05$, ***$p < 0.001$) between study sites, according to post hoc pairwise comparisons. Colonization rates were higher ($p < 0.001$) in spring/June than in summer/August in the three sites.

Discussion

We combined molecular analyses of ECM fungal community composition with patterns of root water uptake, from geographically and climatically contrasting populations of European beech. We had anticipated that seasonal and geographic differences in water availability would underlie changes in the diversity and colonization by ECM fungi as well as in the relative water uptake patterns. Our results showed greater ECM fungal diversity and colonization and higher proportion of long exploration types at the Southern site, where the topsoil was more humid, but the contribution to water uptake of the topsoil did not always change in response to water availability in this soil layer.

The natural distribution of European beech *F. sylvatica* is mainly limited by water availability (Geßler et al. 2007) and there is ample evidence showing that drought impacts negatively on the growth and health of these forests (Zimmermann et al. 2015, Brinkmann et al. 2019, Seeger and Weiler 2021, Neycken et al. 2024). Yet, *F. sylvatica* can

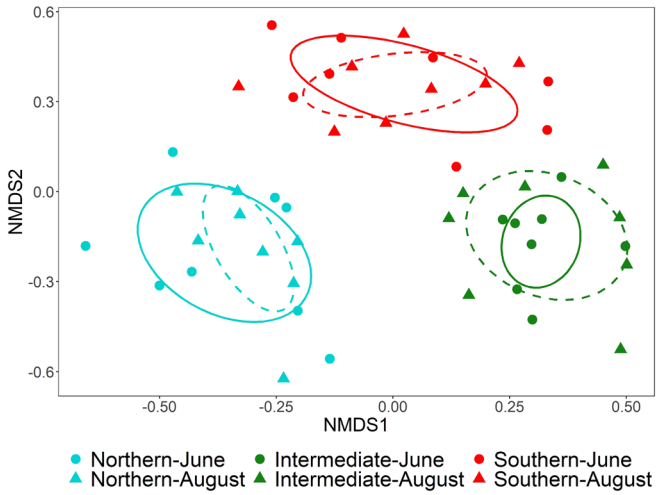

Figure 5. Non-metric multidimensional scaling (NMDS) plot of the ectomycorrhizal fungal communities for the three study sites (Northern, Intermediate and Southern) and the two sampling campaigns (stress value = 0.24). Points and triangles on the ordination space represent spring/June and summer/August sampled communities, respectively, based on Bray–Curtis dissimilarity indices. Solid and dotted lines show the standard deviation ellipses of spring/June and summer/August sampled communities, respectively.

depict some physiological and morphological adaptations that allow this tree to cope with moderate drought. These include, for example, a tight stomatal control in response to both atmospheric (i.e. vapour pressure deficit) and soil drought (Jonard et al. 2011, Leuschner et al. 2022), or reduction of the leaf to sapwood area (Arend et al. 2022). Plasticity in water uptake is another mechanism that would allow beech trees to overcome such drought periods by shifting water uptake from shallower to deeper soil layers (Kinzinger et al. 2024). Here, we found an increase in relative water uptake from deeper soil layers at the Northern and Intermediate sites in summer, in agreement with our first hypothesis and with similar observations from other beech forests (Brinkmann et al. 2019). Yet, here, we did not quantify transpiration, the most direct proxy of tree water uptake, thus we cannot conclude whether the observed shifts in relative water uptake patterns were sufficient to compensate for reduced topsoil water availability (Gessler et al. 2022). Interestingly, at our southernmost and most marginal study site, we did not find evidence of any shifts in relative water uptake depth between seasons. At this site, topsoil moisture was consistently high, maybe due to an evener distribution of precipitation events during the summer in comparison to, for example, our intermediate site (Supporting information); yet the estimated contribution of the topsoil to water uptake at this site was always the lowest, maybe due to an inherently deeper rooting depth (Tumber-Dávila et al. 2022).

We expected patterns of water uptake and of ECM fungal diversity and colonization to be decoupled with low water availability. Our results suggest that topsoil water availability would drive both water uptake patterns and ECM fungal diversity and colonization, but with different implications. In spring, the topsoil contributed significantly to water uptake at the Intermediate and Northern sites, consistent with the higher density of fine roots found in this soil layer in this season. ECM fungal colonization was also greater in spring at all sites, in agreement with our second hypothesis. Given that nutrient uptake depends on soil water availability (Schlesinger et al. 2016) and that ECM hyphae are sensitive to desiccation (Querejeta et al. 2009b), our results suggest that during the early part of the growing season, nutrient and water uptake would be coupled, at least in these sites. In summer, however, we observed a decoupling at all sites and at the Southern site also in spring, as topsoil contribution to water

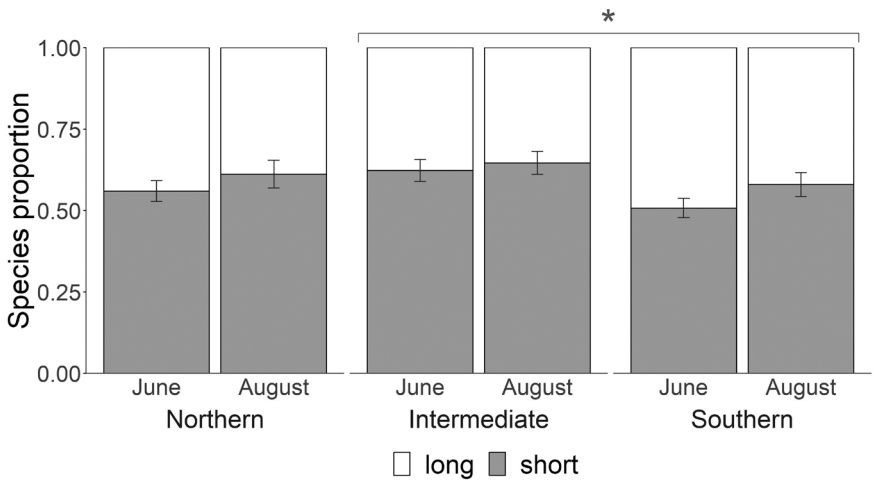

Figure 6. Proportion of ectomycorrhizal fungal species classified according to the exploration type of their hyphae: short-exploration versus long-exploration. Bars depict mean (+SE, n = 8 trees) proportions for the three study sites (Northern, Intermediate and Southern) and the two sampling campaigns (spring/June and summer/August). Asterisk indicates significant difference ($p < 0.05$) between sites according to post hoc pairwise comparisons.

uptake was small, despite the slightly higher VWC and the higher concentration of nutrients and ECM in this layer. This could imply that under moderate drought, water uptake from deep soil layers could still sustain the transpiration demand, but nutrient uptake could be compromised (Querejeta et al. 2021b). It could be argued that hydraulic lift (i.e. passive redistribution of water) could partially alleviate this potential limitation, but experiments in the field and with potted seedlings have suggest that this phenomenon is likely to have a limited quantitative impact in beech (Zapater et al. 2011, Hafner et al. 2021, 2025). Thus, under increasing drought, although trees could compensate for reduced topsoil water availability by shifting water uptake towards deeper soil layers, the associated ECM fungal community dwelling in the topsoil would still suffer from desiccation.

Beyond seasonal differences, across our geographic gradient, we found that our study sites not only differed in ECM fungal colonization, but also in community structure, in agreement with our second hypothesis. However, and contrary to our expectations, analyses of simple metrics (species richness and diversity indexes) did not shed light on how ECM fungal communities varied across the sampled geographic gradient, nor between seasons. We only found that the ECM fungal community was marginally more diverse at the Southernmost site. In contrast, analyses of community composition based on species relative abundances showed that ECM fungal communities clearly differed in their structure across sites but not between seasons within sites. Our forests did not differ in tree species composition (monospecific stands) nor in land-use history (mature forests). Thus, our results indicate that the prevailing climate (Fernandez et al. 2017, Wasyliw and Karst 2020), more than the seasonal variability, together with other topographic and abiotic conditions (soil properties, slope, etc.), determined how ECM fungal communities structured (Miyamoto et al. 2015, León-Sánchez et al. 2018, Querejeta et al. 2021a, 2021b).

We found a greater proportion of short-exploration ECM types at the Intermediate site, which had the highest sand fraction and the lowest soil VWC. These findings are consistent with those of previous studies showing an increase in contact- and short-exploration types under drier conditions (Allen and Kitajima 2014, Castaño et al. 2018, Fernandez et al. 2023). Contact- and short- exploration types are less C-demanding; therefore, these exploration types would be favoured when photosynthesis becomes limiting (Fernandez et al. 2017), for example in sites experiencing periods with low soil water availability (Castaño et al. 2018). The combination of sparse precipitation events during the summer months (Supporting information), together with the lower water holding capacity of the soil, would result in periods of low water availability that could have favoured the selection of ECM fungal partners that would be less costly to replace. On the other hand, experiments with saplings and under controlled conditions have shown that under drought, the proportion of contact-type ECM fungi decreases (Köhler et al. 2018, Castaño et al. 2023). Also, in the field, Nickel et al. (2018) found a rapid decrease of contact-, short- and medium-exploration types in response to drought, and in the longer term (3 years of drought exposure) a relative increase in long-exploration types. Long-exploration ECM fungi usually deploy long hydrophobic hyphae that should allow for a more efficient water and nutrient transport and for the exploration of larger soil volumes (Lehto and Zwiazek 2011, Prieto et al. 2016). The deployment of such structures would be advantageous in environments where water and host photosynthesis would not be limiting, as these long emanating tissues are of more carbon cost to maintain (Fernandez et al. 2017). This could be the case at our Southern site, where we found the highest water availability, both in spring and summer. The deployment of an ECM network with longer emanating exploration tissues could have also been one of the factors that might have contributed to the persistence of this marginal beech population outside its core distribution range. In the long-term and under a climate change scenario, ECM fungal communities with a higher proportion of long-exploration ECM fungi might be able to maintain their functionality to a greater extent than those dominated by contact- or short-type ECM fungi, provided drought did not impair host photosynthesis. Alternatively, under a climate change scenario with increasing frequency and severity of extreme droughts, hosting ECM networks with greater functional diversity (i.e. a mix of long- and short-exploration types) could be a more advantageous strategy conferring greater resistance and recovery capacity.

Conclusions

Our study uniquely combined plant ecophysiology and molecular biology to assess water uptake and soil microorganism biodiversity, moving beyond traditional metrics like growth. Yet, here, we did not assess the contribution of ECM to nutrient uptake, arguably the most relevant function of ECM associations and key for predicting forest functioning under a global change scenario (Terrer et al. 2016). The extent of our conclusions is also limited by the number of sites sampled and their reduced geographic extent within the distribution range of *F. sylvatica*. Overall, our results agreed with previous studies on European beech suggesting that marginal populations or those at the rear-edge of their climatic distribution limit might not necessarily be the most vulnerable to the impacts of climate change (Cavin and Jump 2017, Vilà-Cabrera and Jump 2019, Bert et al. 2022). Instead, our results suggest that beyond their marginality and mean climatic values (e.g. MAT or MAP), maintaining high soil water availability throughout the growth season is more relevant for buffering the impacts of increasing drought. In contrast to previous studies, our approach is not based on the assessment of a single forest function (e.g. growth). Instead, we combined methodologies from plant ecophysiology and molecular biology to assess relative water uptake and the biodiversity of soil dwelling microorganisms, forest properties that depend on tree roots and their associations with symbiotic fungi. Our results suggest that under a climate change scenario, European beech forests could face transient

reductions in topsoil water by shifting water uptake towards deeper soil layers, but these reductions would still be detrimental for the survival of their ECM fungal partners. In a scenario with increased frequency, duration and intensity of drought episodes, as the ECM fungal community contracts and loses diversity, key ecosystem services such as nutrient cycling and forest productivity could be compromised, even in forests traditionally considered resilient to drought stress.

Speculations and alternative viewpoints

Survival and growth of beech forests are predicted to decline in the Mediterranean region in response to increasing drought, at least based on how their radial growth has responded to recent climate change. Unexpectedly, we found that soil water availability in our Mediterranean site was high, even in summer. We believe that this could have been favoured by a more even distribution of precipitation events during summer and by a particular topography (high altitude and north-facing aspect) that would limit evapotranspiration. In the long term, such microclimate could have facilitated the selection of a population of ECM fungi dominated by species with long emanating tissues, more expensive to maintain for the plant, but capable of scavenging larger soil volumes for nutrient uptake and of transporting water through their hyphae. The deployment of such hyphal network would be beneficial for the plant provided that the topsoil is not subject to recurrent desiccation, as we believe is the case in our marginal beech population. In contrast, in sites where soils experience desiccation on a more regular basis, due to sparse summer precipitation and/or to their soil properties, ECM fungi with short mycelia dominate, as in our intermediate site. Paradoxically, short and contact ECM fungi, although cheaper to maintain and replace in terms of carbon (and hence water), would render a small profit for the host plant under drought, as their water-transport capacity is limited and they only allow for exploration of small soil volumes. Still, we have limited knowledge on the actual functionality of these contrasting types of ECM hyphal networks (short versus long dominated). Manipulative experiments assessing drought impacts on the capability to mobilize nutrients and absorb water are the way forward to elucidate the role of ECM functional types in maintaining forest functionality under climate change.

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Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.0gb5mkmdg (Rodríguez-Uña et al. 2025).

Supporting information

The Supporting information associated with this article is available with the online version.

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