ORIGINAL ARTICLE

How beech provenance affects the structure of secondary xylem, leaf traits, and the ectomycorrhizal community under optimal growth conditions

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Abstract

Key message **Provenance controls conductive area of stem secondary xylem, leaf area, and stable isotope (C, N, and O) ratios of beech leaves, while no significant effects were observed for ectomycorrhizal community composition.**

Abstract Beyond growth parameters and drought tolerance, comparatively little is known about the functioning of different beech (*Fagus sylvatica* L.) provenances. We investigated properties of leaves, stem secondary xylem, and ectomycorrhiza (ECM), and explored their interdependencies to identify the best performing beech provenance in optimal growth conditions. The study was conducted on 23-year-old trees in a provenance trial. The investigated provenances originated from Atlantic (Belgium—BE), Alpine (Italy—IT, Slovenia—SI), and continental climates (the Czech Republic—CZ). A significant effect of provenance was observed for stem vessel diameters and conductive area, as well as for foliar %C, δ^{13}C, δ^{15}N, and δ^{18}O. δ^{13}C as a proxy of intrinsic water use efficiency (iWUE) showed that the highest iWUE was achieved in BE provenance. Individuals with a better iWUE had wider growth rings regardless of provenance. Better iWUE was associated with lower specific leaf area (SLA). ECM community composition and diversity indices did not differ significantly among the provenances. Specific ECM taxa were associated with individuals with high SLA, δ^{13}C, δ^{15}N, and δ^{18}O. In optimal growth conditions with no stress events, BE is a promising provenance due to an efficient water conducting system with high vessel diameters and conductive area, and high iWUE, while Alpine provenances showed an adaptation of their water conducting system to freezing conditions at their original locations. Integrating findings from different compartments improves our understanding of functioning of different beech provenances.

Keywords *Fagus sylvatica* · Provenance trial · Stable isotopes · Specific leaf area · Xylem vessels · Ectomycorrhizal fungi

Introduction

Trees respond to changes in their environment by adaptation through natural selection, migration, or phenotypic plasticity. Natural selection and migration are responses to long term changes in the environment and require several

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generations, which is disadvantageous for the pace of the current climate changes, due to long generation times in trees. Meanwhile, phenotypic plasticity is crucial for survival of the fluctuations in the environment that are faced in the lifetime of a tree because of its long lifespan (Gömöry et al. 2020).

The performance of plants is controlled by the equilibrium between water loss and carbon gain (Robson et al. 2012). Leaf ecophysiological traits such as photosynthetic capacity, stomatal conductance, water use efficiency, leaf size, and leaf thickness have a significant influence on net carbon gain (Bresson et al. 2011). However, optimal growth can be sustained only with a functional and efficient water conducting system (Eilmann et al. 2014). The roots of many temperate and boreal tree species are associated with ectomycorrhizal fungi that support tree growth by acquisition of

nutrients from organic matter by colonizing portions of soil that are not accessible to the roots (Rosling et al. 2016) and improved access to soil water (Lehto and Zwiazek 2011). Moreover, there is growing evidence that the diversity of ectomycorrhizal community controls plant performance and N supply (Leberecht et al. 2015; Nguyen et al. 2017).

Some tree species have more phenotypic plasticity than others and are, therefore, more suitable for future forestry. Beech, for example, responds very plastically to environmental conditions (Eilmann et al. 2014) but its response plasticity is very different among provenances (Buhk et al. 2016). Several studies have confirmed wood anatomical, leaf morphometrical, and ecophysiological differences among beech provenances (Peuke and Rennenberg 2004; Bresson et al. 2011; Robson et al. 2012; Sánchez-Gómezet al. 2013; Stojnić et al. 2013, 2022). Although most studies have focused mainly on drought response differences among beech provenances, their functioning in normal years is important too, since these years are crucial for the replenishment of carbohydrate reserves that may help to survive stress events (D'Andrea et al. 2021).

Under controlled conditions, phenotypic provenance responses are commonly studied at the seedling stage in greenhouse experiments. However, the response of older trees, which can differ from the response of seedlings, cannot be studied under greenhouse conditions. These trees must be tested in common garden experiments or transect studies. The advantage of provenance trials over transect studies is in the same climatic and soil conditions at the site of the trial. Trees are exposed to realistic fluctuations in environmental conditions, such as air temperature, precipitation, biotic pressures, etc. Provenance trials or common garden experiments offer insight into the growth and development of different beech provenances at specific sites located in different regions of the current beech distribution (Konnert and Ruetz 2001; Liesebach 2017) and provide valuable data for the investigation of climate adaptation of forest species and intraspecific variation in climate response among tree populations (Kapeller et al. 2013).

Long-term provenance trials are oriented mainly into monitoring traits related to adaptation to specific environmental stresses, such as survival/mortality, growth (height and diameter), phenology (e.g., timing of bud burst, bud set, leaf abscission, pollination, and seed maturation), cold-hardiness, and drought-related traits (e.g., water use efficiency as measured by stable carbon isotope ratios in wood samples) (Aitken 2004; Liesebach 2017) to establish recommendations for the use of suitable provenances in forestry at national or international level (Liesebach 2017). In addition to this important role, provenance trials are increasingly recognized as a valuable source for the study of inter- and intra-population variabilities in phenotypic

plasticity of many anatomical, morphological, and physiological parameters that are crucial for tree functioning (e.g., Cavender-Bares and Ramírez-Valiente 2017; Albaugh et al. 2018; Jankowski et al. 2019; Azuma et al. 2023).

Our study was aimed at assessing suggested differences in beech morphoanatomical and functional traits, ranging from leaf through tree stem to ectomycorrhizal community, among four contrasting provenances under growth conditions of a mesic to wet environment, in an international beech provenance trial at Kamenski vrh, Slovenia. To the best of our knowledge, this is the first study to combine such different aspects of tree growth in a beech provenance trial and to investigate their interdependencies, with the aim of disentangling general patterns and identifying the best performing provenance. We hypothesized that leaf functional traits controlling water, C, and N are correlated with leaf morphological traits through its effects on transpiration and internal CO_2 transport, and that the leaf traits are correlated with the properties of secondary xylem in the stem as appropriate dimensions of water conducting system are needed for leaf expansion and replenishment of water losses due to transpiration. Furthermore, we expected that leaf traits are also correlated with the abundance of ectomycorrhizal fungi due to symbiotic relationship in which photosynthetic C is transported belowground in exchange for nutrients and water. We proposed that the provenances will exhibit different patterns in these relationships due to their different origin. It was also hypothesized that, under optimal growth conditions, the best performance will be associated with a highly efficient water conducting system through the stem and leaf traits that enable best water-use efficiency and carbon gain. We proposed that the carbon gain could affect the composition of the ECM community.

Methods

Location

The beech provenance trial at Kamenski vrh (N 45°47′46″/E 15°02′54″), 544 m a.s.l., Slovenia, was established in 1998 as part of an international network of provenance trials (von Wuehlisch et al. 1998) on a clearcut in a mixed beech-silver fir forest (Božič and Kraigher 2019) in the Pre-Dinaric ecological region, where beech is autochthonous. Two-year-old seedlings of 31 provenances were planted in three blocks, with 31 plots of 10 m × 10 m size per block. Each plot contained five rows of trees at a 2-m distance, and each row was planted with ten trees at 1-m distance. Seedlings of the 31 provenances were grown at the Institute for Forest Genetics and Forest Tree Breeding in Grosshansdorf, Germany (Božič and Kraigher 2019). All seedlings were therefore exposed to the same inoculum of ECM fungi present at the tree nursery.

The soil type at the site of the provenance trial is classified as Eutric Cambisols and Haplic Luvisols. About 15% of the surface is covered with limestone rocks (Božič and Kraigher 2019). Yearly precipitation in the 24-year period from 1995 to 2018 at the nearby precipitation meteorological station Dvor (206 m a.s.l.) averaged 1223 mm, while the average temperature in the 1988–2018 period at the meteorological station Novo mesto (220 m a.s.l.) was 10.9 °C. Total precipitation in 2019 was 1288 mm and the average temperature was 12 °C (ARSO, meteo. arso.gov.si, 4 November 2022) (Fig. S1). Spring and fall frosts sometimes occur but are not common overall, due to the sheltering effect of the surrounding forest. The test site Kamenski vrh is under the influence of the more or less humid continental climate of SE Europe and provides climatically optimal conditions for beech growth according to Ellenberg's climate quotient (Mátyás et al. 2009).

Four provenances were selected for the investigation: P13 (Soignes, Belgium), P37 (Val di Sella, Italy), P54 (Idrija, Slovenia), P64 (Nizbor, Czech Republic), abbreviated as BE, IT, SI, CZ in the next sections (Fig. S2). Meteorological parameters at the origin of provenances are shown in Table S1 and were obtained from E-OBS daily climate data version 27e 0.1° regular grid resolution (Cornes et al. 2018) by extracting mean daily temperatures and sum daily precipitation from the nearest grid point for the period 1960–2022. IT and SI originate from the Alpine region and BE from the Atlantic region. IT and CZ are two extremes in height growth. SI and BE were characterized by the latest flushing among all the provenances. SI flushes unusually late compared to other Slovenian provenances (Robson et al. 2011). It shows adaptation to local climatic conditions at its original site location on a mountain pass (saddle) between two valleys in the border area of the interior and lower Karst regions at an elevation of 940 m a.s.l. The area is characterized by long-lasting snow cover (136 days/year), quite frequent sleet phenomena during the winter and storms during the whole vegetation season (48 days/year and 9 days/month during the summer period) as well as constant winds from the coast along the valleys. The maritime influence differentiates this provenance from IT. SI is among the provenances with the latest flushing on a pan European scale in all analyzed international beech trials (Robson et al. 2011).

Sampling

Sampling was performed on 9th September 2019, after the cessation of the growing season. Nine trees of each provenance were sampled in each block. Six sunny mature leaves per tree were sampled from the upper tree crown with telescopic scissors. Three leaves were frozen in dry ice, while three were put in paper bags and transferred to the laboratory to investigate leaf traits. Sampling of ectomycorrhizal communities was performed by a 274 mL soil corer. Sampling was performed approx. 0.5 m from the tree trunk. Trees at the edge of the plot were generally avoided, but where they were included in the sampling procedure, sampling of the ECM community was performed toward the center of the plot to minimize intermingling with roots of the neighboring provenance.

A subset of six trees per provenance was used for wood anatomical analyses of the xylem growth rings formed in 2019. A smaller subset of trees for wood anatomical analyses was used to avoid excessive damage to the trees in the provenance trial.

Secondary xylem

One increment core from the stem of each tree 1.3 m above the ground was taken for wood anatomy investigations. In the laboratory, the wood cores were split into 3–4 cm long segments. Since only the latest formed xylem growth rings were analyzed, we used the outermost segment of the core for further processing. Thus, from this segment, a 15–20 µm thick transverse section was cut with a WSL sledge microtome using OLFA-80×9 mm spare blades (Gärtner et al. 2015). Since only the latest formed xylem growth rings were analyzed, we used the outermost segment of the core for further processing. Thus, from this segment, a 15–20 µm thick transverse section was cut. The sections were treated with bleaching solution (5–15% chlorine content) to remove sawdust from the cell lumina and to improve the staining intensity in the subsequent staining with a safranin/Astra-blue water mixture. High-resolution images (0.514 pixel/1 µm) of the sections were prepared; image-sequences of the xylem rings were captured with at least 25% of overlapping area and then merged. Panoramic pictures were then processed with Image-Pro Plus 7.1 and ROXAS (v3.0.437) image analysis software to obtain intra-annual variation in wood anatomy (von Arx and Carrer 2014; Arnič et al. 2021). In particular, we examined the width of xylem growth rings, and the anatomy of the water conducting system, i.e., tangential and radial diameter of vessels, vessel density, mean lumen area and percentage of the conductive area.

Leaf traits

Leaf functional parameters in terms of leaf area, leaf weight, C% and N%, stable carbon isotope ratio as a proxy for water use efficiency (Moreno-Gutiérrez et al. 2012), stable nitrogen isotope ratio as an integrator of N cycling (Dawson et al. 2002), and stable oxygen isotope ratio as an integrator of stomatal conductance (Barbour 2007) were investigated.

For leaf area and leaf weight measurements, frozen leaves were scanned on an Epson Perfection V700 Photo Scanner (Seiko Epson Corp., Suwa, Nagano, Japan). Leaf area was measured by ImageJ 1.53e image analysis software (Rasband 1997–2018). After scanning, leaves were dried at 60 °C until constant weight and then weighed on a SCALTEC SBC-31 analytical scale. Specific leaf area (SLA) was calculated by dividing leaf area by weight.

For C%, N%, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ measurements, leaves were dried at 60 °C until constant weight. They were then milled into a powder in a ball mill (Grindomix GM 200, Retsch, Germany). Samples for the determination of carbon and nitrogen isotopes (2.60 ± 0.25 mg) were weighed in tin capsules and samples for determination of the oxygen isotope (0.175 ± 0.020 mg) were weighed in silver capsules using a Sartorius CPA2P micro-analytical balance (Sartorius, Germany). They were analyzed with a Vario Pyro Cube Elemental Analyser (Elementar, Germany) coupled with an IsoPrime100 Isotope Ratio Mass Spectrometer (IsoPrime, UK), running in combustion (950 °C) and pyrolysis mode (1450 °C). Certified reference materials (IAEA-601 and IAEA-602 for oxygen and USGS40, USGS41, IAEA-N-1, and IAEA-N-2 for carbon and nitrogen) and in-house working standards (spruce cellulose, acetanilide, benzoic acid, oak leaves, and corn flour) were used to control the accuracy and precision of the measurements. The analytical precision was <0.1 ‰ for carbon and nitrogen, and <0.3 ‰ for oxygen [expressed as standard deviation of repeated measurements of in-house working standards ($n = 30$)]. Stable isotope results ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) are reported in terms of relative delta (δ) values as the difference between the $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{18}O/^{16}O$ ratio of the sample and international reference material [Vienna PeeDee Belemnite (VPDB), Atmospheric Nitrogen (AIR) and Vienna Standard Mean Ocean Water (VSMOW)], expressed in per mil (‰).

Ectomycorrhizal community on root tips

Soil cores were processed within 48 h after sampling. They were washed under tap water to remove the soil. Fine roots with ectomycorrhizal root tips were removed from coarse roots and kept frozen at – 85 °C until further processing. Before analysis they were freeze-dried (LIO-5 PLT, Kambič, Semič, Slovenia) and milled in a ball mill (MillMix20, Domel, Železniki, Slovenia). Total DNA from freeze-dried samples was extracted using a DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Fungal communities were quantified using Illumina MiSeq NGS sequencing of ITS2 region amplicons. To produce amplicon libraries for Illumina MiSeq NGS, the ITS2 fragment was first amplified by PCR using a Q5® Hot Start High-Fidelity 2X Master Mix (New England BioLabs, Ipswich, Massachusetts, USA) and the primer pair ITS7f

(Ihrmark et al. 2012) and ITS4r (White et al. 1990). Forward and reverse primers were modified to contain Illumina specific overhang adapter sequences. PCR was carried out in a 25 µl reaction volume with 2 µl of DNA template, 12.5 µl of Master Mix and 1.25 µM of each primer. PCR conditions were 98 °C for 30 s followed by 25 cycles at 98 °C for 10 s, 57 °C for 20 s, and 72 °C for 20 s, and final elongation at 72 °C for 2 min on an Applied Biosystems Veriti Thermal Cycler (Thermo Fisher Scientific, Waltham, Massachusetts, USA). PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, California, USA). A secondary semi-nested indexing PCR was then used to attach Illumina sequencing adapters and multiplex indexes, using a Nextera XT Index Kit (Illumina, San Diego, California, USA) following Illumina's recommended protocols. Secondary PCR products were purified using Agencourt AMPure XP magnetic beads before quantification using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). Equimolar concentrations (4 nM) of successfully amplified samples were pooled, and the pooled library was quality checked with an Agilent High Sensitivity DNA kit. Sequencing was performed on an Illumina MiSeq (2×300 cycles, 15% PhiX). For total fungal community, quality filtering of sequence reads and clustering into operational taxonomic units (OTUs) based on 97% similarity was performed using mothur (v.1.44.1; Schloss et al. 2009), as previously described in Mahnic et al. (2020). Taxonomic classification of representative sequences was inferred using the UNITE version 8.2 ITS database for total fungal community (Abarenkov et al. 2020). Ectomycorrhizal fungi were filtered using the FUNGuild database (Nguyen et al. 2016). The analyzed dataset for this study can be found in the NCBI Sequence Read Archive public repository of DNA and RNA sequences under accession number PRJNA892758. Species richness, evenness, and dominance were calculated for each provenance separately.

Statistics

Differences in wood anatomical features and leaf traits among the provenances were quantified using one-way ANOVA in Statistica 13.5.0.30 (Tibco Software Inc., 1984–2018), with provenance as fixed and block as random effect. Equality of variances was checked by the Levene test. Significant differences were calculated using the post hoc Tukey HSD test. In cases in which equality of variances was not achieved, non-parametric Kruskal–Wallis tests by ranks were applied. All statistical analyses of ECM community were completed in R version 3.5.1 (R Core Team 2016), using the community analysis specific package "vegan" (Oksanen et al. 2019) and "stats" (R Core Team 2016). Differences in ECM community composition between sampled provenances

were tested using the ADONIS function (analogous for PerManova) in the "vegan" package for R, which was ran for 9999 iterations using a Bray–Curtis distance matrix. The significance of tree variables as well as a provenance variable ecodistance, which is based on Ellenberg climate quotient (Ellenberg 1988; Mátyás et al. 2009) (see footnote to Table S1), impacting ECM communities was tested with ADONIS function and visualized using the CCA ordination method with cca function in the "vegan" package. Community data were previously standardized with decostand function in "vegan" package, using Hellinger standardization method. One-way ANOVA with Tukey contrasts was used to examine the differences in alpha diversity indices (OTU richness, inverse Simpson's index for OTU evenness and inverse Berger–Perker index for OTU dominance) among the four provenances. The homogeneity of variance assumptions was checked with the Bartlett test ($p > 0.05$). Multivariate generalized linear models (MV-GLMs), specifically, mvabund and manyglm tests from "mvabund" package were used to examine differences in ECM taxa abundances among the provenances, with multivariate and unadjusted univariate p values, determined by the Wald test, both using 10,000 Monte Carlo permutations.

Results

Secondary xylem

Xylem growth rings for 2019 did not significantly differ in vessel density and mean ring width among the four provenances (Table S2, Fig. 1, Fig. S3, supplementary material). However, the widest growth rings were generally observed in CZ and BE provenance, with means of 3.66 and 3.64, respectively. SI provenance showed the lowest mean radial increment with 2.35 mm. With a mean tangential vessel diameter of 38.2 μm (Fig. 1) and radial of 48.9 μm (Fig. S3), BE had the broadest vessels, while IT with 31.4 μm in tangential dimension and 37.2 μm in radial dimension was significantly below. This was also reflected in higher mean lumen area (Fig. S3) and percentage of conductive area (Fig. 1).

Leaf traits

SLA (Fig. S4) and leaf area (Fig. S2) were affected by the provenance (Table S2), where BE showed significantly lower SLA compared to CZ, and the leaf area (Fig. 2) of BE was significantly lower than that of IT. On the other hand, leaf weight (Fig. S4) did not differ among the provenances and appeared to be strongly affected by the microlocation (Table S2).
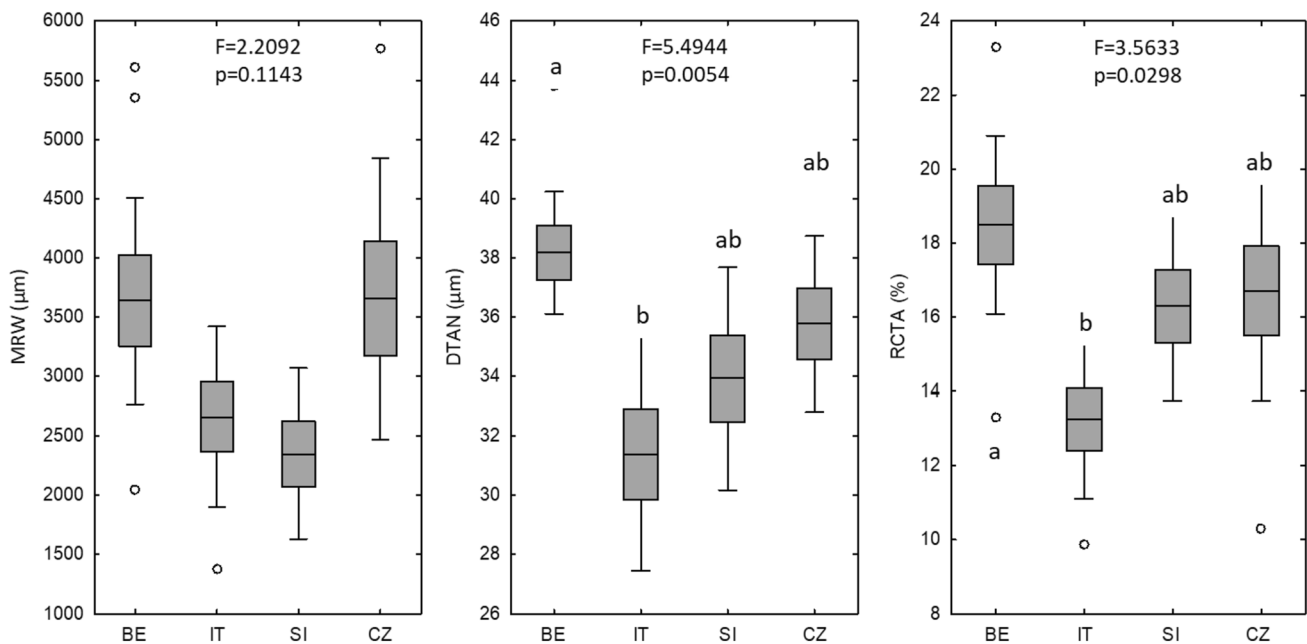

Fig. 1 Box-plots (boxes mean ± stderr, handles 95% confidence interval, circles outliers) showing mean ring width and wood anatomical parameters of tangential vessel diameter (DTAN) and percentage of conductive area (RCTA) in 2019 for four beech provenances (BE, IT, SI, CZ) in Kamenski vrh provenance trial, Slovenia. *F* and *p* values were obtained from one-way ANOVA. Significant differences among the provenances are marked with different letters
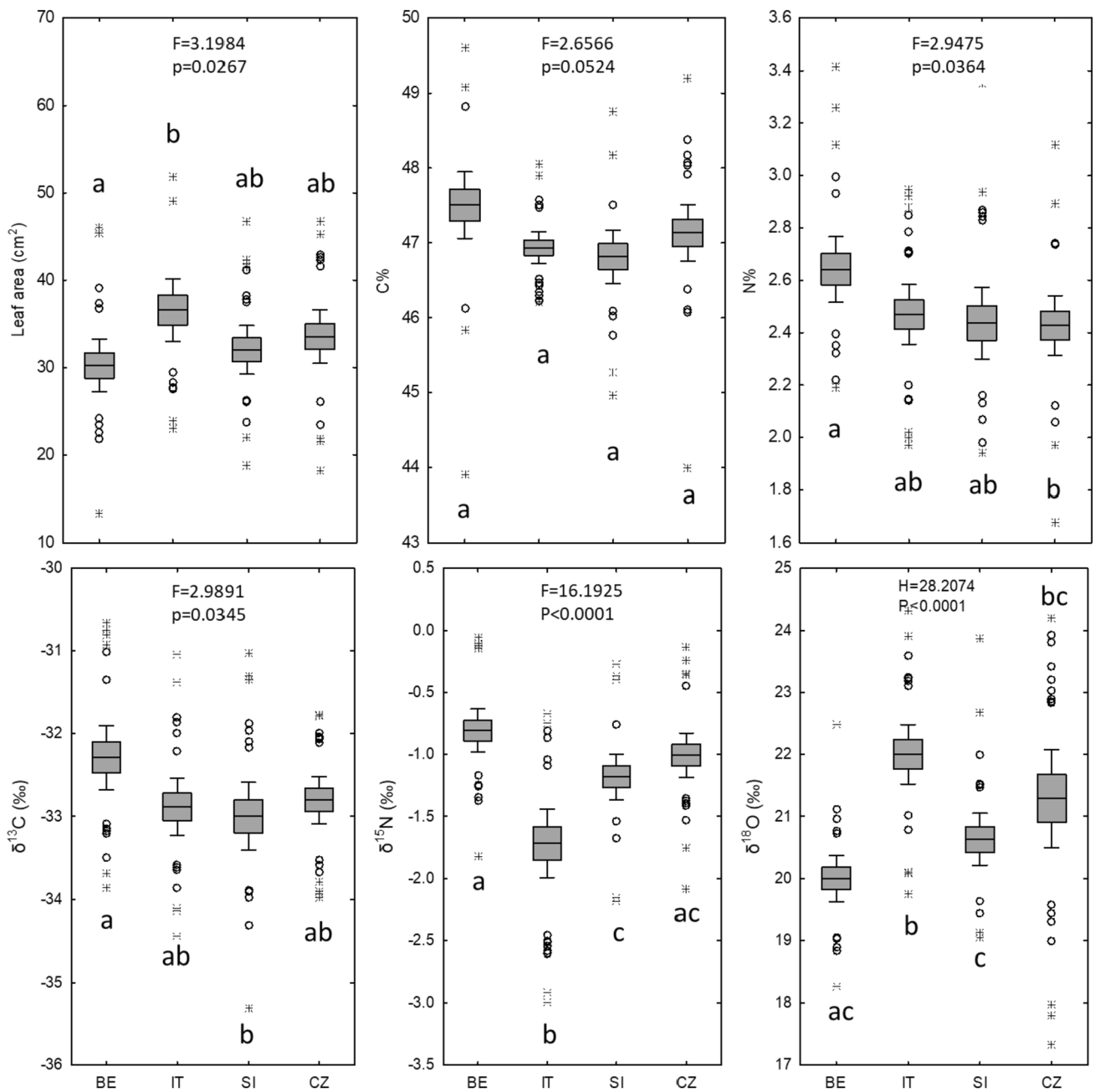
Fig. 2 Box-plots (boxes mean ± stderr, handles 95% confidence interval, circles outliers, asterisks extremes) showing leaf traits of four beech provenances (BE, IT, SI and CZ) in Kamenski vrh provenance trial, Slovenia in 2019: leaf area, C%, N%, and isotopic composition of bulk leaf material ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$). F and p values were obtained from one-way ANOVA, H and p values from the non-parametric Kruskal–Wallis test (in cases in which the criterion on equality of variances was not met). Significant differences among the provenances are marked with different letters

Nitrogen content was significantly higher in BE compared to the CZ provenance, while carbon content did not show any significant differences. With $-32.3 \pm 0.19‰$, $\delta^{13}C$ was significantly higher in BE compared to SI, in which $\delta^{13}C$ was $-33.0 \pm 0.20‰$. IT $\delta^{15}N$ values of $-1.72 \pm 0.13‰$ were significantly lower than those of BE ($-0.81 \pm 0.08‰$), CZ ($-1.00 \pm 0.08‰$) and SI ($-1.18 \pm 0.09‰$). BE and SI were also significantly different. IT, with $22.0 \pm 0.23‰$, had significantly higher $\delta^{18}O$ values than BE ($20.0 \pm 0.18‰$) and SI ($20.6 \pm 0.20‰$). There were also marked differences between BE and CZ (Fig. 2).

Correlations between leaf traits and secondary xylem

There was a significant positive correlation between foliar $\delta^{13}C$ and mean ring width (Table S3). However, this pattern held true only when data from all provenances were considered together (Fig. 3).

When provenances were analyzed separately, specific correlation patterns occurred between leaf traits and wood anatomy in some provenances (Table S3). For BE alone, there was no significant correlation detected at all, whereas IT showed an interconnection between N% and mean lumen area and radial vessel diameter, as well as between SLA and

vessel density, mean lumen area, and radial and tangential vessel diameter. In SI, $\delta^{15}N$ was correlated with mean ring width and vessel density, while $\delta^{18}O$ was correlated with mean ring width, vessel density, mean lumen area, and radial and tangential vessel diameters. In addition, leaf weight was correlated with the percentage of conductive tissue. For the CZ provenance, $\delta^{15}N$ was correlated with mean ring width, while leaf weight was correlated with vessel density, and leaf area with the percentage of conductive tissue (Table S3).

Correlations between SLA and leaf physiological traits

SLA was significantly correlated with C% and N% in IT and CZ provenances (Fig. 4). Except for SI, SLA was negatively correlated with $\delta^{13}C$ (Fig. 5a). With increasing SLA, there was a decline in intrinsic water use efficiency (iWUE). There was no consistent correlation between $\delta^{15}N$ and SLA. However, there was a significant correlation between SLA and $\delta^{18}O$ for BE, IT, and CZ provenances (Fig. 5c).

Ectomycorrhizal community

There was a total of 48 ectomycorrhizal genera associated with beech roots in the provenance trial. The site was dominated by Basidiomycota (80%). Throughout, the most dominant genera at the site were *Russula* (24.37%), *Elaphomyces* (13.00%), *Lactarius* (10.36%), *Clavulina* (8.32%), *Tomentella* (7.7%), *Hygrophorus* (6.92%), *Inocybe* (6.3%), *Sebacina* (4.48%), *Cortinarius* (4.45%), and *Hysterangium* (4.45%) (Fig. 6a, b). Generally, ectomycorrhizal community
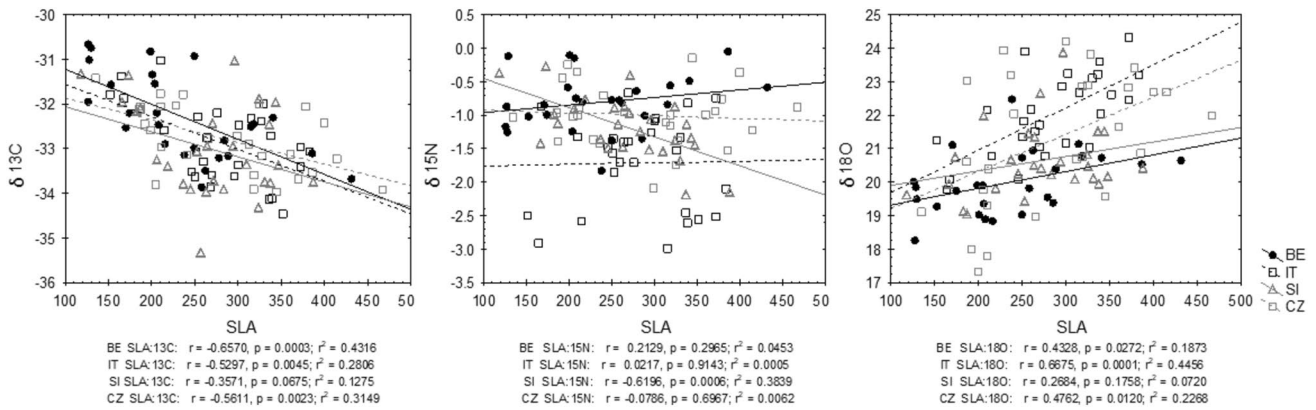

Fig. 3 Correlation between foliar $\delta^{13}C$ and mean ring width (MRW) for all provenances together in Kamenski vrh provenance trial, Slovenia in 2019
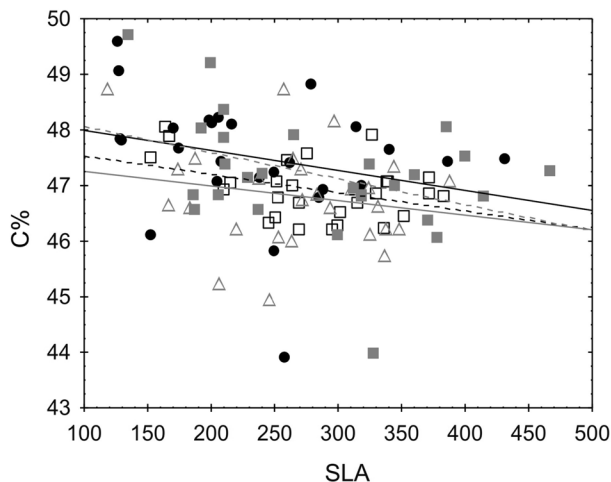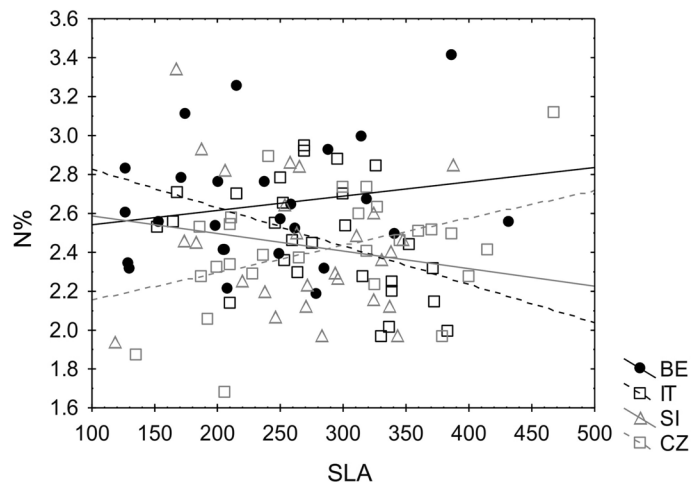
Fig. 4 Relationships between SLA and leaf C% and N% of four beech provenances (BE, IT, SI, and CZ) in Kamenski vrh provenance trial, Slovenia, in 2019

Fig. 5 Relationships between SLA and bulk leaf δ¹³C, δ¹⁵N, δ¹⁸O of four beech provenances (BE, IT, SI, and CZ) in Kamenski vrh provenance trial, Slovenia, in 2019

composition did not differ among the provenances (PER-MANOVA, $p = 0.102$). Diversity indices (Table S4) were comparable ($p = 0.308$ for species richness, $p = 0.778$ for evenness and $p = 0.658$ for dominance). Species richness ranged from 12.22 to 13.92 (Table S4).

Although the general community composition did not differ significantly among the provenances, abundances of 25 genera were significantly different, both among abundant and rare taxa (18 abundant and 7 rare genera) (Fig. 7, Table S5). Provenance similarity according to ECM community composition showed a grouping of BE and CZ in one cluster and IT and SI in a second cluster (Fig. S5).

CCA ordination (Fig. 8) provided an explanation for 9.46% of the total variability through the first axis and 6.83% through the second. Altogether, the first two CCA axes explained 16.29% of the total variability. We observed statistically significant effect of several tree variables, e.g., mean leaf area ($p = 0.038$), δ¹³C ($p = 0.001$), δ¹⁵N ($p = 0.001$), and δ¹⁸O ($p = 0.007$) in separating of ECM in general, where ECM taxa *Byssocorticium*, *Balsamia*, and *Pachyplodes* showed tendency to occur with trees with higher values of δ¹³C and higher mean leaf area. Meanwhile, *Rhizopogon* showed a tendency to occur with trees with higher values of δ¹⁵N in contrast to *Hebeloma* and *Tuber* which occurred with trees with higher values of δ¹⁸O.

Discussion

Beech is believed to respond very plastically to environmental conditions (Eilmann et al. 2014). Our study focused on the response of four provenances originating from three different climates, Alpine, Atlantic and continental, to optimal growth conditions.

Secondary xylem

Only insignificant differences in radial increment between the provenances were observed in 2019, with BE and CZ at the upper and IT and SI at the lower point. Consistent with this result, in the Netherlands, variation in radial growth was mainly controlled by the environment and only slight differences were attributed to genetic background (Eilmann et al. 2014). On the other hand, it has been pointed out that wood anatomical traits are mainly under genetic control (Eilmann et al. 2014). In the year of the present study, with no extreme weather conditions, BE had larger vessel diameters, higher mean lumen area and relative conductive area compared to IT. Larger diameters allow for better xylem water transport, but they are prone to xylem embolies (Sperry 1993; Tyree and Zimmermann 2002). BE originates from a humid Atlantic area, where precipitation events are more evenly distributed over the whole growing season, drought events with an elevated risk of xylem embolism are less probable and mild temperatures are characteristic all year-round. IT and SI originate even from more humid areas, but they had to adapt to freezing conditions at their original locations since they are both from Alpine regions. Freezing of xylem sap in winter induces the formation of air bubbles, which may lead to cavitation (Sperry 1993). On thawing the air bubbles merge, expand and embolize the complete conduit (Charrier et al. 2017). In alpine areas, freeze–thaw cycles occur daily in late winter to early spring. When air temperatures increase while the soil is still cold or frozen, this promotes aboveground dehydration and hydraulic failure, so-called winter drought. Low soil temperatures, not necessarily below 0 °C, limit root water uptake (Charrier et al. 2021). Apparently, beech provenances from the Alps avoid these threats through small diameter vessels, which are less prone to embolism.
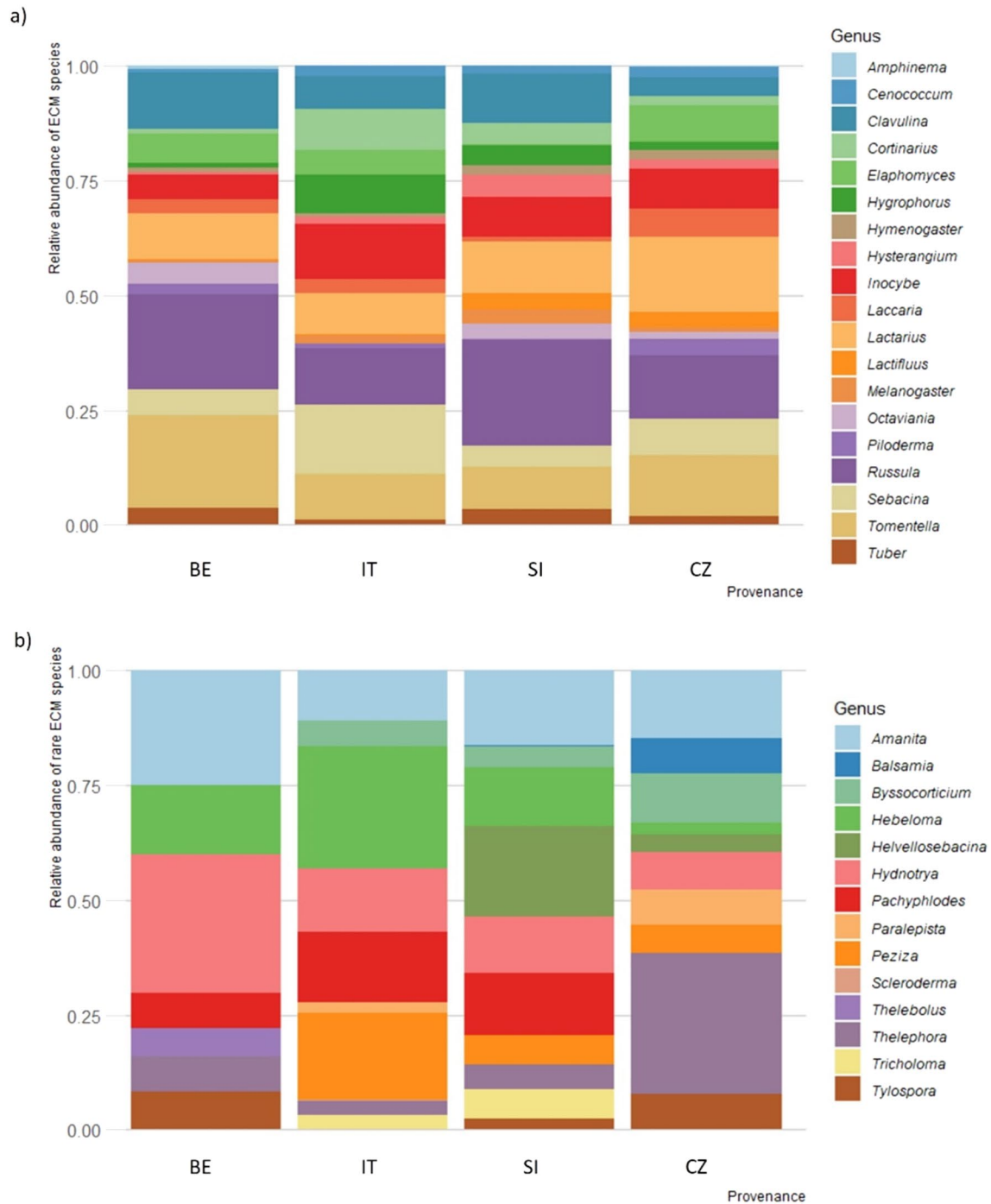
Fig. 6 Relative abundances of abundant (**a**) and rare (**b**) ECM taxa for four beech provenances (BE, IT, SI, and CZ) from beech provenance trial in Kamenski vrh, Slovenia. Abundant ECM taxa appear at least 10-times in each provenance and in a min. of 20% of samples, while rare ECM taxa occur in less than 20% of samples, but at a minimum limit of at least 5% of samples

Leaf traits

As shown for beech, leaf area adjustments to the growth site are limited, to a certain extent leaf area responds to the meteorological conditions of the previous year and the current year meteorological characteristics (average temperature and precipitation sum in April–May) (Petrik et al. 2022). In Polish provenance trial, early flushing beech provenances developed larger leaf areas (Kempf et al. 2018), which could be associated with better efficiency in terms of use of water resources in early spring, when water supply is usually ample. Although leaf phenology was not recorded
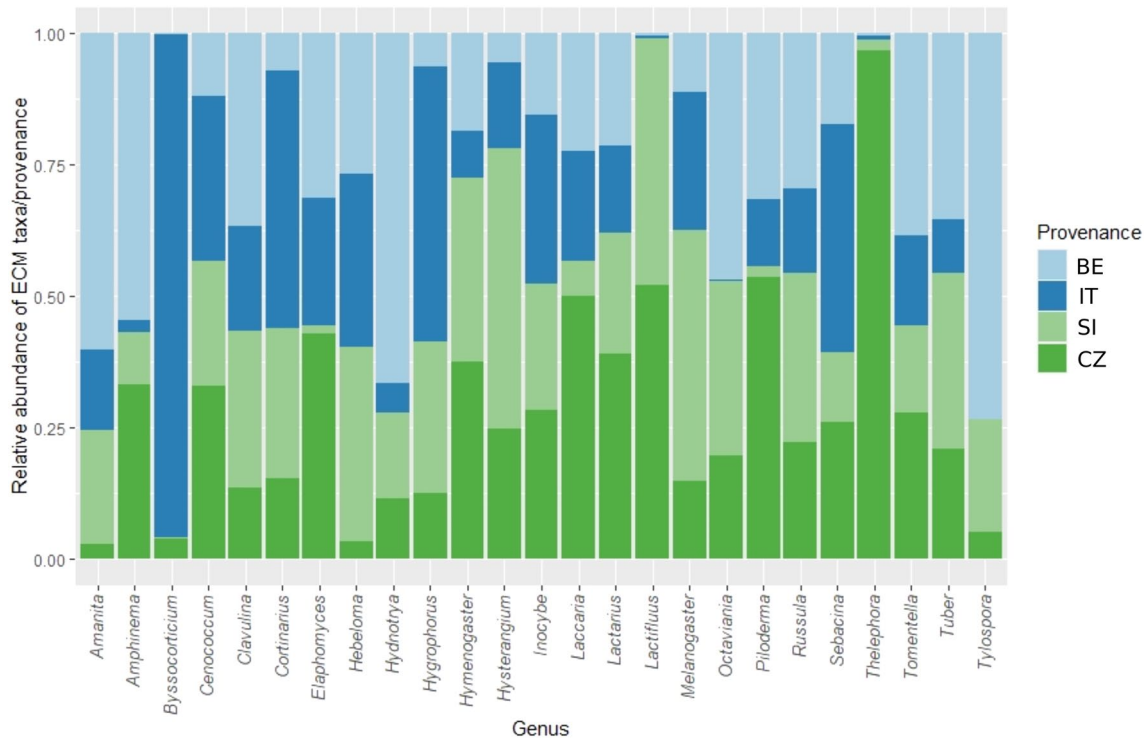
Fig. 7 Relative abundance of ectomycorrhizal taxa for four beech provenances in Kamenski vrh provenance trial. Only taxa with statistically significantly different abundances among the provenances are shown. See Table S5 in supplementary material for marked differences
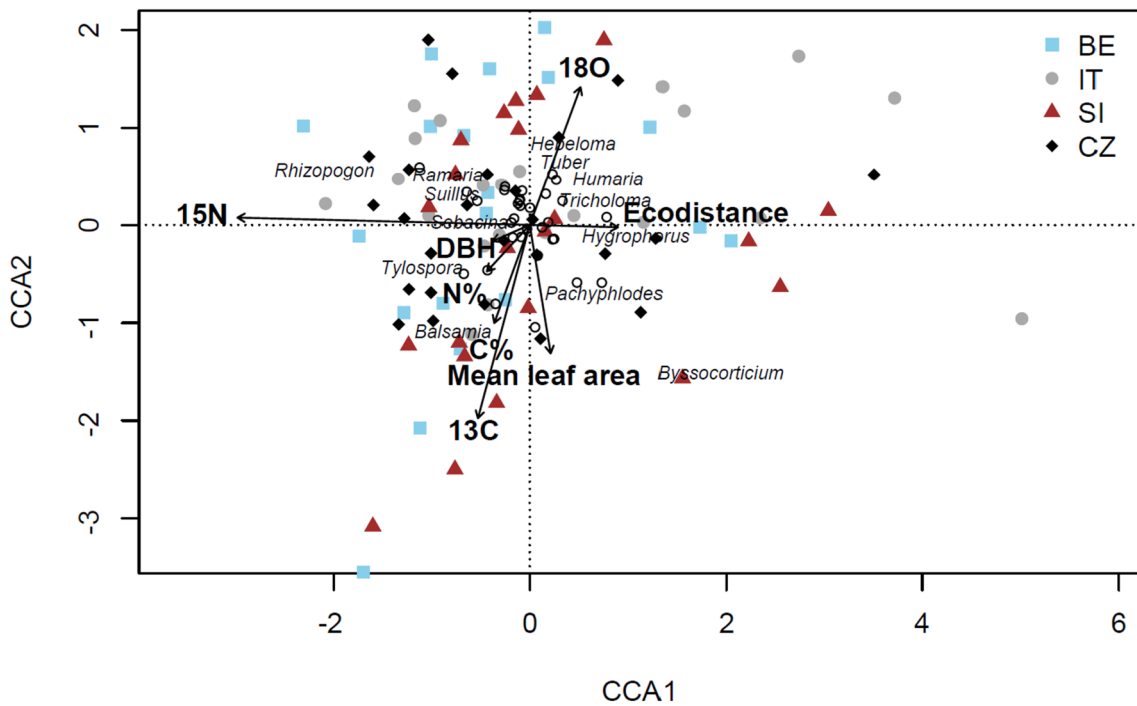

Fig. 8 CCA ordination for ECM community in beech provenance trial in Kamenski vrh, Slovenia. The first CCA axis explains 9.46% of the variability, while the second CCA axis explains 6.83% of the variability. Different shapes in the ordination represent ectomycorrhizal samples collected from the specific provenance (BE, IT, SI, and CZ). Only statistically significant tree performance indicators ($p < 0.05$) and representative ECM fungal taxa with the highest priority are shown (open circles)

in our research, data from other provenance trials support the correlation of leaf area with the time of flushing. In a Spanish provenance trial, the same IT provenance (Val di Sella), that developed the highest leaf area in our study, flushed at day 114, while BE provenance Soignies flushed at day 124 (Robson et al. 2011). Early flushing provenances might be under threat due to late spring frosts (Robson et al. 2012), the frequencies of which are suggested to increase in the future (Zohner et al. 2020).

The foliar $\delta^{13}C$ in C3 plants depends on the ratio between the partial pressures of CO_2 at the sites of carboxylation in the chloroplast and in the ambient air, and it is a good proxy for leaf-level intrinsic water use efficiency (iWUE) (Dawson et al. 2002; Moreno-Gutiérrez et al. 2012). Plants may achieve high iWUE through lower transpiration or high net photosynthesis, or both (Farquhar et al. 1989). Since $\delta^{18}O$ is believed to be independent of variation in net photosynthetic rate, it can help to distinguish the independent effects of net photosynthetic rate and stomatal conductance on $\delta^{13}C$ Moreno-Gutiérrez et al. 2012).

Foliar $\delta^{13}C$ values in the selected provenances were the most negative for SI, i.e., discrimination against ^{13}C was high. This is indicative of low iWUE (Moreno-Gutiérrez et al. 2012), which is in line with the highest precipitation at the origin of this provenance (Table S1), which could result in adaptation to an ample water source with no need to save water. On the other hand, BE provenance, achieved the highest iWUE. BE provenance, although from the Atlantic region where water through the growing season is always available, still receives almost three time less precipitation compared to the original location of SI. Concerning the results based on $\delta^{13}C$, some caution should be taken when drawing conclusions on genotypic differences in iWUE from the $\delta^{13}C$ signals of bulk leaves, as reported in the study of poplar provenances, mainly due to discrepancies found between $\delta^{13}C$ in bulk leaves and wood cellulose in some of the investigated genotypes (Verlinden et al. 2015). In their study, the highest iWUE was associated with genotype with the earliest leaf phenology, which was not the case in our study, where BE was late flushing. These contradictory results indicate that larger datasets should be investigated to confirm whether iWUE could be deduced from the precipitation of the original location or any other trait.

There were striking differences in the $\delta^{18}O$ of the beech provenances, with BE showing the lowest and IT the highest values. As leaf $\delta^{18}O$ negatively correlates with stomatal conductance in oaks (Cocozza et al. 2020) and other plants (Barbour et al. 2000), we infer that IT had the lowest seasonal stomatal conductance and BE the highest. BE apparently achieved the highest iWUE in another way but limiting stomatal conductance or alternatively, $\delta^{18}O$ might not be appropriate indicator of stomatal conductance in beech.

There was considerable differentiation among the four provenances in $\delta^{15}N$. $\delta^{15}N$ reflects differences in N source, N fractionation during uptake or its internal re-allocation after absorption (Aranda et al. 2017). However, since the trees grew on a uniform site, differences in N source can be ruled out. Plant $\delta^{15}N$ may additionally be affected by ectomycorrhizal fungi (Hobbie and Högberg 2012). In fact, more positive values of $\delta^{15}N$ appeared to be related to the ECM genus *Rhizopogon* (see below). In our study, IT had the lowest $\delta^{15}N$ values. In beech, low $\delta^{15}N$ values in leaf bulk tissues are associated with drought sensitivity (Peuke et al. 2006). On the other hand, foliar $\delta^{15}N$ also changes with phenological phase, as shown for *Fagus crenata*, in which values decreased from green leaf to pre-abscission (Enta et al. 2020). Since our knowledge of multiple effects on foliar $\delta^{15}N$ values are still limited, our results require further studies to disentangle differences in the foliar $\delta^{15}N$ values of our provenances.

Correlations between leaf traits and secondary xylem

There was a significant positive relationship between foliar $\delta^{13}C$ and mean ring width, although this pattern was detected only when data from all provenances were considered together. Individuals with less negative values of foliar $\delta^{13}C$ had wider growth rings, regardless of provenance, which means that they achieved a better ratio between water loss relative to carbon assimilation (i.e., the definition of water use efficiency). However, since this observation is based on only one growing season, without considerable weather extremes, years with extreme events might differentiate among provenances.

Various correlations between leaf morphological and physiological traits and secondary xylem anatomy that were found within IT, SI, and CZ provenances are supported by the finding that leaf transpiration and carbon gain are coupled to the xylem water transport system in trees, as seen by scaling between leaf and xylem area (Petit et al. 2018). We found correlations among SLA, leaf weight or leaf area and vessel density, vessel dimensions, and percentage of conductive tissue. Individuals with larger vessel diameters (and smaller vessel density) within IT provenance developed larger SLA, while individuals with higher percentage of conductive tissue developed higher leaf weight within SI provenance, but higher leaf area within CZ provenance. On the other hand, individuals with higher vessel density within CZ provenance developed higher leaf weight. Efficient xylem water supply either through increased vessel size, or increased conductive area positively affects leaf expansion in either dimension through the improvement of cell turgor. The absence of this relationship within BE provenance indicates that its highly efficient water conducting system does not

represent any limitation to leaf growth under investigated conditions with sufficient precipitation. Correlation of $\delta^{18}O$ to xylem anatomical characteristics within SI provenance indicated that individuals with the highest vessel lumen area experienced the lowest stomatal conductance. SI provenance was the only one in this study that was transferred to drier environment and apparently the individuals with the highest water supply to leaves had to close their stomata more often; whereas in provenances transferred to wetter environments (Table S1), the extent of stomatal regulation was so low that did not result in statistically significant numbers. Since the number of samples per provenance investigated for correlations between leaf traits and secondary xylem was relatively low, further evidence is needed as to disentangle the control of water supply to leaves in beech.

Correlations between SLA and leaf physiological proxies

In addition to environmental factors, $\delta^{13}C$ in plants and leaf iWUE are also controlled by plant characteristics, such as specific leaf area and leaf N content (Seibt et al. 2008). There was a significant negative correlation between SLA and $\delta^{13}C$ for all provenances except SI. A higher iWUE within some provenances was apparently associated with lower SLA (i.e., thicker leaves). This is consistent with findings for various woody species by Mediavilla et al. (2001). Furthermore, within a species, thicker leaves have a lower tissue density, with more air volume which enhances internal CO_2 conductance to the site of carboxylation (Mediavilla et al. 2001). On the other hand, low SLA had lower $\delta^{18}O$ values which suggests that the stomatal conductance of thicker leaves was better. Accordingly, it can be concluded that thicker leaves achieve better iWUE, despite relatively high stomatal conductance, obviously through improved internal CO_2 conductance to the site of carboxylation. Indeed, it has been reported for beech that higher maximum carboxylation rates and better iWUE are achieved in thicker leaves along the vertical canopy profile (Scartazza et al. 2016). A positive association between leaf mass area (inverse of SLA) and iWUE appears to be a general pattern in plants (Petrík et al. 2023) and is based on a positive relationship between leaf thickness and mesophyll conductance to CO_2 (Tomás et al. 2013). In the light of climate change, thicker leaves would be beneficial from the viewpoint of water deficit, but not from the viewpoint of higher temperatures, since thicker leaves dissipate heat less efficiently (Leigh et al. 2017).

In beech and plants in general, leaf thickness is usually correlated with N content per area, with thinner leaves (high SLA) exhibiting lower N contents per leaf area (Mediavilla et al. 2001; Reich 2014; Scartazza et al. 2016; Li et al. 2016) and lower photosynthetic capacity per area (Reich 2014, and high SLA exhibiting higher N per mass and higher photosynthetic capacity per unit of leaf mass (Reich 2014). We found contrasting patterns among our provenances for SLA-N% relationship (Fig. 4) which requires further studies.

Ectomycorrhizal community

Ectomycorrhizal taxa encountered in our study corresponded to ECM communities typically reported for beech forests (e.g., Pena et al. 2010). In comparison to values recorded in the same provenance trial when the trees were 7 years old (Westergren et al. 2004), species richness increased. The improved values correspond to the succession of ectomycorrhizal fungi with stand age (Fernandez-Toiran et al. 2006; Twieg et al. 2007) in a combination with colonization of previously clearcut area in the forest (Jones et al. 2003) where the trial was established.

Studies on conifers showed that the effect of tree provenance on ECM community is relatively small compared to the effect of the environment (Pérez-Izquierdo et al. 2019; Kujawska et al. 2023), while studies on broadleaved species are largely missing. Present study confirms findings for conifers as we observed no significant variation in the diversity indices of ECM fungi among the provenances and the community composition appeared to be relatively consistent across the provenances, although we were able to detect differences in abundances in more than half of the present ectomycorrhizal genera (Fig. 7, Table S5). This finding is probably due to relatively similar abundances of the most dominant genera. As our investigation was done on a genus level, even bigger differences could be encountered if species level was considered. Two clusters of provenances with greater similarity of ECM communities were detected, a first cluster with IT and SI, and a second one with BE and CZ. IT and SI both originate from the Alpine region, but are not genetically related (Marjana Westergren, personal communication). However, they were both transferred to the environment which is similar to their original locations.

In this provenance trial, CZ was the slowest growing provenance (Železnik et al. 2019) and it hosted almost all *Thelephora* ectomycorrhizae. Similarly, *Thelephora* was significantly more abundant in slow growing (height) clones of *Picea abies* with different origins (Korkama et al. 2006). We, therefore, conclude that *Thelephora* may be less conducive for height growth than other genera of ECM. This could be due to high respiration rates of root system colonized by this fungus which drains carbohydrates belowground. However, this finding should not be generalized as under some circumstances this fungus may be beneficial for tree growth (Dixon et al. 1984; Colpaert 1999). Negative effects on tree growth by *Thelephora* may relate to specific nutrient conditions, e.g., low N or specific forms of N (Colpaert et al. 1996; Finlay et al. 1992). On the other hand, IT, as the best growing provenance

(Krajnc et al. 2022), hosted almost all *Byssocorticium* ectomycorrhizae. *Byssocorticium* has been reported to be one of the taxa contributing most to the enzymatic activity of an ECM community and may therefore be very important for nutrient uptake (Rineau and Courty 2011), even if its relative abundance is low (Courty et al. 2008), as in our case. With its important role in nutrient release from organic compounds, it could support the IT provenance with its demand for fast growth.

In our study, there was higher impact from tree morphology and physiology compared to provenance on the structure of the ECM community. Symbiont-specific response of iWUE in needles was observed in conifers inoculated with different species of ectomycorrhizal fungi and was in any case higher compared to non-mycorrhizal controls (Guehl et al. 1990; Guehl and Garbaye 1990; Hobbie and Colpaert 2004). The mechanism behind the improved iWUE in mycorrhizal plants compared to non-mycorrhizal controls is in the improvement of nutrient availability which increases the amount of delivered nutrient per amount of transpired water (Hobbie and Colpaert 2004). Fungal symbionts might provide plants with access to forms of N through enzymatic activity which would otherwise be completely unavailable (Smith and Read 2008). The association of *Byssocorticium*, which was reported as an important contributor to tree nutrition (see previous paragraph), with trees expressing higher values of $\delta^{13}C$ (better iWUE) and higher leaf area is, therefore, reasonable; while for other two taxa associated with these traits (*Balsamia, Helvellosebacina*), there are no existing reports on their function up to our knowledge. Ectomycorrhizal fungi may modulate $\delta^{15}N$ values in leaves (Hobbie et al. 1999, 2000), which was attributed to enzymatic reactions within the fungi producing isotopically depleted ammonia and amino acids, which are subsequently passed on to plant symbionts (Hobbie et al. 1999; Hobbie and Högberg 2012). ECM fungi that take up organic nitrogen, exhibit higher $\delta^{15}N$ values (Trudell et al. 2004). *Rhizopogon*, associated with higher $\delta^{15}N$ values in our study is an ECM fungus which forms highly differentiated rhizomorphs (threads of hyphae which extend far into soil and increase the volume of exploited soil), therefore belonging to the so-called long-distance exploration type (Agerer 2001). This exploration type is adapted to explore for organic N (Lilleskov et al. 2011), which supports our finding. Inoculation with ectomycorrhizal fungi may also increase stomatal conductance (Nardini et al. 2000; Wang et al. 2021), thus affecting $\delta^{18}O$ values. The reported improvement in stomatal conductance in *Quercus ilex* plants inoculated with *Tuber* (Nardini et al. 2000) is consistent with our results. The increase of stomatal conductance was through the expansion of fine root surface area which could supply more water to aboveground parts (Nardini et al. 2000) or alternatively,

some ectomycorrhizal fungi may increase the absorptive capacity of roots by forming extensive mycelia (Wang et al. 2021).

The strong correlation between $\delta^{15}N$ values in leaves and the presence of *Rhizopogon* is rather surprising since *Rhizopogon* is mainly reported from Pinaceae. However, the present provenance trial was established in a beech-silver fir forest. Even today, some regeneration of silver fir is found below the trial beech trees. The extensive network of rhizomorphs that *Rhizopogon* can form (Molina et al. 1999) probably extends into the rhizosphere of beech trees. Rhizomorphs can also provide an interplant exchange of nutrients (Simard et al. 1997) which could explain its association with high $\delta^{15}N$ values in beech leaves, but further evidence is needed to confirm this finding.

Conclusions

Under average weather conditions, all the investigated provenances showed comparable radial growth in the investigated year. However, there was a very general pattern that individuals with high iWUE had better radial growth. High iWUE was associated with lower SLA (thicker leaves). Thicker leaves had better stomatal conductance as indicated by $\delta^{18}O$, so high iWUE must have been achieved through higher carboxylation efficiency or net photosynthesis.

Vessel diameter variation among the provenances indicated an adaptation of this trait to the site of provenance origin. Wide vessels were found in BE which originates from an Atlantic climate with evenly distributed precipitation and only minor frost events. On the other hand, narrow vessels occurred in SI and IT which face a climate with more severe frost events. Despite a rich water supply for all provenances in the trial, their strategies for water use, as indicated by $\delta^{13}C$, were different, with BE gaining more carbon per unit of water used compared to SI. Obviously, in normal years without drought events and frosts, BE would flourish due to its efficient water transport system, good water use efficiency, and high carbon gain, but its wide vessels pose a risk for embolism in the case of extreme events.

Provenance was not a strong determinant of the ECM fungal community composition. Some taxa of ECM fungi were correlated with individuals with specific morphological and physiological traits, making it worth testing whether the inoculation with these taxa could modulate the efficiency of trees in their resource use.

To obtain an insight into the plasticity of the researched provenances the study should encompass longer period, ideally experiencing contrasting weather conditions, or alternatively, more locations of provenance trials in contrasting site conditions in the single year should be investigated.

Declarations

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