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Aerial and underground organs display specific metabolic strategies to cope with water stress under rising atmospheric CO$_2$ in *Fagus sylvatica* L.

Brígida Fernández de Simón [ORCID] | **Estrella Cadahía** | **Ismael Aranda** [ORCID]

Grupo de Ecología Funcional de Especies Forestales, Centro de Investigacion Forestal (CIFOR-INIA) CSIC, Madrid, Spain

Correspondence
Brígida Fernández de Simón and Ismael Aranda, Grupo de Ecología Funcional de Especies Forestales, Centro de Investigacion Forestal (CIFOR-INIA), CSIC, Carretera de A Coruña Km 7.5, 28040 Madrid, Spain.
Email: fdesimon@inia.csic.es and aranda@inia.csic.es

Abstract

Beech is known to be a moderately drought-sensitive tree species, and future increases in atmospheric concentrations of CO$_2$ ([CO$_2$]) could influence its ecological interactions, also with changes at the metabolic level. The metabolome of leaves and roots of drought-stressed beech seedlings grown under two different [CO$_2$] (400 (aCO$_2$) and 800 (eCO$_2$) ppm) was analyzed together with gas exchange parameters and water status. Water stress estimated from predawn leaf water potential (Ψ_{pd}) was similar under both [CO$_2$], although eCO$_2$ had a positive impact on net photosynthesis and intrinsic water use efficiency. The aerial and underground organs showed different metabolomes. Leaves mainly stored C metabolites, while those of N and P accumulated differentially in roots. Drought triggered the proline and N-rich amino acids biosynthesis in roots through the activation of arginine and proline pathways. Besides the TCA cycle, polyols and soluble sugar biosynthesis were activated in roots, with no clear pattern seen in the leaves, prioritizing the root functioning as metabolites sink. eCO$_2$ slightly altered this metabolic acclimation to drought, reflecting mitigation of its effect. The leaves showed only minor changes, investing C surplus in secondary metabolites and malic acid. The TCA cycle metabolites and osmotically active substances increased in roots, but many other metabolites decreased as if the water stress was dampened. Above- and belowground plant metabolomes were differentially affected by two drivers of climate change, water scarcity and high [CO$_2$], showing different chemical responsiveness that could modulate the tree adaptation to future climatic scenarios.

1 | INTRODUCTION

Beech (*Fagus sylvatica*), a significant tree species in European natural forests, might increasingly be threatened by the ongoing climate change, particularly in its southernmost distribution range (Chen et al., 2015). Drought stress is the most important abiotic factor limiting plant performance in the Mediterranean region (Morales et al., 2005), which is experiencing its warmest period of the last millennium (Guiot & Corona, 2010), together with a decrease in summer rainfall. These trends are expected to continue in the next decades (IPPC, 2018), affecting woody vegetation in the mountains of the Mediterranean basin, especially broad-leaved species best adapted to the climate in higher latitudes. Beech is known to be a moderately drought-sensitive forest tree species (Backes & Leuschner, 2000;

Aranda et al., 2002) when compared to other temperate forest tree species from the *Acer*, *Picea*, *Quercus*, *Carpinus*, *Tilia*, *Sorbus*, or *Fraxinus* genera (reviewed in Leuschner, 2020). This sensitivity is partly related to functional traits, reflecting a low capacity to deploy mechanisms of drought tolerance (Aranda et al., 2002, 2005). Besides, the location of their intense fine root system in the upper soil layer (with less than 5% below 1 m) (Meier et al., 2018), together with its poor responsiveness to climatic gradients (Förster et al., 2021), would limit its ability to explore deeper and wetter soil areas and to adapt to environmental situations with limited soil water availability.

Beech responses to drought are complex processes due to the important morphological and physiological alterations that arise in the face of water scarcity (reviewed in Bréda et al., 2006, and Leuschner, 2020) but also due to the molecular changes that also happen at the metabolic level. Water stress modifies carbon and nitrogen metabolism in beech leaves (El Zein et al., 2011; Peuke et al., 2006) in pace with decreases in the predawn water potential (Ψ_{pd}) (He et al., 2020). Some of the changes imply the accumulation of non-structural carbohydrates (NSC) (Aranda et al., 2018; Liu et al., 2017; Peuke et al., 2002; Schraml & Rennenberg, 2000). These increases in the pool of carbohydrates are linked to their osmotic adjustment capacity (Aranda et al., 2021). Other metabolites with potential osmoprotectant properties, and that also build up in response to drought are some amino acids, mainly proline, but also valine, aspartic acid, and γ-aminobutyric acid (Aranda et al., 2018). The foliar levels of secondary metabolites, mainly phenolic compounds, are also affected differently by water stress depending on the specie. Drought limits their accumulation in beech (Aranda et al., 2018), as in other tree species such as eucalyptus (Correia et al., 2016) or willow (Turtola et al., 2005), while the impact of unfavorable environmental conditions is usually related to higher levels of foliar terpenic and phenolic compounds in other species (Fernández de Simón et al., 2017, 2021; Sumbele et al., 2012; Tattini et al., 2004). Foliar phenolics in trees are related to the chemical defense, but they also play a role as antioxidants and in photoprotection (Agati et al., 2012; Agati et al., 2013; Noctor et al., 2015).

Although the metabolomic changes triggered by drought in beech leaves have received much attention in recent years, studies on roots have focused almost exclusively on mineral nutrients, given their reduced availability for roots on dry soils and the impairment of water and nutrients uptake (Brunner et al., 2015; Gessler et al., 2017), and on non-structural carbohydrates (Hagedorn et al., 2016). More specifically, decreased levels of K, Mg, Ca, P, and N macronutrients were detected in beech roots during drought (Peuke & Rennenberg, 2011; Zang et al., 2021), with soil type and provenance having a great influence (Leberecht et al., 2016; Netzer et al., 2016). Decreased starch concentrations and an increase in C, N, and soluble carbohydrates, mainly driven by sucrose levels, were found in fine beech roots of young and adult water-stressed trees (Hagedorn et al., 2016; Nikolova et al., 2020). However, to our knowledge, the study of drought-triggered changes in the overall metabolome of beech roots has not yet been addressed.

Drought is the abiotic stress most studied in beech trees, but other climate and global change drivers, such as high levels of atmospheric CO_2 (eCO_2), might modulate and mitigate the functional

response of beech trees to drought (Heath & Kerstiens, 1997). The current CO_2 enrichment of the atmosphere, a process inherent to climate change, has increased in a very short time to levels above 400 ppm, compared to a relative constancy in the previous 100,000 years of between 200 and 300 ppm (NOAA, 2021). Therefore, understanding plant responses to future eCO_2 will become increasingly important as CO_2 levels rise, as they trigger the stimulation of carbon net uptake, modifying the carbon balance and the growth of species and individuals (Huang & Xu, 2015). Enrichment of CO_2 in the atmosphere might also trigger other response mechanisms under drought influencing plant performance (Becklin et al., 2017), such as water-saving effects and enhancement of photosynthesis (Aranda et al., 2020; Kelly et al., 2016; Je et al., 2018). This response of trees to eCO_2 may be influenced by drought duration and severity (Birami et al., 2020; Duan et al., 2013; Peñuelas et al., 2017), as well as being species- and ecosystem-specific (Xu et al., 2015).

From a metabolic point of view, a series of chemically driven changes were previously observed in trees coping with water scarcity under eCO_2, although the impact of drought was greater than that of eCO_2, which only delayed or eliminated some of the changes produced by drought (Niinemets, 2010). This suggests that plants growing under eCO_2 perceive lesser stress during drought, eliciting a differential response according to each specific organ (Bachofen et al., 2018; Birami et al., 2020). These changes ranged from increased levels of C-rich compounds that may be coupled to an increase in carbon stored as starch (Duan et al., 2013), to an antioxidant protective effect of eCO_2 under drought conditions, due to a slower rate of ROS production together with a stronger antioxidant metabolism (Fernández de Simón et al., 2020; Schwanz & Polle, 2001; Sekhar et al., 2017). Enhanced levels of soluble sugars were found in the roots of pine species (Bachofen et al., 2018; Duan et al., 2015; Runion et al., 1999), lemon (Paudel et al., 2018), and eucalyptus (Duan et al., 2013), while the amount of starch was decreased or unchanged in lemon saplings (Paudel et al., 2018) and eucalyptus (Duan et al., 2013), respectively. Leaves were also found to have contrasting responses in carbohydrate metabolism depending on tree species (Bachofen et al., 2018; Duan et al., 2015; Picon-Cochard & Guehl, 1999). In some cases, surplus carbon was also funneled into secondary metabolites, reinforcing their antioxidant system (Fernández de Simón et al., 2020; Runion et al., 1999). On other hand, differential stoichiometric and metabolomic shifts have been observed according to the organ. N, P, and other elements also showed an asymmetrical relationship between leaves and roots (Gargallo-Garriga et al., 2015; Sardans et al., 2017), as well as it was observed for the overall primary metabolome in *Pinus halepensis* (Birami et al., 2020).

We have established several working hypotheses for a better understanding of the molecular responses in beech under CO_2 enriched atmospheres: (1) the beech metabolome will differ between leaves and roots due to their different functions as sources and sinks of carbon; (2) drivers of global climate change, such as drought and eCO_2 triggers a differential metabolite accumulation in leaves and roots, implying the up−/down- regulation of different metabolic pathways; (3) drought sensitivity of beech trees could be alleviated by increasing atmospheric [CO_2] through a series of chemically driven

changes in both leaves and roots. We analyzed the metabolome of leaves and fine roots of drought-stressed beech seedlings grown under two different levels of atmospheric $[CO_2]$ (ambient (aCO_2) (400 ppm) and enriched (eCO_2) (800 ppm)). We provide novel insights on not only the root metabolome but also on the impact of eCO_2 on leaf and root drought responses in *F. sylvatica* seedlings. This global analysis of plant leaves and roots is crucial to project the impacts of global change in the metabolic composition of plant communities, as it can trigger possible imbalances between aerial and underground processes.

2 | MATERIALS AND METHODS

2.1 | Experimental setting and plant growth

Seedlings from three open-pollinated families of contrasted performance, texted on a previous essay under greenhouse conditions (Aranda et al., 2017), were selected for the experiment. One-year-old seedlings were transplanted in 2015 in 6 L pots filled with a 3:1 (*v:v*) mixture of peat/sand. Pot substrate was fertilized with 2 kg m^{-3} of 6 months slow-release fertilizer (Osmocote Plus fertilizer, 16-9-12 NPK + 2 micronutrients, Scotts) added to the growth substrate. Twelve seedlings from each family were arranged according to a full random design in two walk-in growth chambers with ambient (400 ppm) and enriched (800 ppm) CO_2 atmospheres (Fitoclima 10000EHHF, Aralab Ltd.), and under optimal conditions of lighting (16/8 h light/dark photoperiod). Maximum PPFD was 700 μmol photons m^{-2} s^{-1} at the top of plants during the light period. Relative humidity and day-night temperature were maintained at 60%–65% RH and 20–25°C, respectively. To overcome a possible chamber effect, the location of the seedlings within each growth chamber was changed every 2 weeks, and all plants were moved at monthly intervals between chambers. The $[CO_2]$ of 800 ppm was reached by injecting CO_2 in the inlet air to the growth chamber from a bullet of N-CO_2 mixture. Seedlings were well-watered from one and a half month after the beginning of the bud burst. Subsequently, under both CO_2 treatments, half of the seedlings continued to be well watered (C plants) while irrigation was progressively reduced in the other half (D plants). Water use by individual plants was different because of the differential plant water consumption, related to the atmospheric CO_2 of the growth chamber and initial plant size. Therefore, watering was adjusted plant per plant to ensure that effective water stress endured by seedlings was imposed at the same rate and intensity in both CO_2 environments. Accordingly, the water used by the plants was controlled by weighting individual pots three times a week and by the additional measurement of volumetric soil water content (SWC) using TDR probes (CS650-L, Campbell Scientific Inc.) connected to a datalogger (CR1000, Campbell Scientific). Watering was adjusted by analyzing the water consumption between two consecutive watering dates. Effective water stress, and the rate of imposition, resulted in only differences given by the moderate drought during the period of stress. The precise control of watering began 2 weeks before the beginning of the water stress period imposition, up to the end of the experiment 40 days from the beginning of drought imposition. In addition, the water status of the seedlings from predawn water potential (Ψ_{pd}) measured with a Scholander chamber (PMS Instrument Co. 7000, Corvallis) was assessed at the peak of the stress in DOY196 and 1 h before beginning the dial light period in the growth chamber.

2.2 | Leaf ecophysiological traits

Gas exchange was measured in leaves that unfolded and developed under the two CO_2 treatments and 2 h after the beginning of the light cycle of the growth chamber. Net photosynthesis (A_{net}), stomatal conductance to water vapor (g_{wv}), and chlorophyll fluorescence were recorded with a portable photosynthesis system (LiCor 6400 XP, Li-COR Inc.) coupled with an integrated fluorescence chamber (Chamber Li-6400-40, Li-COR Inc.). The environmental conditions of the gas exchange chamber were 1200 μmol m^{-2} s^{-1} PPFD, previously tested to be saturating for A_{net}, a constant VPD of ~1.2 KPa, optimum leaf temperature was set to 25°C, and the growing CO_2 conditions were 400 and 800 ppm, respectively. The intrinsic water use efficiency (iWUE) was estimated from the quotient A_{net}/g_{wv}. The actual photochemical efficiency of photosystem II (φPSII) was estimated according to Genty et al. (1989) by measuring the steady-state fluorescence (Fs) and maximum fluorescence during a light-saturating pulse (Fm') just after recording the gas exchange. The electronic transport rate through PSII (ETR) was calculated as in previous studies with beech seedlings (i.e. Aranda et al., 2017).

2.3 | Sampling and metabolome extraction

Leaves (L) and fine roots (R) were harvested from 36 studied plants. At the peak of the stress (40 days from the beginning of drought imposition), two leaves were harvested from each plant just after the gas exchange measurement, maintained under dark conditions, and immediately frozen in liquid N_2 and stored at −80°C until freeze-drying. Fine (≤1 mm in diameter) root samples were carefully harvested from each plant by opening a window at the bottom of the pots without destroying the rooting media and stored and freeze-dried, following as for the leaves. These roots are generally considered to lack a lignified structure and are expected to be more active. Freeze-dried plant material was ground to powder in a ball mill (Retsch Mm 300) and stored in a dry and dark atmosphere for no more than 2 months until metabolomics analysis. Metabolite extraction was performed in duplicate, as in Cadahía et al. (2015).

2.4 | Metabolome analysis by GC–MS

An Agilent 6890 N GC system equipped with a quadrupole mass spectrometer Agilent 5973 N was used according to the conditions of Agilent G1979AA Fiehn GC/MS Metabolomics RTL Library Software

(2013). An in-house made reference library with more than 300 mass spectra from known compounds that were analyzed under the same conditions, along with commercial mass spectral libraries (Wiley10/Nist2017 GC/MS Library; Agilent Fiehn GC–MS Metabolomics RTL Library), were used for the identification of compounds, in which a match greater than 95% was sought. Peak areas obtained from selected ion monitoring (SIM) and calibrations made with pure reference compounds analyzed under the same conditions were used to calculate the concentrations of each metabolite, using the internal standard method.

2.5 | Statistical analysis

The open-source software MetaboAnalyst 4.0 (Chong et al., 2018) was used to carry out the statistical analysis of metabolomes. The raw data were logarithmically transformed, Pareto scaled, and subjected to univariate and multivariate analysis.

For each metabolite, a univariate test (ANOVA) was performed to evaluate significant changes regarding organ (O), watering treatment (W), and growth [CO_2] (CO2). The interaction between the factors (O × W, O × CO2, W × CO2 and O × W × CO2) were also included. To analyze significant differences, we applied the Tukey-HSD post hoc tests and False Discovery Rate (FDR), obtaining adjusted p (q-values), and establishing a threshold q-value <0.05 from FDR correction to consider means as significantly different.

To find key metabolites that could discriminate among organs and growth conditions, different multivariate analyses were carried out: Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) to compare the quantitative metabolome of studied organs (leaves and roots); and Partial Least Squares Discriminant Analysis (PLS-DA) to evaluate differences in quantitative metabolite profiles in each organ in relation to watering. Separately in each organ, we also performed an OPLS-DA using the watering treatment as the predictive component to detect those metabolites that undergo the most intense concentration changes triggered by drought. Afterwards, PLS-DA was applied, considering the drought and [CO_2] factors together to obtain models that grouped samples according to the four different classes considered a priori, in each organ separately. We identified the metabolites with higher loading on the predictive models from S-Plots, taking those

showing higher p loadings, and in OPLS-DA, taking into account mainly those with p correlations higher than 0.5 regarding the predictive component. The predictive ability (Q^2) of the model was used as a criterion of model quality in PLS-DA or OPLS-DA models.

3 | RESULTS

3.1 | Water stress and gas exchange

The SWC was higher in potted control plants than in drought-stressed plants, regardless of the atmospheric [CO_2] (Table 1). The Ψ_{pre} confirmed that the effective water stress endured by plants grown under both [CO_2] environments was the same, as it only changed according to watering (Ψ_{pre} lower in D plants than in C). Gas exchange was affected by both watering and [CO_2] in the atmosphere. The A_{net} was higher in plants under eCO_2 for both irrigation levels, C and D (Table 1). The leaf A_{net} in drought-stressed plants grown under eCO_2 was even higher than that of C plants in aCO_2. The ETR changed similarly to A_{net}, with higher values under eCO_2 and a slight decrease under water stress (Table 1). The g_{wv} changed mainly in response to watering, with significantly higher values in control plants than in the drought-stressed and without a clear impact imposed by the CO_2 level. The iWUE was highly stimulated in plants C and D by the atmosphere enrichment, as it was higher under the eCO_2 environment regardless of watering (Table 1). Moreover, water stress also brought about an increase in iWUE beyond the effect of CO_2.

3.2 | Leaf and root contrasting metabolomes

Of the three factors studied, the organ was the most relevant in determining differences in the metabolome, followed by the water stress endured by the plants and with the CO_2 of the growth environment only having low relevance (Table S1). Leaves and roots displayed qualitative and quantitative contrasting metabolomes, with roots having a higher variability, as from a total of 201 quantified compounds, 173 were found in roots and 110 in leaves, and only 74 were detected in both organs (Supplementary Table S1). These qualitative differences affected almost all metabolic pathways (Figure 1), standing out

TABLE 1 Gas exchange variables evaluated in beech seedlings growing under two [CO_2]—400 (aCO_2) and 800 (eCO_2) ppm—and watering regimes—well-watered (C) and water-stressed (D)

Growth ambient	Watering	A_{net} (µmol m^{-2}s^{-1})	g_{wv} (mol m^{-2} s^{-1})	ETR (µmol m^{-2} s^{-1})	iWUE (µmol mol^{-1})	Ψ_{pre} (MPa)	SWC (%)
aCO_2	C	9.98 ± 0.49 C	0.247 ± 0.021 A	80.36 ± 4.47 B	43.77 ± 4.04 C	-0.37 ± 0.12 A	27.05 ± 3.25 A
	D	7.11 ±0.77 D	0.094 ± 0.012 B	63.41 ± 5.46 C	79.11 ± 6.56 B	-1.07 ± 0.14 B	9.18 ± 1.06 B
eCO_2	C	17.95 ± 0.76 A	0.218 ± 0.017 A	100.9 ± 3.77 A	85.60 ± 4.36 B	-0.35 ± 0.08 A	26.23 ± 1.31 A
	D	13.15 ± 1.33 B	0.117 ± 0.012 B	85.06 ± 6.55 B	122.6 ± 13.26 A	-1.21 ± 0.18 B	9.56 ± 1.14 B

Note: A_{net}—net leaf photosynthesis; g_{wv}—stomatal conductance to water vapor; ETR—electronic transport rate; iWUE—intrinsic water use efficiency; Ψ_{pre}—predawn water potential; SWC—volumetric soil water content. Average values ± SE are presented. Different capital letters in a column denote significant differences after fisher post hoc test ($p < 0.05$).
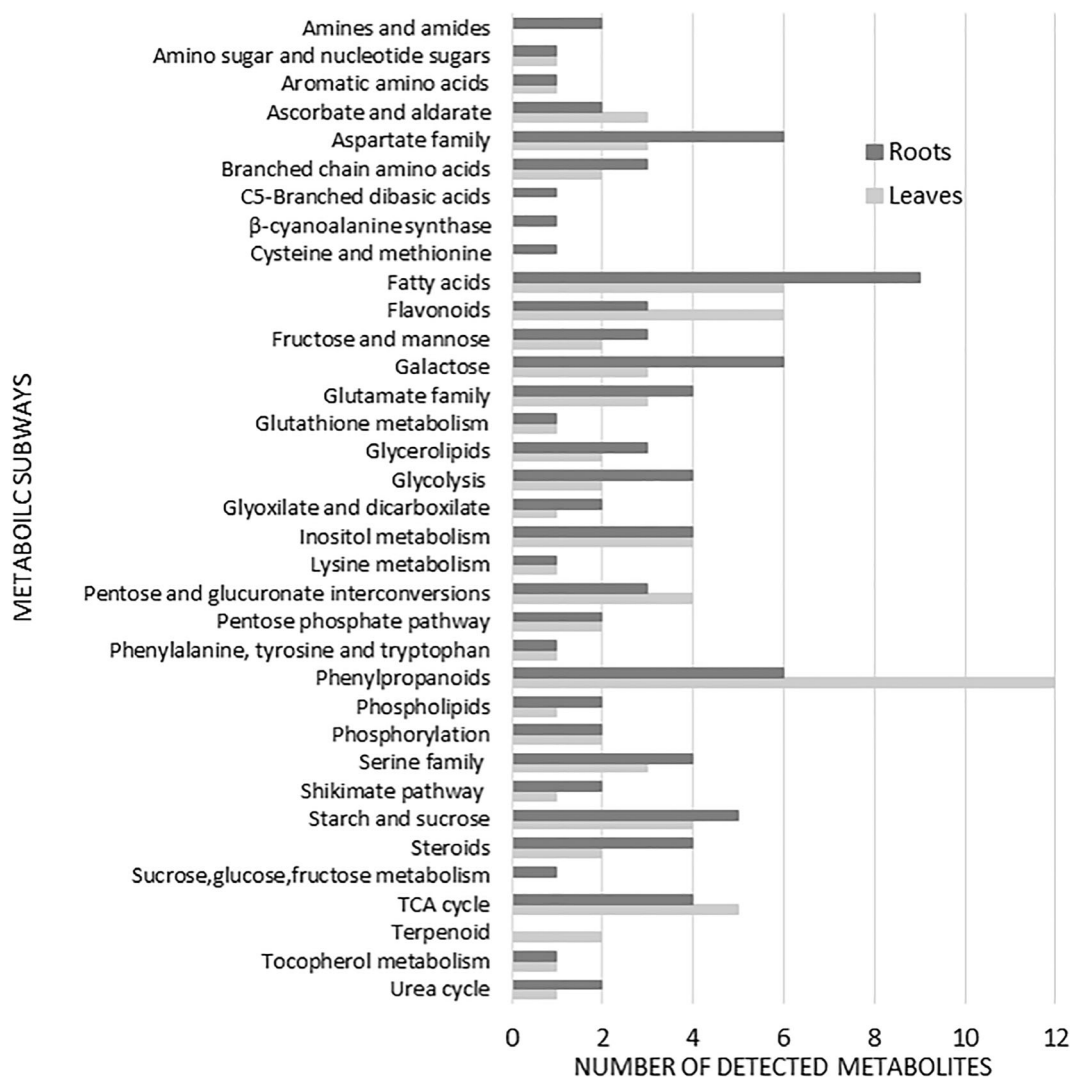
FIGURE 1 Metabolic sub pathway classification of 122 identified metabolites in at least one of the studied plant organs

in the leaves, due to the high number of identified metabolites, the phenylpropanoid and the flavonoid metabolism pathways, while in the roots the metabolites of the routes of fatty acids, galactose, aspartate and almost all amino acid-related sub-pathways were more numerous.

In addition to the qualitative differences, we also found clear quantitative differences between the organs. Almost all the detected levels were higher in leaves, particularly C compounds such as sugars, carboxylic and sugar acids, sugar alcohols, and phenolic compounds, while nitrogen compounds were mainly accumulated in roots. Of the 74 compounds detected in both organs, only seven differed quantitatively significantly between them (Table S1- Factor Organ). The OPLS-DA carried out using the organ as the dependent variable ($Q^2 = 0.975$) resulted in a clear separation between leaves and roots in the first predictive component (T-score = 55.1%) (Figure 2). Among those metabolites that were significantly accumulated in the leaves, some phenolic compounds (epicatechin, gallocatechin ferulic, and caffeic acids, 4-hydroxy-3-methoxyphenyl derivatives [4H3MPD] not fully identified), organic acids (shikimic, threonic, quinic, xylonic), carbohydrates (maltose,

sucrose, *myo*-inositol, galactinol), lipids (linolenic acid, glycerol-3-phosphate), and other compounds such as 3,4-dihydroxy-dihydro furanone, dehydroascorbic acid or α-tocopherol stand out. All these showed high *p* values and negative loading values in the OPLS model (Table S2). In roots, phosphoric acid levels were much higher than in the leaves, being the metabolite contributing the most to the leaf-root differentiation. In addition, mainly amino acids (L-threonine, L-aspartic acid, pyroglutamic acid, GABA, L-alanine, L-glutamic acid, L-valine, and L-serine) but also mannitol, citric, and lactic acids, *myo*-inositol phosphate, and glycerol were the metabolites that preferentially accumulated in roots, with high *p* values and positive loading values in the OPLS model (Table S2).

3.3 | Leaf and root contrasting drought-triggered metabolomes

The drought treatment resulted in increases and decreases in the concentrations of many of the metabolites detected in both the leaves

Scores Plot
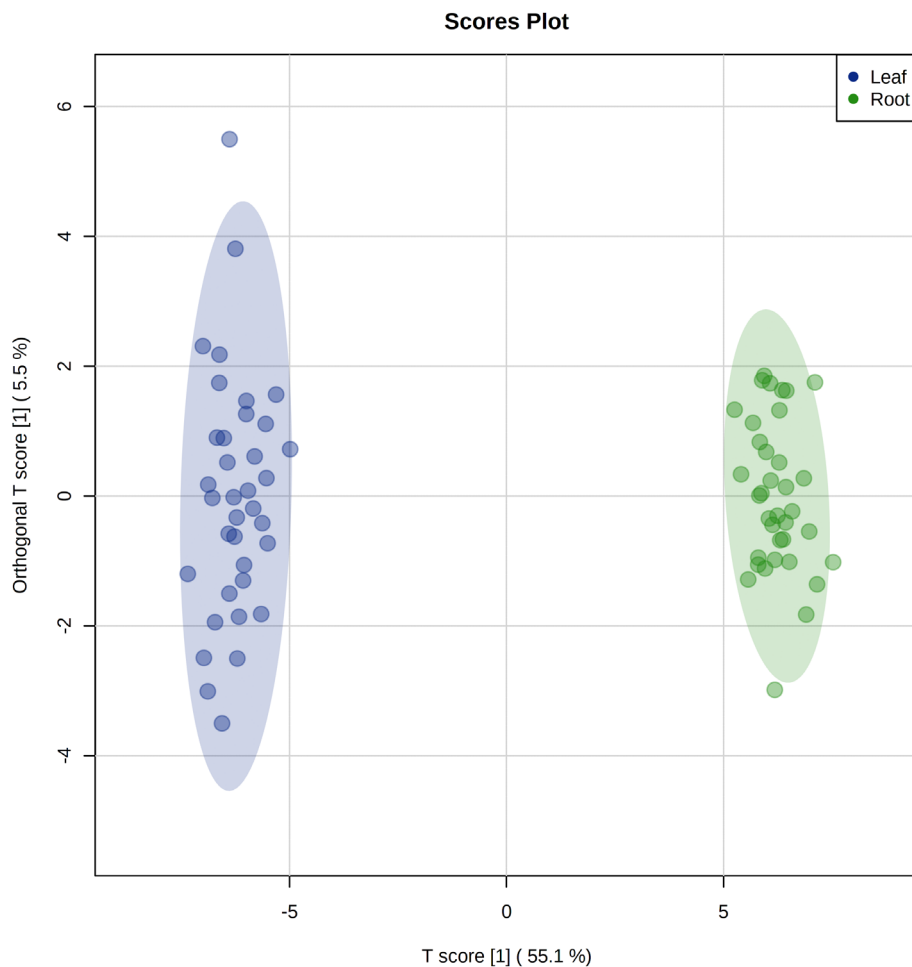
FIGURE 2 Score scatter plot of the first predictive component (x-axis) vs the first orthogonal component (y-axis) of OPLS-DA model from metabolome in *Fagus sylvatica* leaves ($n = 36$) and roots ($n = 36$) ($R^2X = 0.551$; $R^2Y = 0.987$; $Q^2 = 0.975$). Shadings indicate 95% confidence region. S-plot and numerical values of higher p values and p correlations are shown in Table S2

and roots. Still, only 30 of the 74 quantified metabolites detected in both organs showed q-values <0.05 regarding watering (W) or Organ × Watering (O × W) interaction factors (Table S1—Watering and O × W factors) according to the ANOVA. This, again, confirmed that the organ was the most determining factor in the metabolome features, above those the response due to drought. This led to the identification of three distinct clusters in the PLS-DA model ($Q^2 = 0.625$) performed considering the quantified compounds in both organs and the different watering treatments (Figure 3). The distribution of the samples within Component 1 (64.9% of variance) separated the leaves and roots samples, with positive and negative scores, respectively. As expected, metabolites with high loadings in Component 1 were the same as in the OPLS-DA model above (Table S3). In addition, root samples were significantly separated into two groups according to water treatment across Component 2 (11.9%). Samples from well-watered plants (RC—dark green) located to the negative zone while samples from water-stressed plants (RD—light green) aggregated to the positive zone. Leaf samples were located very close to zero in relation to this Component. Similar results were obtained throughout Component 3 (2.6%), with the leaf samples (dark and light blue) shifting to positive and negative zones
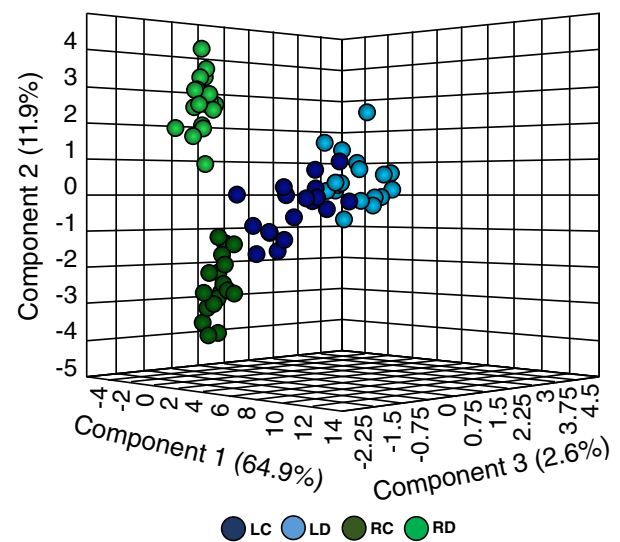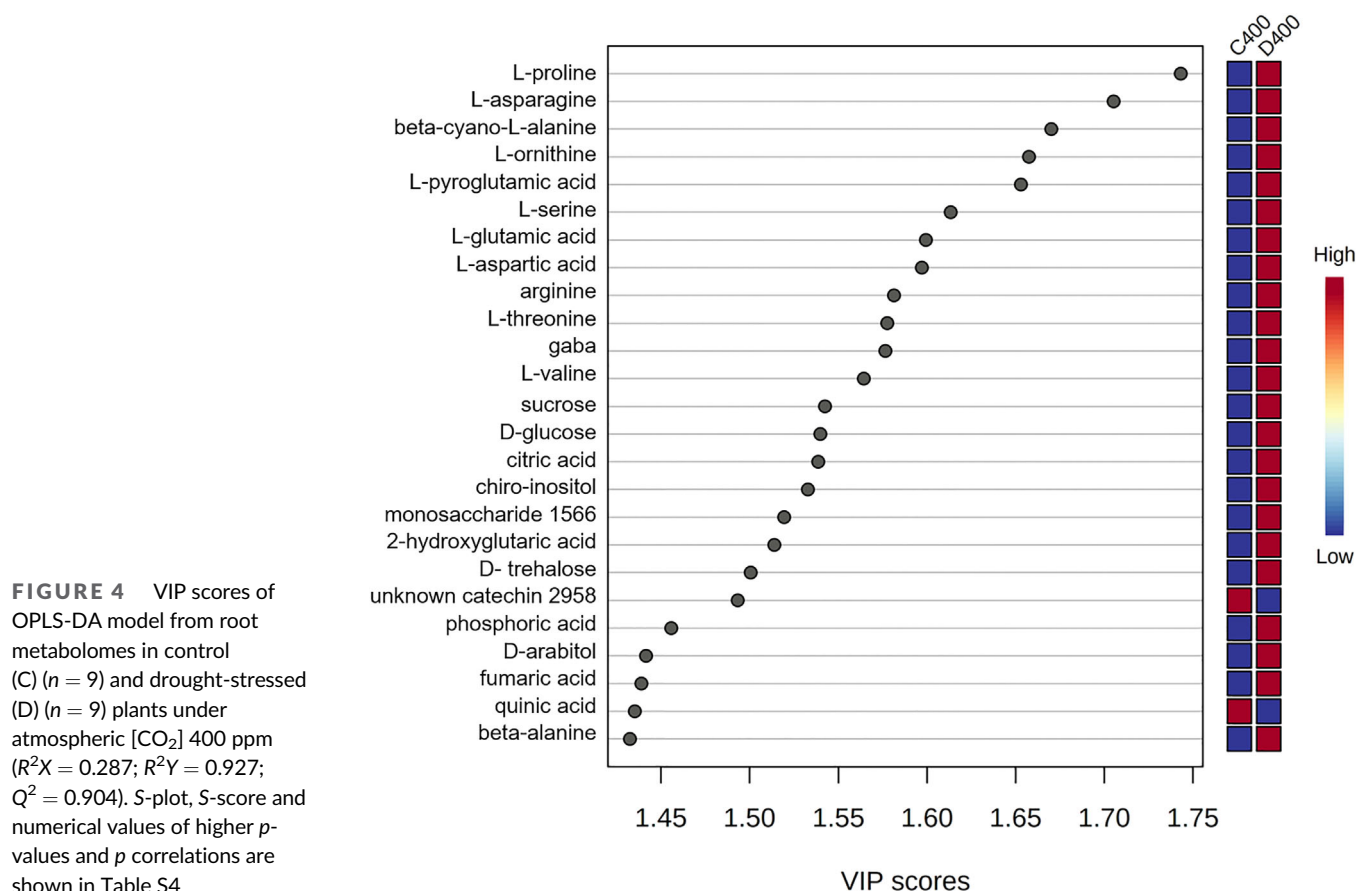

FIGURE 3 Score plot of PLS-DA model from leaf (L) and root (R) metabolomes in well-watered (C) and water-stressed (D) *Fagus sylvatica* plants ($R^2 = 0.7552$, $Q^2 = 0.6254$ for the three first components). $n = 18$ in all groups. The numerical values of higher p loadings are shown in Table S3
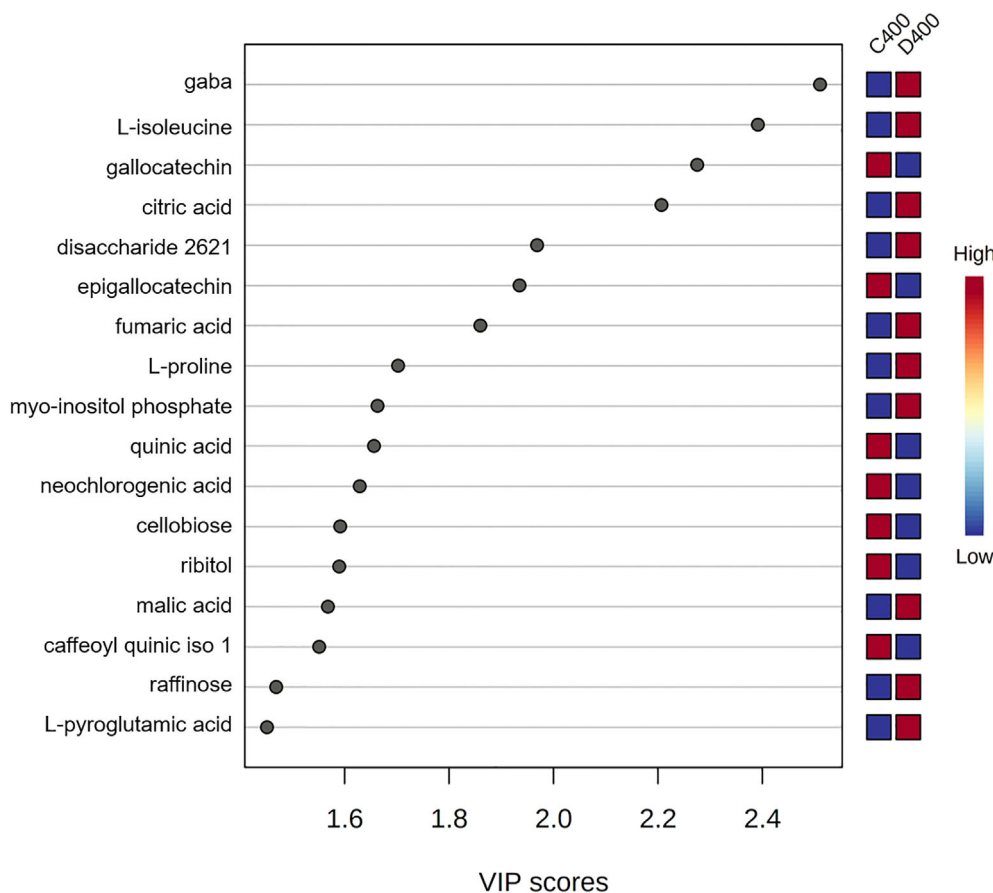
according to the watering treatment. Still, there was no clear discrimination between the two groups, and the root samples were located very close to zero. The metabolomics changes triggered by drought were different in leaves and roots and more significant in the latter as more metabolites were significantly affected. In the roots, as a consequence of water stress, the increases were particularly significant in the levels of amino acids, highlighting L-proline, L-alanine, pyroglutamic acid, L-serine, aspartic acid, L-valine or GABA, as well as in the levels of the TCA cycle metabolites, fumaric, malic and citric acids, all of them with high positive loadings in Component 2. In leaves, drought also brought about significant increases in some amino acids such as L-isoleucine and GABA, and TCA cycle metabolites, with positive loadings in Component 3, but there were also some decreases in the levels of some flavonoids (gallocatechin, catechin), polyols (scyllo-inositol, chiro-inositol), and sugars (cellobiose, arabinose, galactose) with negative loadings. Only a few metabolites showed important loadings in both Components (2 and 3) in the PLS-DA model: amino acids such as L-isoleucine and GABA, citric acid, 4H3MPD 2003, glyceric acid, methyl phosphoric acid, and chiro-inositol, the last three showing loadings with opposite sign according to each Component.

More details about drought-triggered changes in the metabolome of each organ were obtained from statistical analyses performed independently in leaves and fine roots, considering all detected metabolites of each organ. We found a clear separation between the control and water-stressed samples in roots throughout the first predictive component (T-score 32%) from the OPLS-DA model obtained by using watering treatment as a dependent variable ($Q^2 = 0.904$) (Table S4). In addition to those previously mentioned, other compounds showed a significant accumulation in water-stressed samples (Figure 4). There was a significant increase in many amino acids, such as L-asparagine, L-ornithine or β-cyano-alanine, and many carbohydrates (chiro-inositol, arabitol, sucrose, trehalose, etc.) as well as other compounds (2-hydroxyglutaric acid, glyceric acid, glycerol-2-phosphate, phosphoric acid, etc.). However, other compounds showed decreased levels after drought, also contributing to control-drought differentiation of the root samples. It highlights the quinic acid, some flavanols ((−)-epicatechin, (+)-catechin), and some not fully identified compounds (unknown catechins 2958 and 2924, and unknown sugar 3517).

The drought-triggered changes in the leaf metabolome were less noteworthy since a lower predictive value was obtained in the OPLS-DA model to separate drought from well-watered plants ($Q^2 = 0.561$), in which only 17 compounds showed a p(corr) > 0.05 regarding the first predictive component (T-score = 12.4%) (Figure 5 and Table S5). Among them, the drought caused an increase in the concentrations of the amino acids GABA, L-isoleucine, pyroglutamic acid, and L-proline, the organic acids citric, malic, and fumaric, the sugars disaccharide 2621 and raffinose, and myo-inositol phosphate in leaves. However, some compounds showed a decreased response to drought, such as ribitol, quinic acid, cellobiose, and the phenolic compounds gallocatechin,

FIGURE 4 VIP scores of OPLS-DA model from root metabolomes in control (C) ($n = 9$) and drought-stressed (D) ($n = 9$) plants under atmospheric [CO_2] 400 ppm ($R^2X = 0.287$; $R^2Y = 0.927$; $Q^2 = 0.904$). S-plot, S-score and numerical values of higher p-values and p correlations are shown in Table S4

FIGURE 5 VIP scores of OPLS-DA model from leaf metabolomes in control (C) ($n = 9$) and drought-stressed (D) ($n = 9$) plants under atmospheric [CO_2] 400 ($R^2X = 0.124$; $R^2Y = 0.798$; $Q^2 = 0.561$). S-plot, S-score and numerical values of higher p values and p correlations are shown in Table S5

epigallocatechin, neochlorogenic acid, and caffeoylquinic isomer, with negative loading values in the model.

3.4 | Rising [CO_2] effect on leaf and root drought-triggered metabolomes

Metabolome analysis showed that the combined effect of rising [CO_2] and drought was again higher in the roots than in the leaves of *F. sylvatica*, prevailing drought-triggered changes over those induced by the higher levels of atmospheric [CO_2] (Figure 6).

In the leaves of both control and stressed plants, it was not possible to clearly distinguish the impact of [CO2] growing conditions as samples from plants grown under [CO_2] of 400 or 800 ppm were not clearly separated in the PLS-DA model ($R^2 = 0.6649$; $Q^2 = 0.2647$) (Figure 7 and Table S6). However, some metabolites such as kaempferol, quercetin, malic acid, glycerol-3-phosphate, and *myo*-inositol phosphate increased in concentrations under eCO$_2$, particularly in control plants (Figure 8). Other metabolites (ribitol, 4-hydroxycinnamic, pipecolic and ferulic acids, cellobiose, raffinose, levoglucosan, and some unknown compounds) decreased under eCO$_2$, particularly in control plants (Figure 8). These changes lead to a tendency for the control plants to be in the positive zone of Component 2 (8.1% of variance) under conditions of eCO$_2$ and in the negative zone under aCO$_2$. A similar but opposite trend was also observed

regarding the distribution of drought-stressed plants through Component 4 (7.8% total variance). Only GABA and L-arabinose showed decreased levels at high [CO_2] under drought (Figure 8), while many metabolites increased their concentrations under these environmental conditions, such as caffeoylquinic isomer, glycerol-3-phosphate, disaccharides 2605 and 2022, phytol, epigallocatechin and gallocatechin, and oleic acid, among others (Figure 8).

In roots, most metabolites that showed some significant change in concentration under drought did not show significant differences in their level in response to the [CO_2]. However, in an important percentage of metabolites (44.5%), the drought-triggered changes were modulated by the [CO_2], as for GABA or malic acid that showed in response to water stress a lower and higher increase respectively under eCO$_2$ (Figure 8). These changes in response to drought were even opposite according to the [CO_2], such as those of caffeic and linoleic acids or alpha-tocopherol (Figure 8). These differences in the root metabolome make it possible the discrimination between samples depending on treatment in the obtained model from PLS-DA ($R^2 = 0.9049$, $Q^2 = 0.5305$) (Figure 9 and Table S7). Through Component 1 (44.5% of variance), the samples were distributed regarding water treatment, clearly discriminating between control and drought-stressed plants. The Component 2 (6.4% of variance) allowed that the samples were distributed concerning the level of atmospheric CO$_2$, particularly for roots of drought-stressed plants in which [CO_2] effect was somewhat clearer. Sugar alcohols such as
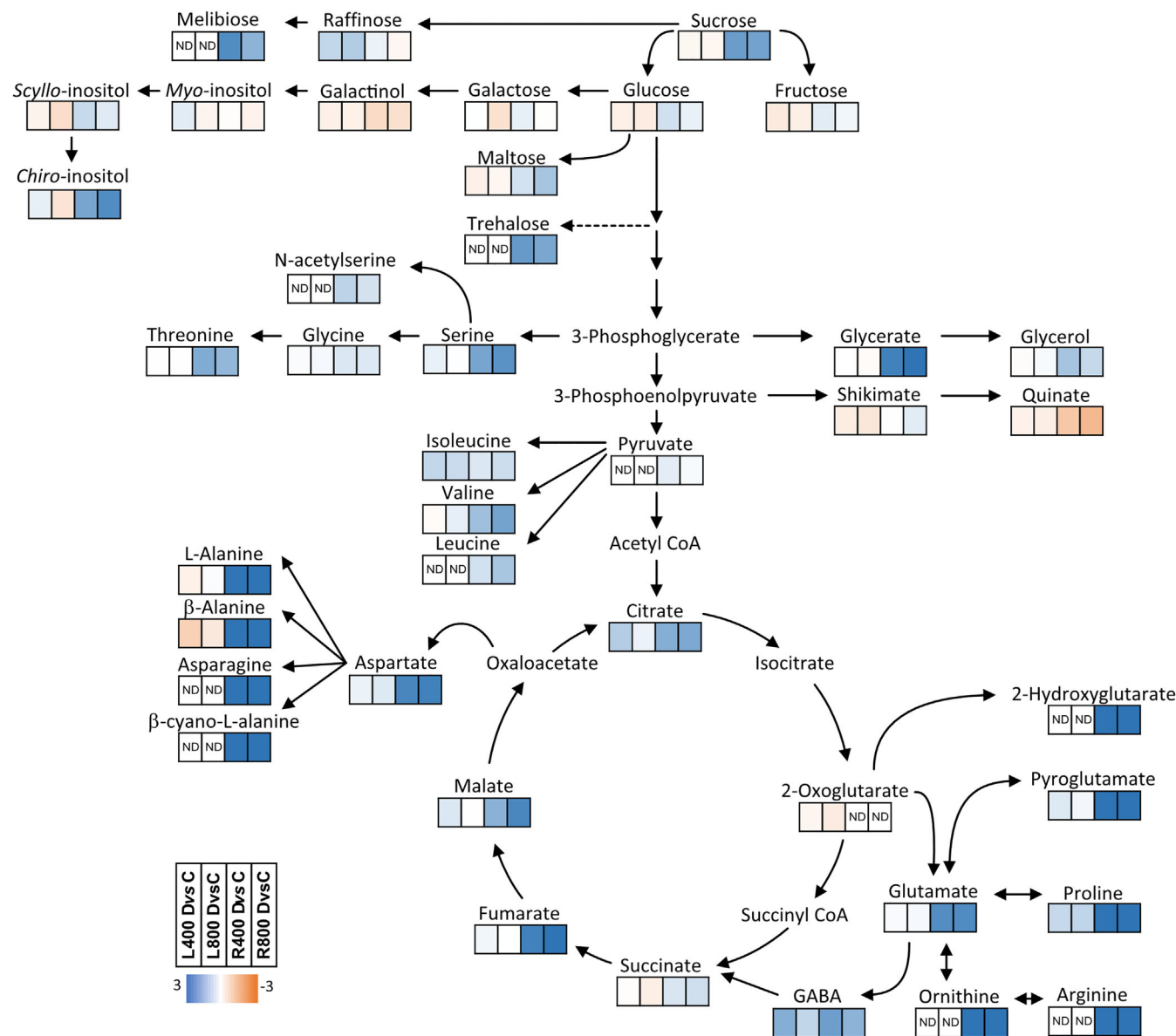
FIGURE 6 Simplified primary metabolic pathways associated with differentially changing metabolites in leaves (L) and roots (R) from control (C) and drought-stressed (D) plants under atmospheric [CO_2] of 400 or 800 ppm. Different box colors show log-2 transformed fold of change calculated as the ratio between metabolite values in drought-stressed and control plants. $n = 9$. ND = not detected

D-threitol and D-mannitol, lipids such as glycerol-2-phosphate, and linoleic and 2-hydroxyglutaric acids increased their concentration under rising [CO_2], particularly in water-stressed plants (Figure 10), showing negative coefficients in Component 2, while caffeic acid, erythritol, levoglucosan, lactic acid and some not fully identified catechins and sugars showed decreased concentrations under eCO$_2$ (Figure 10) (positive loadings in this Component). The CO_2-triggered changes in the metabolome of roots from well-watered plants were also reflected in Component 2, but also partly by the distribution of the samples along Component 3 of this PLS-DA model. Some carbohydrate and phenolic metabolites, besides alpha-tocopherol and linoleic acid, were the most correlated to this Component, with negative and positive loadings (Table S7).

4 | DISCUSSION

### 4.1 | Water stress and gas exchange

Beech has been considered as one of the temperate species that could benefit most from the CO_2 enrichment of the atmosphere in the future (Medlyn et al., 1999). Water stress brought about a decrease in gas exchange rates in response to the mild water stress endured by the plants, as stomatal closure is the main mechanism in trees to limit water losses and avoid hydraulic failure (Martínez-Vilalta & Garcia-Forner, 2017). Although the photosynthetic capacity can suffer a down-regulation of the maximum carboxylation capacity in the long term, from a biochemical acclimation to a CO_2 enriched
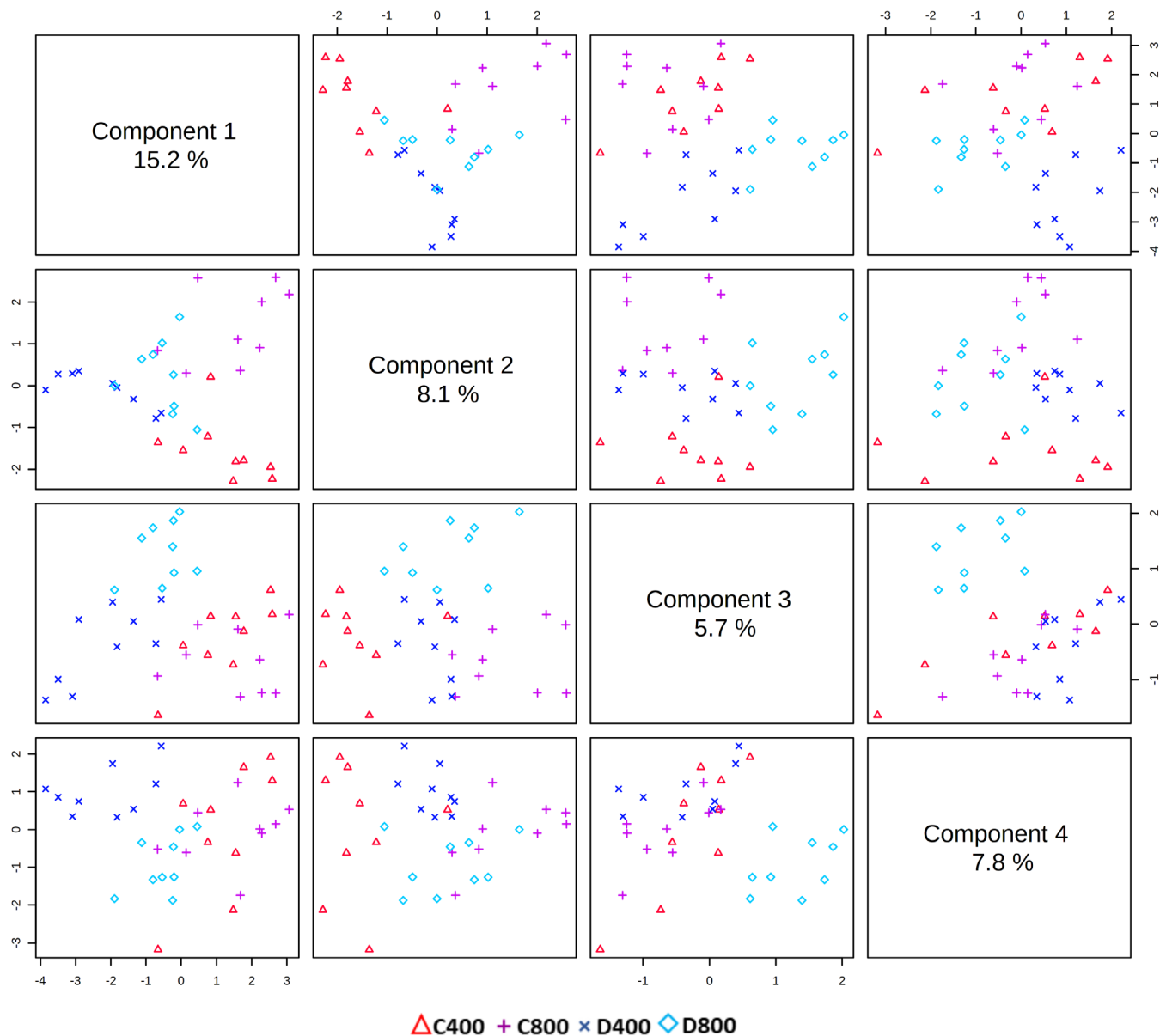
FIGURE 7 Score plot of PLS-DA model from leaf metabolomes in control (C) and drought-stressed (D) plants under atmospheric $[CO_2]$ of 400 or 800 ppm ($R^2 = 0.6649$; $Q^2 = 0.2647$ for the four first components). The numerical values of higher p loadings are shown in Table S6

atmosphere, as observed in previous studies carried out in beech (Leverenz et al., 1999; Urban et al., 2014), under our enriched CO_2 condition of the atmosphere a stimulation of A_{net} of beech was observed. The A_{net} was higher under enriched CO_2 regardless of the water stress endured by the seedlings. Thus, the increase in the source of CO_2 was more relevant than the limitations imposed by the decrease in stomatal conductance in response to drought, as higher sub-stomatal $[CO_2]$ will be maintained for a given g_{wv} in comparison to aCO_2 conditions (Menezes-Silva et al., 2019). This points to a higher impact of CO_2 as a carbon source of the whole leaf carbon uptake capacity than to the potential photosynthetic capacity adjustment in response to the CO_2 enrichment, as observed in other species (Aranda et al., 2020; Bauweraerts et al., 2013). Therefore, iWUE

increased in response to water stress, but this increase was much more relevant under eCO_2 because of the higher C source at the fixing sites (Epron et al., 1996; Urban et al., 2014). The higher increase in iWUE under eCO_2, regardless of soil water availability, was consequence mainly of a higher net leaf photosynthesis. However, this surplus of carbon did not significantly impact the quantitative changes of the metabolome. This raised the question about the final allocation of the extra carbon fixed by plants that could have been allocated to growth or increase of root exudates as additional carbon sinks. Apart from the observed changes in the A_{net} due to the CO_2 enrichment of the atmosphere, most of the molecular changes were elicited by the water stress endured by plants mirrored in the metabolic changes in leaves and roots (see later).
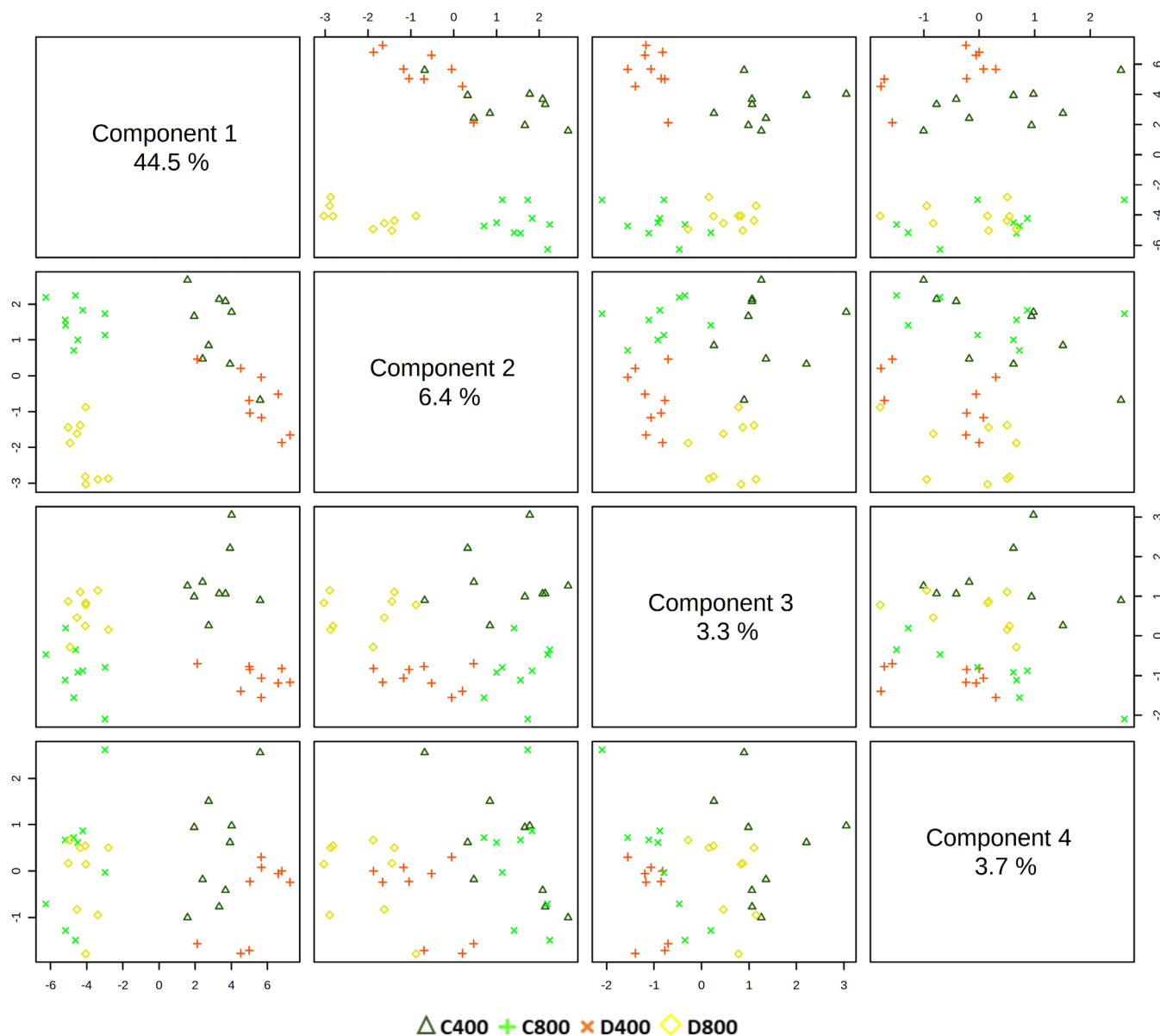
FIGURE 8 Score plot of PLS-DA model from root metabolomes in control (C) and drought-stressed (D) plants under atmospheric [CO$_2$] of 400 or 800 ppm ($R^2 = 0.9049$; $Q^2 = 0.5305$ for the three first components). The numerical values of higher p loadings are shown in Table S7

4.2 | Leaf and root metabolomes are shaped by their functions

Leaves have been the main focus of the studies of the metabolome in trees, although roots also play an important role in plant-environment interactions (Klimesova et al., 2018; Wang et al., 2021). The aerial and underground organs in plants display different metabolomes (de Miguel et al., 2016; Merchant et al., 2006; Sardans et al., 2017) due to their different physiological functions and roles as carbon source and sink organs in plants, which correlate with their high metabolic specialization (Li et al., 2016). Qualitatively, beech roots showed a wider variety of primary metabolites than leaves, such as in other tree species (de Miguel et al., 2016. However, quantitatively most metabolites accumulated in leaves, especially those related to C

metabolisms such as sugars, sugar acids, sugar alcohols, fatty acids, phenylpropanoids, and flavonoids, while N and P metabolites such as amino acids, other nitrogen compounds, and phosphoric acid accumulated in higher concentrations in the roots. In beech leaves, the allocation of C causes a significant accumulation of non-structural carbohydrates (NSC) and many other C metabolites as well as starch, although to a lesser extent (Blessing et al., 2015; Desalme et al., 2017). In beech roots, the soluble sugars are only a short-term storage pool, fueling root exudates and root litter (Brüggemann et al., 2011), since most of the C accumulates as starch in a long-term investment strategy (Blessing et al., 2015; Gomez et al., 2020; Plavcova et al., 2016). Even so, C metabolites, including sugars, organic acids, sugar alcohols, and polyphenols, were the most abundant group of metabolites found in beech roots.
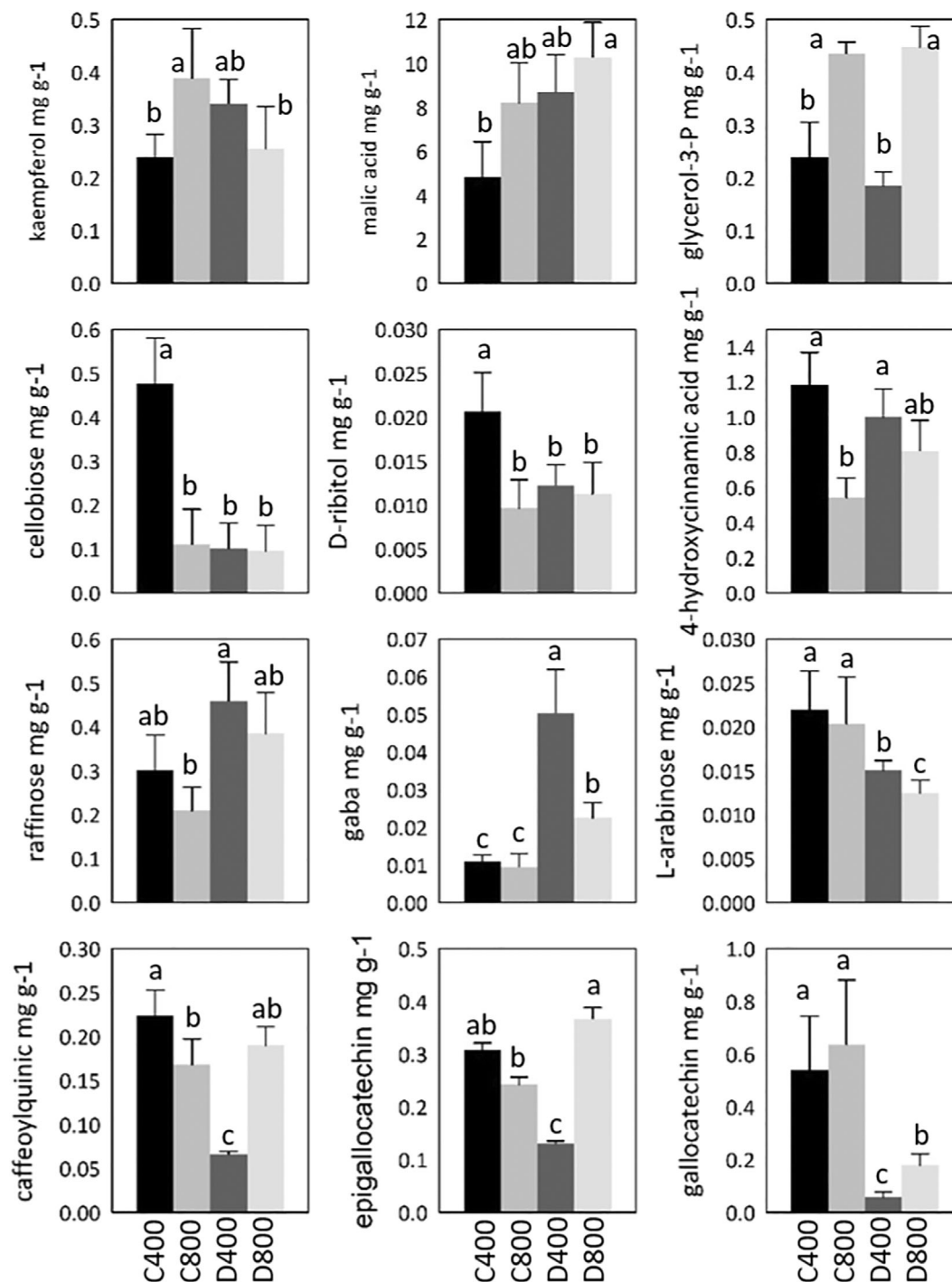
FIGURE 9 Average concentration (mg g^{-1}) and standard error of some of the most significant metabolites in leaves from control (C) and drought-stressed (D) plants under atmospheric [CO$_2$] 400 or 800 ppm. Different letters for each metabolite denote a statistical difference with a 95% confidence level after q-false discovery rate calculation, with "a" indicating the highest concentration

About P metabolites, as the absorption of inorganic phosphate (PO$_4^{3-}$), the absorbable form of P, occurs through the roots, a higher concentration is expected in this organ. Indeed, the difference in phosphoric acid level was the most contributing feature to leaf-root metabolic differentiation. Netzer et al. (2016) also found higher P$_i$ levels in fine roots than in beech leaves from Balkan, Bulgarian, and

Greek provenances. However, trees from Central European showed a more balanced distribution between the organs (Netzer et al., 2016; Zang et al., 2021), highlighting the importance of soil P availability in P allocation (Meller et al., 2019). Soil phosphate represents the only source of phosphorus for plants, a crucial component in phospho-lipids, nucleic acids, and ATP (Péret et al., 2014), so the uptake of

FIGURE 10 Average concentration (mg g^{-1}) and standard error of some of the most significant metabolites in roots from control (C) and drought-stressed (D) plants under atmospheric [CO_2] 400 or 800 ppm. Different letters for each metabolite denote a statistical difference with a 95% confidence level after q-false discovery rate calculation, with "a" indicating the highest concentration
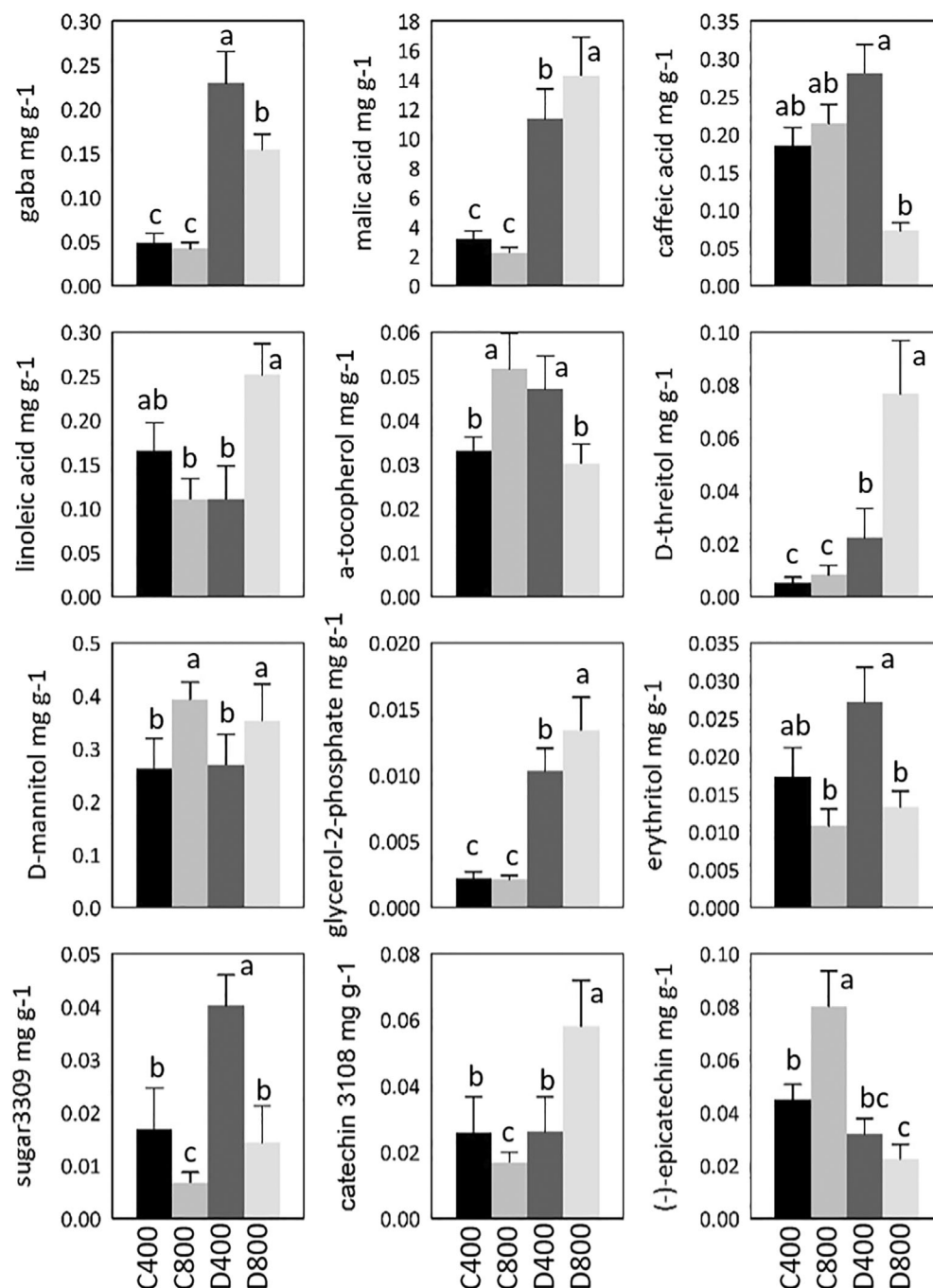
PO_4^{3-} from the rhizosphere maintains intracellular homeostasis during growth (Baek et al., 2017).

There were also important differences between the root and leaf metabolomes in relation to N metabolism, with a higher abundance of amino acids in roots, and many of them were only detected in this organ. It may appear that unstressed trees accumulate significant amounts of N in their roots, setting up a possible lag event between demand and external availability, such as spring growth (Joseph et al., 2021). Although soil N is available to plants in the form of amino acids only to a small extent (Liu et al., 2017), nitrate and ammonium absorbed by roots are converted into amino acids (Miller et al., 2007), resulting in its accumulation preferentially in

roots, which is the predominant form of N reserve in many trees (Gomez et al., 2020). In this N reserve in beech roots, asparagine and arginine, with four and two N atoms, respectively, were the most abundant amino acids. Both are relevant amino acids for N storing of the assimilated N, among other functions of these amino acids, such as N transport or osmotic pressure maintenance (reviewed in Gomez et al., 2020). Other N compounds with relevant levels in beech roots, all of them with one N atom, were hydroxylamine, an intermediate in ammonium nitrification, betaine, and aspartic and pyroglutamic acids, probably with less involvement in the storage of assimilated N, but with influence on other physiological functions.

4.3 | Drought-triggered metabolomics changes are organ-specific

In both organs, the main source of variation in the pool of metabolites was determined by the water stress endured by the seedlings. Secondly, eCO_2 modulated the concentration of some metabolites. Both, the metabolomics changes triggered by drought and by eCO_2 were different in leaves and roots, with a higher impact in the latter. The early response to low levels of drought in roots, in a fast ecological strategy (Nikolova et al., 2020) is related to its function since it is the first sensory tissue that is responsible for transmitting chemical signals to the aerial tissues to inform about the hydric status of the soil (Schachtman & Goodger, 2008) and regulates the expression of genes that control molecular pathways of the drought response (Janiak et al., 2016). However, it was not only the intensity of the response to the drought that differed between organs. We also found organ-specific drought-triggered metabolomics changes, as in other tree species, both deciduous, such as *Eucalyptus* (Merchant et al., 2006), and evergreen, such as *Pinus pinaster* (de Miguel et al., 2016; Fernández de Simón et al., 2021).

Water stress modifies nitrogen and carbon metabolism in beech leaves (El Zein et al., 2011; Peuke et al., 2006) with an increased degree of change in specific metabolites as the drought intensifies. This was previously observed from the progressive changes in the predawn leaf water potential as surrogate of water stress, and the changes in the concentration of some metabolites (Aranda et al., 2018). Under drought conditions, an accumulation of amino acids is triggered, highlighting proline, valine, aspartic acid, and γ-aminobutyric acid, as well as soluble carbohydrates, both mono and disaccharides (Aranda et al., 2018; Liu et al., 2017; Peuke et al., 2002; Schraml & Rennenberg, 2000). In some cases, changes are dose-dependent in a non-linear fashion as the exponential increase in raffinose trisaccharide, which is activated when a water stress threshold is exceeded (Aranda et al., 2018). In our moderate drought stress conditions ($\Psi_{pre} = -1$ MPa), the accumulation of amino acids in leaves was significant for only three of them: GABA, isoleucine, and proline, in order of accumulation. These amino acids could play a role as osmoprotectants (Aranda et al., 2021; Joshi et al., 2010; Lehmann et al., 2010) or signaling metabolites in the runway processes related to stomatal closure (Krasensky & Jonak, 2012; Ramesh et al., 2017). In roots, the drought-triggered accumulation of amino acids was much more important, both qualitatively and quantitatively, in spite that nitrogen uptake may be limited under water stress in beech (Bueno et al., 2021; Fotelli et al., 2009), but it could also be a result of an impairment to mobilize nitrogen from roots to leaves linked to the water stress. Among all of them, proline accumulation was the most pronounced drought response in beech roots (5.6-fold change as compared to 1.02 in leaves), higher than values previously reported in water-stressed young beech trees (Hagedorn et al., 2016) and in other species (Brunner et al., 2015; Yaish, 2015). This increase in proline levels was probably related to its roles described in the literature, such as acting as osmolyte and osmoprotectant, reducing oxidative damage, enhancing signal transduction pathways, or stabilizing DNA and protein complexes (Hayat et al., 2012; Szabados & Savouré, 2010). Besides proline, the drought-triggered increases of other amino acids implied in proline biosynthesis (arginine and proline metabolism pathway), such as arginine, ornithine, glutamate, and even pyroglutamate, were among the most significant in beech roots (Figure 6). A possible explanation could be that the root act as nitrogen storage to cope with potentially higher levels of water stress, providing a source to maintain and stimulate proline synthesis, which could be available to be allocated to leaves or shoots if required. This would contribute to the maintenance of the osmotic potential and radical scavenging in different organs, increasing the chances of plant survival (Bhaskara et al., 2015; Hayat et al., 2012; Kavi Kishor & Sreenivasulu, 2014). Other amino acids that showed important increased levels in roots under drought were asparagine and beta-cyano-L-alanine (fold change >4). These amino acids along with arginine would function as stored assimilated N since they are N-rich (four or two N atoms in their structure), also to reinforce N transport from roots to other organs, or to maintain osmotic pressure (Gomez et al., 2020; The et al., 2021). However, unlike in leaves, the increases of GABA and isoleucine were much less prominent, especially for the latter.

Regarding C metabolism, drought-triggered changes in the leaf metabolome were significant only for the increases in the levels of TCA cycle intermediates (citric, malic, and fumaric acids), and the sugars disaccharide 2621 and raffinose, and myo-inositol phosphate. The increases in raffinose levels were slight but statistically significant. Much more remarkable was the drought-triggered belowground C accumulation with carbohydrates such as melibiose, sucrose, trehalose, and two monosaccharides, but also polyols such as chiro-inositol, arabitol, and threitol, among others, as it has also been reported in beech trees (Blessing et al., 2015; Hagedorn et al., 2016; Nikolova et al., 2020; Zang et al., 2014) and other tree species, previously (Birami et al., 2020; de Miguel et al., 2016; Merchant et al., 2006). It seems that beech adjusted its leaf/root soluble sugar accumulation according to the effective water stress endured by plants, probably with the strategy of prioritizing the root functioning to tolerate moderate drought stress, reflecting the drought status of trees (Brunner et al., 2015; He et al., 2020), and reinforcing the key role played by beech roots in their resilience to drought, with a sink driven carbon balance (Hagedorn et al., 2016).

In both organs, the levels of secondary metabolites were also affected by water stress that limited their accumulation, decreasing flavonoids and phenylpropanoids, together with its precursor, the quinic acid, although those of benzoic and cinnamic compounds increased slightly in the roots. This has been reported in beech trees (Aranda et al., 2018) and other tree species such as eucalyptus (Correia et al., 2016), willow (Turtola et al., 2005) or oak (Suseela et al., 2020), although unfavorable environmental conditions are usually related to higher foliar levels of terpene and phenolic compounds (Fernández de Simón et al., 2017, 2021; Sumbele et al., 2012; Tattini et al., 2004). As in the leaves, the organic acids involved in the TCA cycle (citric, malic, fumaric, and succinic) also showed a high drought-triggered accumulation level in the roots, implying that this metabolic pathway was more active in beech trees under a mild–moderate

drought. Together with the proline accumulation and the flavonoid and quinic acid decrease, this trait was the shared way of coping with water stress in both organs. In leaves, a more active TCA cycle was expected in drought conditions due to the importance that this pathway acquires when less photosynthesis is brought about because of the stomatal closure (Zhang & Fernie, 2018). In the roots, the conversion of absorbed nitrate and ammonium into amino acids (Miller et al., 2007) required energy and C skeletons substrates, accomplished through the glycolysis-TCA cycle (Fernie et al., 2004), mainly 2-oxoglutarate for glutamate biosynthesis, and oxaloacetate for that of aspartate. They are the first metabolites in the biosynthetic pathways that produce glutamine, proline, ornithine, and arginine, or asparagine, alanine, threonine, and isoleucine, respectively, as well as many of the other amino acids showing drought-triggered increased levels in roots. These two intermediates in the TCA cycle were not detected in roots, probably because they were fueling these pathways. The increased levels of malate or citrate could be also related to their role in root exudates (Gargallo-Garriga et al., 2018), taking part probably in plant nutrition, communication with microorganisms, and releasing unavailable P forms (reviewed in Maurino & Engqvist, 2015), although more in-depth research is needed in this area (Williams & de Vries, 2020). P uptake was also significantly improved during drought in the roots, but not changed in the leaves, which reinforced the asymmetric allocation of resources in the tree for growth and energy metabolism, as has been described in woody and non-woody plants (reviewed by Sardans et al., 2017).

4.4 | Rising [CO_2] slightly mitigates the drought effect on leaf and root metabolomes

The high levels of atmospheric CO_2 altered the metabolic response to drought, since a greater carbon availability led to variations in foliar and root metabolomes, both in primary and secondary metabolic pathways. Though, its effect on the roots was clearer. In leaves, we expected that rising [CO_2] in the atmosphere would result in the building up of the carbohydrate pool under drought (reviewed in Misra & Chen, 2015), because of the increase in the leaf carbon uptake. But no drought-triggered increases were detected under eCO_2 for sucrose, glucose, or fructose, the most abundant soluble carbohydrates, although there were small increases in some minor disaccharides. However, the foliar increases of secondary metabolites in response to eCO_2 stood out over primary metabolites, mainly benzoic and phenylpropanoid derivatives, and some flavan-3-ols. It seems that under drought the carbon surplus at eCO_2 was invested in the leaves, further from allocation to new growth, to fuel carbon-based secondary metabolites biosynthesis. Gunthardt-Goerg and Vollenweider (2015) suggested as the best explanation the imbalance between C and N supply, as well as the perception of the rising [CO_2] as an unfavorable change in the environmental conditions, where the plant improve the facing of oxidative signaling in response to drought (Fernández de Simón et al., 2020; Noctor & Mhamdi, 2017; Schwanz & Polle, 2001; Sekhar et al., 2017). Some

other metabolic changes could also be related to a possible carbon: nitrogen imbalance. This is the case of the increase in the level of malic acid as a significant fraction of the fixed carbon, which can function as a temporary carbon storage molecule (reviewed in Maurino & Engqvist, 2015), or with other functions as osmotic adjustment. Increased levels of foliar malate under eCO_2 have been detected in both woody (Fernández de Simón et al., 2018) and no-woody (Barnaby et al., 2015; Maurino & Engqvist, 2015) plants. In roots, malate levels also slightly increased under eCO_2 but only when beech saplings grew water-stressed, as happened with citric and fumaric acid levels. Under these environmental conditions, increases of other osmotically active substances, such as threitol and mannitol, but also glycerol-2-phosphate, linoleic, and 2-hydroxyglutaric acids and some minor saccharides and polyphenols were also detected in roots. However, many more metabolites decreased their concentrations when comparing to the levels reached under drought at aCO_2. These were mainly phenolic compounds, carbohydrates, and some N compounds, thus reflecting a buffering of the drought effect under eCO_2. Furthermore, some phenolic compound and carbohydrate levels showed contrasting variations in roots due to the eCO_2 depending on whether the plant is subjected to drought conditions or not. For example, under eCO_2, increased epicatechin levels were only found in well-watered plants, while threitol only increased in those water-stressed. And similar contrasting variations were detected for phenolic compounds such as caffeic acid, catechin, and some other catechins, flavonoids, and carbohydrates not fully identified. The slightly lower levels in some N compounds under eCO_2 in both, leaves and roots, indicate that their accumulation could be modulated by the water status of the plant (Barnaby et al., 2019) confirming the decrease in elemental N content under eCO_2 in the tree leaves (McMurtrie et al., 2008), and particularly in the fine roots (Leakey et al., 2009). However, Uchytilová et al. (2019) detected a non-significant N increase in the roots of beech saplings after two years under an eCO_2 environment. Paudel et al. (2018) associated these changes in the C:N in leaf and root, with the accumulation of biomass due to the change in the proportion of fine roots that can occur under a moderate soil drought (Zang et al., 2021), which helps improve tolerance to drought endured under eCO_2.

5 | CONCLUSIONS

The aerial and underground organs in beech saplings displayed different metabolomes. The roots showed a higher variety of primary metabolites although quantitatively most metabolites accumulated mainly in leaves, especially those related to C metabolism. Whereas N and P metabolites accumulated at higher concentrations in roots. Among all of them, the contrasting phosphate level between both organs was the feature most contributing to leaf-root metabolic differentiation. Moreover, not stressed beech accumulates significant amounts of N rich compounds in their roots, standing out asparagine and arginine, with four and two N atoms, respectively, as the most abundant amino acids. This can make up a suitable way to store

assimilated N, among other functions of these amino acids (reviewed in Gomez et al., 2020).

In both, leaves and roots, the main source of variation in the pool of metabolites was the water stress endured by seedlings. On a second term, eCO_2 modulated the concentration of some metabolites in both organs, being the metabolomics changes boosted by drought and eCO_2 different in leaves and roots, and more remarkable in the latter.

The prompt response to low levels of drought in roots could contribute to a rapid ecological strategy (Nikolova et al., 2020). In this early response, the important increase in proline concentration through the activation of arginine and proline metabolic pathways was the main feature of the drought response in beech roots, probably getting ready to cope with a potential intensification in the level of water stress. In this strategy, roots also activated the biosynthesis of N-rich amino acids related to storing, while in leaves the amino acid increases were lower. The drought-induced activation of C metabolic pathways was also more intense in roots, particularly the TCA cycle and polyols and soluble sugar biosynthesis, which suggest the importance of root functioning to tolerate moderate drought stress, maximizing its resource uptake functions under unfavorable soil conditions.

The high levels of atmospheric CO_2 altered this metabolic adaptation to drought, reflecting a slight mitigation of its effect under eCO_2, though with a different response according to organ. The leaves showed only minor changes in their metabolome, investing the carbon surplus in carbon-based secondary metabolites and malic acid biosynthesis. In roots, although increases in the TCA cycle and some osmotically active substances were detected, most of the metabolites reached lower concentrations, as if the level of water stress were lower and their accumulation was based on the hydric state of the plant. This is a first study of the metabolic changes that occur in the leaf and root of beech subjected to water stress under an atmosphere enriched in CO_2, but broader studies (number of trees, genotypes, drought intensity, etc.) will be necessary to confirm these eCO_2–drought interactions.

AUTHOR CONTRIBUTION

Brígida Fernández de Simón, Estrella Cadahía, and Ismael Aranda designed the research, Brígida Fernández de Simón and Estrella Cadahía designed, performed, and interpreted the GC–MS analysis, Brígida Fernández de Simón performed and interpreted the statistical analysis, Ismael Aranda designed the physiological research and performed and interpreted physiological analyses, and Brígida Fernández de Simón led the writing of the manuscript. All authors have read, critically reviewed the intellectual content, corrected, and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Brígida Fernández de Simón https://orcid.org/0000-0002-2731-4128

Ismael Aranda https://orcid.org/0000-0001-9086-7940

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