forests

MDPI

Article

Effects of Extreme Drought and Heat Events on Leaf Metabolome of Black Alder (*Alnus glutinosa* L.) Growing at Neighboring Sites with Different Water Availability

Lijun Zhu [1,†], Zhengqiao Liao [1,†], Lei Liu [1] and Baoguo Du [1,2,*]

[1] College of Life Science & Biotechnology, Mianyang Normal University, Mianxingxilu 166, Mianyang 621000, China
[2] Chair of Tree Physiology, Institute of Forest Sciences, Albert-Ludwigs-Universität Freiburg, Georges-Koehler-Allee 53, 79110 Freiburg, Germany
* Correspondence: baoguo.du@ctp.uni-freiburg.de; Tel.: +49-(0)761-2038308
† These authors contributed equally to this work.

Abstract: Riparian tree species are thought to be sensitive to the more frequent and intensive drought and heat events that are projected to occur in the future. However, compared to waterlogging, information about the responses of these tree species to water limitation and heat is still scare. Black alder (*Alnus glutinosa* L.) is a riparian tree species with significant ecological and economic importance in Europe. In the present study, we investigated the physiological responses of black alder (*Alnus glutinosa* L.) to different water availabilities growing at neighboring sites. Compared to trees with unlimited water source, trees with a limited water source had 20% lower leaf hydration, 39% less H_2O_2 contents, and 34% lower dehydroascorbate reductase activities. Concurrent with dramatically accumulated glutathione and phenolic compounds, leaf glutathione contents were two times higher in trees with limited water than in trees with sufficient water. Limited water availability also resulted in increased abundances of sugars, sugar acids, and polyols. Serine, alanine, as well as soluble protein related to nitrogen metabolism were also accumulated under limited water conditions. In contrast to sulfate, leaf phosphate contents were significantly increased under limited water. No significant effects of water conditions on malondialdehyde and ascorbate contents and fatty acid abundances were observed. The present study improves our understanding of the physiological responses of black alder to different water conditions. Our findings highlight this riparian species is at least to some extent resistant to future drought with a well-regulated system including antioxidative and metabolic processes and its potential as an admixture candidate for afforestation in either water-logged or dry areas, particularly in nitrogen limited habitats.

Keywords: black alder (*Alnus glutinosa* L.); drought; flooding; antioxidants; carbohydrates; amino acids; metabolic regulation; nitrogen fixation tree species

Citation: Zhu, L.; Liao, Z.; Liu, L.; Du, B. Effects of Extreme Drought and Heat Events on Leaf Metabolome of Black Alder (*Alnus glutinosa* L.) Growing at Neighboring Sites with Different Water Availability. *Forests* **2023**, *14*, 151. https://doi.org/10.3390/f14010151

Academic Editors: Mateja Germ and Ivan Kreft

Received: 14 December 2022
Revised: 9 January 2023
Accepted: 10 January 2023
Published: 13 January 2023

1. Introduction

Riparian forest ecosystems are extremely important corridors due to their high productivity, biodiversity, and ecological services [1,2]. Nowadays, they are under threat from projected global climate change and changes of land-use by exacerbating aridification and altering hydrological regimes [3]. Dioecious riparian trees are usually waterlogging-tolerant species but sensitive to water shortage [4,5]; therefore, they may be particularly vulnerable to the projected warming climate and altered precipitation [6]. Steadily increased greenhouse gas emissions have significantly changed the global climate, and will continue to do so in the future, with more frequent and intensive climate extremes such as heat and drought events projected [7]. These extreme climate events could fundamentally alter the composition, structure, and biogeography of forests in many regions [8,9]. Apparently, the complex physiological process will be impacted by fluctuated soil water availability as well

as high temperatures, for instance reducing transpiration and photosynthesis, invoking antioxidative and osmoprotective system, regulating metabolic pathways, adjusting carbohydrates and amino compounds partitioning and allocation, and consequently impairing growth and causing mortality [10–14]. Increased tree mortality and die-offs triggered by drought and/or heat have been well documented in some locations, e.g., southern Europe, western North America, and northern Australia [15–17]. Moreover, significant expansion of drought-tolerant taxa in riparian ecosystems in semi-arid to arid regions around the world is expected [8]. Compared to intensively addressed responses of trees to waterlogging, less is known about the physiological effects of water shortage on riparian species, which are vulnerable to hydrogeomorphological changes [8,18–20].

In summer 2018, central and northern Europe were stricken by extreme drought and heat [21]. Germany had never experienced such hot and dry conditions from March to November as in 2018 [22]. The negatively impacted areas of the 2018 summer drought are 1.5 times larger and significantly stronger compared to August 2003 [23], not only in terms of crop production, but also for the forest ecosystems [21,24,25]. These disasters again highlight the emerging climate change risks for forests. On the other hand, each of the recent extreme drought and heat events also provide a unique opportunity to study the response of tree species to heat and drought waves and evaluate its fate under such a changing climate [21]. *Alnus glutinosa* L., also known as black alder, naturally distributes in most of Europe, from central Scandinavia to the southern coast of the Mediterranean Sea, and is often found growing in wetlands, as well as near ponds, lakes, and rivers. It represents about 5% of the forest area and forms large highly productive stands in north and south parts of Central Europe [26]. It is not only an economically important species for timber production, but also frequently used as a potential tree for brackish and saline habitats [27], and as a valuable admixture species for improving soil properties due to its robust root system and promising nitrogen fixation ability [26,28]. Unlike most hygrophilous tree species, black alders are often found in drier environments as a pioneer species [29], although they are sensitive to drought [4,5,30]. Previous studies have shown alder had much weaker stomatal regulation than European beech (*Fagus sylvatica* L.) and oak (*Quercus petraea* L.) in response to limited soil water content [31,32], and its leaf level water relations were hardly influenced by in situ water conditions [33]. Little information is available regarding the leaf level physiological responses of black alder to different water conditions.

In the present study, to explore the leaf metabolic responses of black alder to different water conditions, we compared the hydration, reactive oxygen species (ROS) levels and antioxidants characteristics, profiles of carbohydrates, nitrogen compounds, as well as other low molecular weight water soluble metabolites and anions in leaves of black alder trees grown at neighboring sites with different water availability. Specifically, we tested the following hypotheses: (1) trees with limited water supply have lower leaf hydration, and consequently higher ROS levels and upregulated antioxidants contents; (2) water limitation also induced accumulation of osmoprotectants as well as altered carbon and nitrogen metabolic pathways. The study will help to unfold the physiological responses of black alder to limited water availability, and provide valuable information for forest management in the future with projected warmer and drier conditions.

2. Materials and Methods

2.1. Experimental Conditions and Plant Material

The experimental site is located at the Moosweiher lake of Freiburg, Baden-Württemberg, Germany (7°48′16.484″ E, 48°1′43.828″ N) (Figure 1). The mean annual temperature is 11.4 °C and mean annual rainfall is 662.1 mm (Deutscher Wetterdienst, DWD). The total area of the lake is ca. 7.6 ha with a maximum depth of 8 m and elevation of 272 m asl. Water conductivity was between 337 and 299 μS cm^{-1} [34]. The surrounding tree species are mostly black alder, with some scattered *Tilia cordata* and *F. sylvatica*. In the present study, 10 adult *Alnus glutinosa* trees were selected: 5 trees along the lake shore

with unlimited water availability were selected as control (SW) group, while the other 5 trees > 20 m away from the lake (which is farther than the roots distribution limit of the trees [35,36]) served as the limited water availability (LW) group. The diameters at breast height (DBH) were measured before sampling. DBH were 27.4 ± 1.9 cm and 26.8 ± 2.1 cm for control and low water availability groups, respectively. Data of air temperature and precipitation (Figure 2) of the study area were obtained from the Deutscher Wetterdienst (https://www.dwd.de/EN/Home/home_node.html (accessed on 16 September 2021)). Sampling took place on 31th August 2018 between 12:00 to 14:00 during the extreme summer drought event across Europe [21,23]. Twigs from the southwest side of the middle crown of the 10 alder trees were cut, leaves were immediately harvested and frozen in liquid nitrogen and transported to lab, then stored in −80 °C until homogenized in liquid nitrogen for further analysis.

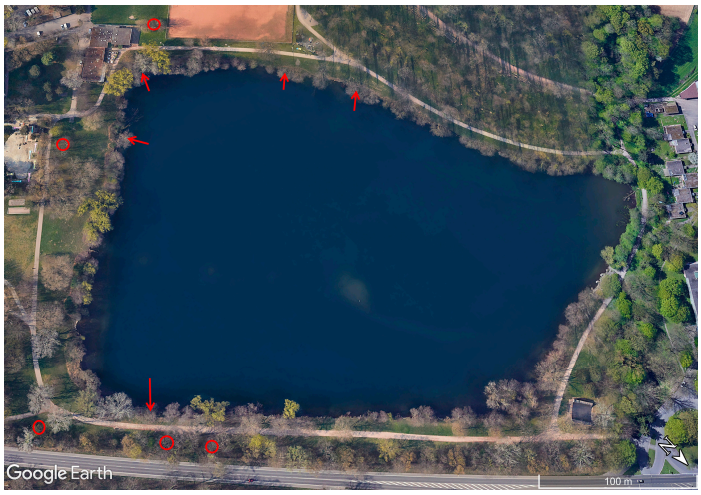

Figure 1. Map of sampling trees at the lake. Arrows and circles indicate the sample trees with sufficient and limited water resource, respectively.

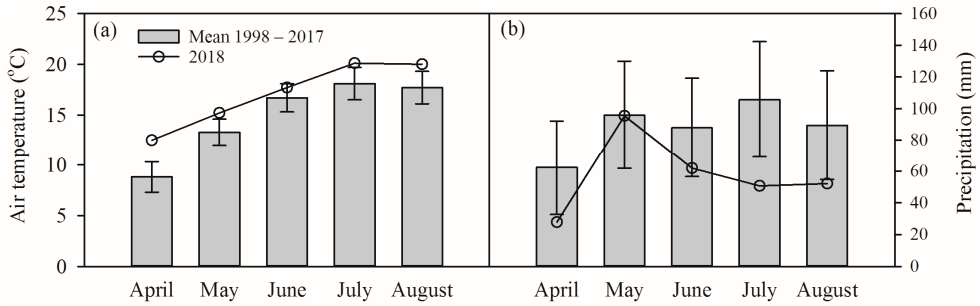

Figure 2. Monthly air temperature (**a**) and precipitation (**b**) in 2018 (in line) and the average from 1998 to 2017 (in bar plot, mean ± standard deviation). Data from the Deutscher Wetterdienst.

2.2. Leaf Hydration Determination

Leaf hydration (g H_2O g^{-1} DW) was determined as (FW-DW)/DW, where FW is the fresh weight and DW is the dry weight. DW was obtained by drying the samples in an oven at 60 °C to constant weight [37].

2.3. Determination of Hydrogen Peroxide (H_2O_2), Malondialdehyde (MDA) Contents, and In Vitro Activities of Glutathione Reductase (GR) and Dehydroascorbate Reductase (DHAR)

Leaf H_2O_2 was extracted and determined as described in [37]. Frozen leaf powder was extracted in 0.1% (*w*/*v*) trichloroacetic acid (TCA). After centrifugation at 120,000× *g* for 15 min, aliquots of 300 µL supernatant were combined with 300 µL of 10 mM potassium phosphate buffer (pH 7.0) and 600 µL of 1 M KI. The absorbance of H_2O_2 was measured at

390 nm (UV-DU650 spectrophotometer, Beckman Coulter Inc., Fullerton, CA, USA). H_2O_2 concentration was quantified using a standard curve ranging from 0 to 200 µM H_2O_2.

The malondialdehyde content was determined as described by Tariq et al. [5]. Briefly, 50 mg frozen leaf powder was extracted with 1.5 mL 10% (*w*/*v*) trichloroacetic acid (TCA) in 95 °C water bath for 30 min. After centrifugation at 120,000× *g* for 5 min, 0.75 mL supernatant was mixed with 0.75 mL 0.6% thiobarbituric acid solution, and the mixture was again incubated at 95 °C for 30 min. The mixture was then cooled in an ice bath, and its absorbance (OD) at 450, 532, and 600 nm were read with a UV-DU650 spectrophotometer (Beckman Coulter Inc.). The MDA concentration was calculated using the following equation:

$$\text{MDA}(\text{mol g}^{-1}) = 6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \times \text{OD}_{450}$$

In vitro GR (EC 1.8.1.7) and DHAR (EC 1.8.5.1) activities of leaves were determined as described previously [38]. GR activity was quantified by monitoring glutathione dependent oxidation of 1.25 mM NADPH at 340 nm; DHAR activity was analyzed directly by following the increase in absorbance at 265 nm, resulting from GSH-dependent production of ascorbate [39].

2.4. Thiols and Ascorbate Measurement

Thiols, i.e., total and oxidized glutathione (GSH), cysteine, and γ-glutamylcysteine were extracted with 1 mL 0.1 M HCl containing polyvinylpoly-pyrrolidone (PVP 6755, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as previously described by Schupp and Rennenberg [40]. Quantification of oxidized glutathione (GSSG) was based on the irreversible alkylation of the free thiol groups of the GSH present with N-ethylmaleimide (NEM) and the subsequent reduction of GSSG with dithiothreitol (DTT) [41]. Reduced thiols were derivatized with monobromobimane and separated on an ACQUITY UPLC® HSS (Waters GmbH, Eschborn, Germany) with a C-18 column (2.1 × 50 mm; 1.18 µm mesh size, Agilent Technologies, Palo Alto, CA, USA), applying a solution of potassium acetate (100 mM, pH 5.3) in methanol (100%) for elution. Concentrations of thiols were quantified according to a mixed standard solution consisting of GSH, cysteine, and γ-glutamylcysteine subjected to the same procedure [42].

Leaf total and reduced ascorbate were determined using a colorimetric method previously described [37]. Concentrations of total and reduced ascorbate were calculated according to a standard curve using dilutions of 1.5 mg ml^{-1} L-ascorbic acid (Sigma-Aldrich, Steinheim, Germany).

2.5. Soluble Protein and Sugar Determination

Total soluble protein contents were determined as previously described [43]. Absorbance at 595 nm was measured by a Sunrise Microplate Reader (Tecan Austria GmbH, Groedig, Austria). Contents were quantified according to a standard curve using bovine serum albumin standards (BSA; Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany).

Soluble sugar was extracted and determined as previously described [37]. Fifty mg frozen leaf powder was extracted with 1.5 mL of milliQ water at 95 °C for 5 min. After centrifugation, 200 mL of 10 times diluted supernatants were mixed with 1 mL anthrone reagent (50 mg anthrone and 1 g thiourea in 100 mL 70% H_2SO_4). The reaction was boiled for 15 min and the absorbance was measured at 578 nm after cooling down [44]. Sucrose (Sigma-Aldrich Chemie GmbH) was used as a standard for quantification.

2.6. Determination of Anions

Anions of phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) were determined in aqueous extracts from homogenized frozen material by automated anion chromatography as described previously [45]. Separation of anions was achieved on an ion exchange column (AS12A, 4 mm, Dionex, Idstein, Germany) with 2.7 mM Na_2CO_3 and 0.3 mM $NaHCO_3$ as the mobile phases. Detection and quantification were performed with a pulsed amperometric detector

(Electrochemical detector ED 40 Dionex). Sodium salts of phosphate and sulphate were used as standards.

2.7. Low Molecular Weight Soluble Metabolites Analyzed by Gas Chromatography-Mass Spectrometry (GC-MS)

Relative abundances of water soluble low-molecular-weight metabolites in leaves were analyzed by a gas chromatography–mass spectrometry system (GC-MS, Agilent GC 6890N coupled to a 5975C quadrupole MS detector; Agilent Technologies, Palo Alto, CA, USA). Metabolites were extracted, derivatized, and separated according to a method previously described [37]. Peak identification and deconvolution of chromatograms were performed using the quantitative analysis module of the MASSHUNTER software (Agilent Technologies). For metabolite identification, the Golm metabolome database [46] were used. Peak areas were normalized using the peak area of the internal standards ribitol (Sigma-Aldrich) and the dry weight of the samples. Abundance of metabolites was indicated by normalized peak areas. Artefact peaks and common contaminants were identified by analysis of "blank" samples prepared in the same manner as biological samples. Signals corresponding to these artefacts were omitted from interpretation.

2.8. Statistical Analysis

Significant differences of trees between SW (trees with sufficient water) and LW (trees with limited water availability) groups were examined by t-test using SigmaPlot 12.0 (Systat Software GmbH, Erkrath, Germany). To have an overview of the water condition effects, partial least square discriminant analysis (PLS-DA) was conducted using a public web tool (MetaboAnalyst 5.0, http://www.metaboanalyst.ca/ (accessed on 7 January 2023)) [47] after $\log_{10}$ transformation and mean-centering. Missing values were replaced by half the minimum abundance of respective compounds, assuming that their concentrations were below detection limit. Data shown in figures and tables represent means ± standard error (n = 5) on a dry weight basis.

3. Results

Compared to trees under sufficient water condition (SW), trees grown under limited water condition (LW) had 20% lower leaf hydration (p = 0.002) and 39% decreased leaf hydrogen peroxide contents (p = 0.03) (Figure 3a,b), but similar malondialdehyde content (Figure 3c). Trees with LW had 37% and 59% higher leaf soluble sugar (p = 0.02) and soluble protein contents (p = 0.03), respectively (Figure 4a,b). Leaf sulfate content of LW trees was 25% lower (p = 0.02) than SW trees, whereas, phosphate content was 46% higher (p = 0.007) (Figure 4c,d).

Water availability had no significant effects on leaf total, reduced ascorbate, dehydroascorbate (DHA), as well as the ratio between reduced ascorbate and DHA (Figure 5). Leaf cysteine and γ-glutamylcysteine (γ-EC) contents did not change between SW and LW conditions (Figure 6a,b). Whereas, total and oxidized GSH were dramatically accumulated in leaves of alder trees at LW, i.e., 5.3 and 5.0 folds higher than SW, respectively (Figure 6c,d), compared to SW trees, LW trees had 34% lower dehydroascorbate reductase (DHAR) activity ($p < 0.05$), but similar glutathione reductase activity (Figure 7).

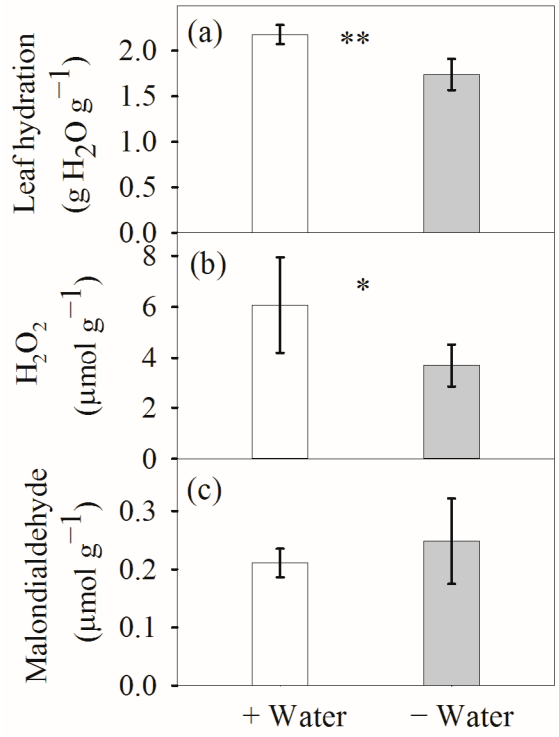

Figure 3. Leaf hydration (**a**), hydrogen peroxide (**b**), and malondaldehy contents (**c**) of *Alnus glutinosa* grown under sufficient (+ water, blank bars) and limited water conditions (− water, grey bars). Data shown represent mean ± standard deviation (n = 5) on a dry weight basis. Asterisks indicate significant differences between trees with sufficient and limited water supply at $p < 0.05$ (*) and 0.01 (**) respectively.

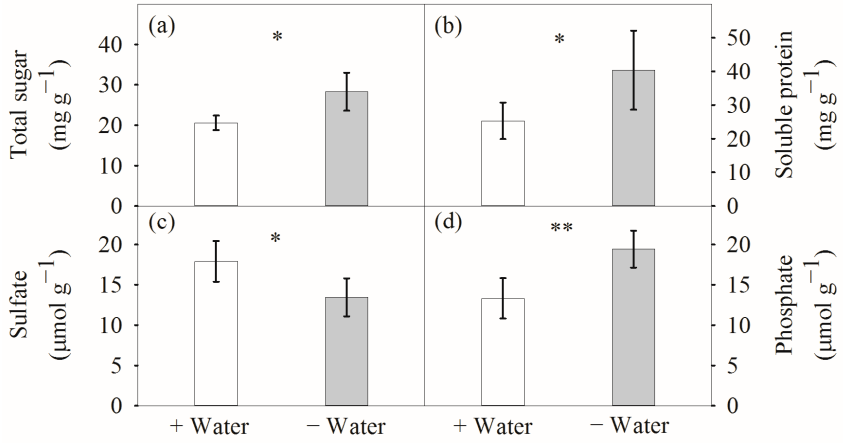

Figure 4. Leaf total sugar (**a**), soluble protein (**b**), sulfate (**c**) and phosphate contents (**d**) in leaves of *Alnus glutinosa* grown under sufficient (+ Water, blank bars) and limited water conditions (− Water, grey bars). Data shown represent mean ± standard deviation (n = 5) on a dry weight basis. Asterisks indicate significant differences between trees with sufficient and limited water supply at $p < 0.05$ (*) and 0.01 (**) respectively.

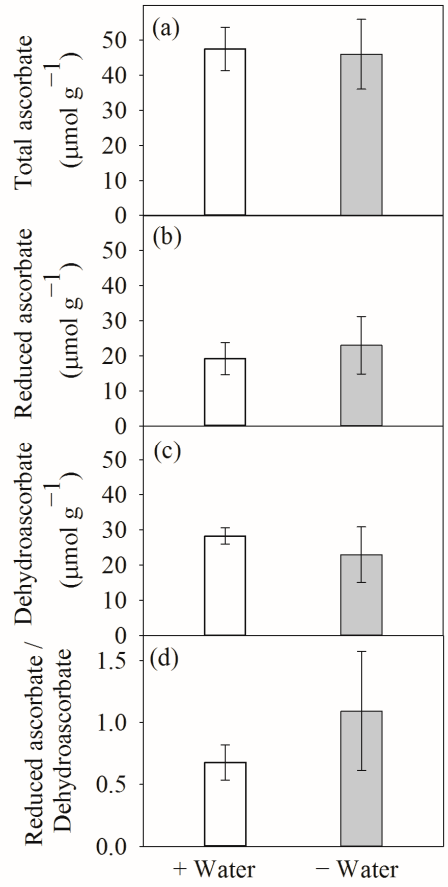

Figure 5. Contents of total (**a**), reduced ascorbate (**b**), dehydroascorbate (**c**), and ratio between reduced and dehydroascorbate (**d**) in leaves of *Alnus glutinosa* grown under sufficient (+ water, blank bars) and limited water conditions (− water, grey bars). Data shown represent mean ± standard deviation (n = 5) on a dry weight basis. No significant differences ($p < 0.05$) between trees with sufficient and limited water supply were found in all parameters.

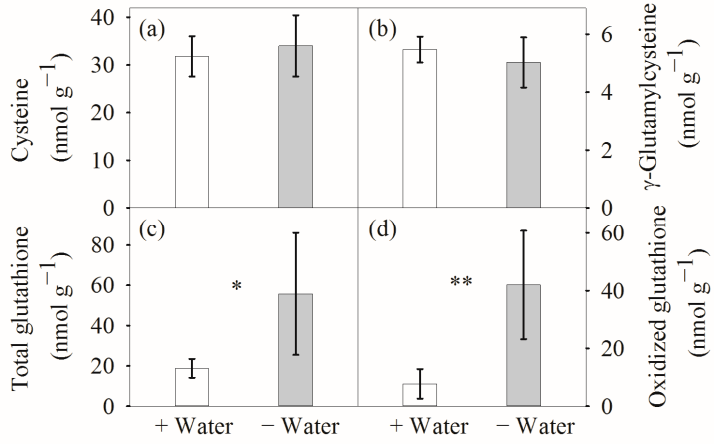

Figure 6. Thiols of cysteine (**a**), γ-glutamylcysteine (**b**), total (**c**), and oxidized glutathione (**d**) contents in leaves of *Alnus glutinosa* grown under sufficient (+ water, blank bars) and limited water conditions (− water, grey bars). Data shown represent mean ± standard deviation (n = 5) on a dry weight basis. Asterisks indicate significant differences between trees with sufficient and limited water supply at $p < 0.05$ (*) and 0.01 (**) respectively.

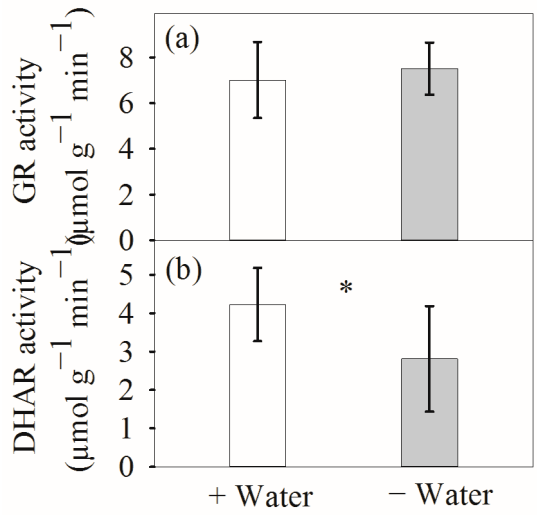

Figure 7. Activities of glutathione reductase (GR) (**a**) and dehydroascorbate reductase (DHAR) (**b**) in leaves of *Alnus glutinosa* grown under sufficient (+ water, blank bars) and limited water conditions (− water, grey bars). Data shown represent mean ± standard deviation (n = 5) on a dry weight basis. Asterisk (*) indicates significant differences between trees with sufficient and limited water supply at $p < 0.05$.

Generally, abundances of sugars, e.g., β-D-allose and lyxose, D-xylobiose, and arabinose; sugar acids, e.g., ribonic acid, gluconic acid, glyceric acid, and gulonic acid; as well as polyols of sorbitol, arabitol, and cellobiitol were higher in LW trees than in SW trees (Figure 8). Similarly, higher abundances of amino acids were also documented under LW, particularly for serine, alanine derived from 3-phosphoglycerate and pyruvate, respectively. Limited water availability also resulted in accumulation of most phenolic compounds, i.e., catechin, tyrosol, 4-methylcatechol, piceatannol, cis-4-hydroxy-cinnamic acid, hydroquinone, and their precursors of quinic acid, shikimic acid, and phenylalanine derivatives. Abundances of sugar alcohols of galactinol and viburnitol, two proline derivatives of N-methyl cis-4-hydroxymethyl-L-proline and N-methyl trans-4-hydroxy-L-proline 2S,4R-4-hydroxy-1-methyl pyrrolidine-2-carboxylic acid (R002953), two phenolics of 4-hydroxy-benzoic acid and threo-guaiacylglycerol, as well as fumarate, were significantly ($p < 0.05$) declined under limited water conditions (Figure 8).

To have an overall view of the drought effects, a PLS-DA analysis based on the 46 parameters with significant differences (Table S1) in the present study was performed. A significant separation between LW and SW plants (R^2 = 0.974, Q^2 = 0.849) were presented in the scores plot, and component 1 explained 67.8% of variance (Figure 9). The sugars of D-Xylobiose, β-D-allose, lyxose and dihydroxyacetone, GSSG and total GSH, phenolics of dihydroxyphenylalanine, tyrosol and piceatannol, and galactinol were the top 10 important compounds of component 1 according to their VIP scores (Variable Importance for Projection) (Figure 9b). These compounds were significantly ($p < 0.01$) increased under LW except for galactinol (Figures 8 and 9b).

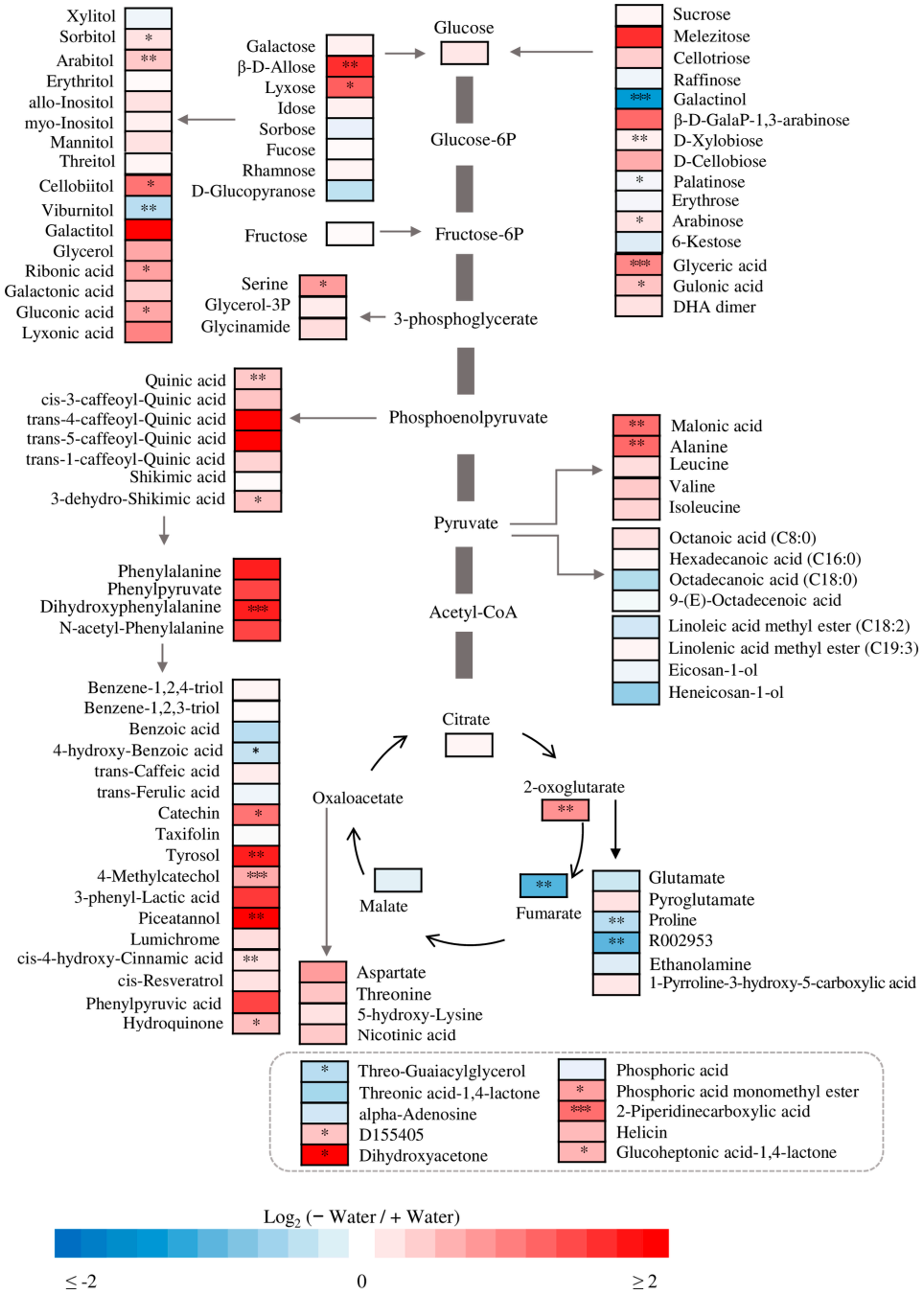

Figure 8. Changes (log_2 limited water/sufficient water) of low molecular weight metabolites in leaves of *Alnus glutinosa* grown under sufficient (+ water) and limited (− water) water conditions. *, **, and *** indicate significant differences between trees with sufficient and limited water supply at $p < 0.05$, 0.01, and 0.001, respectively. R002953 and D155405 are codes of N-methyl trans-4-hydroxy-L-proline (2S,4R)-4-hydroxy-1-methyl pyrrolidine-2-carboxylic acid and an unknown metabolite, respectively, in Golm library.

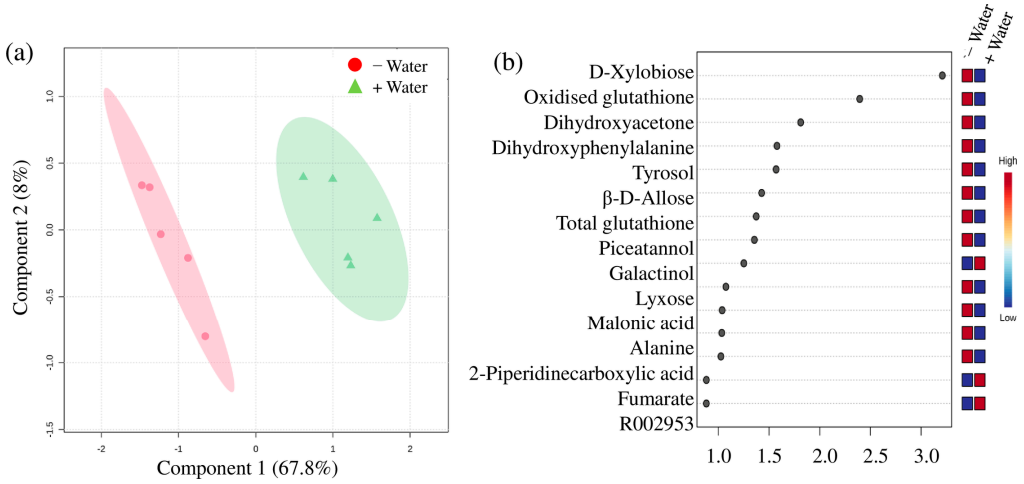

Figure 9. Clustering (**a**) of *Alnus glutinosa* grown under sufficient (+ water, triangle) and limited (− water, circle) water conditions. PLS-DA analysis was performed based on 46 with significant differences. Semi-transparent shadings indicate 95% confidence regions. (**b**) The most important 15 parameters according to VIP (Variable Importance for Projection) scores generated from PLS-DA analysis.

4. Discussion

4.1. Leaf Hydration and Antioxidative Systems at Different Water Conditions

Drought is a misfortune for both forest and agriculture since water is crucial for plant survival and growth [48]. Plants have strategies to cope with water limitation. In addition to the fastest processes of the abscisic acid (ABA)-mediated stomatal closure to reduce water loss, under prolonged drought stress or increased stress intensity, plants can also increase root water uptake from the deeper soil, adjust osmotic processes, and activate the antioxidative systems [12,48]. The latter are fundamentally important to protect the photosynthetic apparatus from photo-oxidative destruction [12,49,50]. Plenty of studies have demonstrated that the ascorbate-glutathione pathway plays a vital role in detoxifying ROS in many plant species [51]. In the present study, although we could not determine and exclude the ground level soil water supply, compared to trees with sufficient water, significantly decreased leaf hydration in LW trees may indicate water shortage [52] and/or a possible signal for longer-term acclimation processes [53] during a long term drought and heat event [21,23]. However, severe damage of plant cells was not speculated as indicated by the stable MDA contents and lowered H_2O_2 contents (Figure 3) [5,50].

Ascorbate and glutathione are differentially influenced by environmental factors [51]. In the present study, leaf total and oxidized glutathione contents were increased in LW trees compared to SW trees. Similarly, increased leaf glutathione contents have been documented in drought treated apple (*Malus domestica*), European beech, and poplar (*Populus nigra* × *deltoides*) [54–56]. The enhanced glutathione concentrations are thought to provide better protection under abiotic stresses [50,52]. The relatively low capacity of oxidized glutathione reducing systems as seen from the dramatically accumulated GSSG contents could be partly attributed to changes in NADP(H) redox status as well as the stable glutathione reductase (GR) activity [50]. Feasibility of DHA reductase activity and DHA pool size as indication of oxidative stress is still under debate [57], although many studies have shown increased DHAR activity in concert with enhanced ascorbate contents under drought conditions [58,59]. In the present study, little effects of water conditions were observed in both reduced and total ascorbate contents (Figure 5), but a 34% declined DHAR activity was observed under drought (Figure 7b). Similarly, declined DHAR activities were also observed in apple (*Malus prunifolia* and *M. hupehensis*) [60] and *Pinus densata* [61] leaves after long term drought treatment. Conserved leaf total and reduced ascorbate contents were also observed in date palm (*Phoenix dactylifera* L.) seedlings, even though

DHAR activities were significantly increased under drought [38]. The regeneration of ascorbate in the plant takes place in two ways: the Mehler APX reaction mainly reducing monodehydroascorbate (MDHA) to ascorbate, and the Halliwell-Foyer-Asada cycle mainly reducing DHA to ascorbate [62]. The contribution to the reduction of oxidized ascorbate of the latter is estimated to be much lower than that of the Mehler APX reaction [63]. In the present study, DHAR activity was significantly decreased in LW trees (Figure 7); however, the decreased DHAR activity had little effects on different forms of ascorbate contents (Figure 5), which was probably due to either the enhanced biosynthesis of ascorbate or the stimulated reduction via the Mehler APX reaction. Moreover, recent studies in *Arabidopsis thaliana* found that both DHAR activities and glutathione contents determine the ascorbate accumulation, and GSH itself can reduce DHA nonenzymatically [64]. Compared to trees with sufficient water supply, lower leaf H_2O_2 contents in trees with less water availability probably indicated to some extent drought resistance of *A. glutinosa*, and, apart from the ascorbate-glutathione cycle [50,51,65], other protective mechanisms may exist, most likely from the significantly increased abundances of secondary metabolites, i.e., tyrosol, catechin, hydroquinone, as well as 4-methylcatechol and piceatannol with antioxidant activity (Figure 8) [66]. Therefore, our first hypothesis was only partly supported because trees with limited water availability and lower leaf hydration did not translate to higher ROS levels and apparently upregulated antioxidants contents.

4.2. Compatible Solutes at Different Water Conditions

Soluble sugars, sugar alcohols, protein, and amino acids are notable osmolytes playing crucial roles in maintaining osmotic equilibrium and protecting macromolecules, as well as membranes, thereby providing resistance against drought and cellular dehydration [65]. Tariq et al. [5] found soluble sugars were accumulated, whereas the soluble proteins was decreased in drought-stressed 2-year-old *A. cremastogyne* seedlings. In the present study, consistent with our second hypothesis, monosaccharide of lyxose, β-D-allose, arabinose, and disaccharides of D-xylobiose were significantly increased under LW conditions (Figure 8) and total sugar content was 17% higher than trees with sufficient water, but not statistically significant ($p = 0.16$, Figure 4a). On the contrary, leaf soluble protein contents were significantly accumulated in trees with limited water supply (1.6 folds of SW trees). Abundances of sugar alcohols and sugar acids were largely increased under drought, particularly for sorbitol, arabitol, and cellobiitol of sugar alcohols, as well as ribonic acid, glyceric acid, and gluconic acid of sugar acids [65]. Instead of drought-induced higher proline contents [5], we found proline derivates were significantly decreased in LW trees. However, abundances of alanine, serine, and 2-piperidinecarboxylic acid, as well as dihydroxyphenylalanine, the precursor of dopamine, were significantly enhanced in the present study (Figure 8). Together with significantly accumulated soluble protein, an altered nitrogen metabolism is speculated. Similar effects were also reported in other plant species in response to abiotic stresses [67–70].

Galactinol and raffinose function as antioxidants and/or osmoprotectants, which may lead to the increased tolerance of oxidative damage caused by drought [66], as observed previously in date palm, *A. thaliana*, and *Zea mays* leaves [68,71–73]. However, in the present study, abundance of galactinol was significantly decreased in LW trees (Figure 8), which was probably due to the concurrent high temperature [72], as also observed in *Betula pendula* [74]. Similar effects of water shortage on foliar galactinol contents were also observed in Douglas fir (*Pseudotsuga menziesii*) needles [67], which could be the effects of enhanced consumption for the synthesis of osmolytes of the raffinose family, and function as antioxidants [75]. The latter has been identified as an endogenous mediator of defense amplification and priming in *Arabidopsis thaliana*, and its accumulation was critical for systemic acquired and local resistance to bacterial pathogens [76]. Like drought-treated Douglas fir and date palm, European beech, cork oak (*Quercus suber*) [67,68,77,78], as well as anoxia exposed *Sebastiania commersoniana*, *Erythrina speciosa*, and *Sesbania virgate* [79], and pathogen-infected silver birch [80], no significant effects of water conditions were observed in fatty acid composition

and concentration, probably indicating stable membrane structures, as also indicated by the conserved MDA contents [81]. Although we observed significantly decreased leaf hydration, we could not conclude the LW trees were stressed, since we did not determine the plant and soil water potential.

4.3. Responses of Anions to Water Availability

Studies have proved that sulfate can trigger ABA production and regulate stomatal closure in Arabidopsis (*A. thaliana*) [82,83]. Moreover, it was the only macronutrient that increases in the xylem sap of maize (*Zea mays*) in a drought [84]. The declined leaf sulfate contents of LW trees may reflect drought-induced declined roots uptake and xylem transport and higher demand of sulfate for synthesis of the ROS scavenger glutathione, as well as a result of the regulatory function of ABA signaling to maintain stomatal conductance at a certain level [82,83,85], therefore, to prevent carbon starvation [86]. Moreover, the foliar phosphate concentration was also significantly increased in LW trees (Figure 4d), which has been shown to significantly improve the drought resistance of *A. cremastogyne* seedlings [5].

5. Conclusions

In conclusion, although the black alder is often observed in humid habitats and was thought to be drought sensitive, our study suggests that it has at least to some extent tolerance to water shortage, partially due to the protection from the accumulated nonenzymatic antioxidants and compatible solutes including sugars, sugar alcohols, sugar acids, and nitrogen compounds. The current study also highlights the potential of black alder as a pioneer tree species in forestation and as an intercropping species in soil improvement due to its prominent ability of drought resistance and nitrogen fixation. In the future, more detailed experiments under well controlled conditions as well as long-term field investigations are recommended to have a deep understanding of the effects of projected drought and heat events on this tree species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14010151/s1, Table S1: Variable Importance for Projection (VIP) scores of partial least square discriminant analysis (PLS-DA).

Author Contributions: Conceptualization, B.D.; investigation and data curation, L.Z., Z.L. and B.D.; original draft preparation, L.Z., Z.L., L.L. and B.D.; review and editing, L.L. and B.D.; funding acquisition, B.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Science and Technology Department of Sichuan Province, China (2019YJ0508), and the National Natural Science Foundation of China (32271863). We acknowledge support by the Open Access Publication Fund of the University of Freiburg.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dybala, K.E.; Matzek, V.; Gardali, T.; Seavy, N.E. Carbon Sequestration in Riparian Forests: A Global Synthesis and Meta-analysis. *Glob. Chang. Biol.* **2019**, *25*, 57–67. [CrossRef] [PubMed]
2. Kozlowski, T.T. Physiological-Ecological Impacts of Flooding on Riparian Forest Ecosystems. *Wetlands* **2002**, *22*, 550–561. [CrossRef]
3. Rodríguez-González, P.M.; Colangelo, M.; Sánchez-Miranda, Á.; Sánchez-Salguero, R.; Campelo, F.; Rita, A.; Gomes Marques, I.; Albuquerque, A.; Ripullone, F.; Camarero, J.J. Climate, Drought and Hydrology Drive Narrow-Leaved Ash Growth Dynamics in Southern European Riparian Forests. *For. Ecol. Manag.* **2021**, *490*, 119128. [CrossRef]
4. Hu, H.; Chen, H.; Hu, T.; Zhang, J. Adaptability Comparison between the Seedlings of *Eucalyptus grandis* and *Alnus cremastogyne* under the Condition of Continuous Drought Stress. *J. Agric. Sci.* **2012**, *4*, 75–86. [CrossRef]

5. Tariq, A.; Pan, K.; Olatunji, O.A.; Graciano, C.; Li, Z.; Sun, F.; Zhang, L.; Wu, X.; Chen, W.; Song, D. Phosphorous Fertilization Alleviates Drought Effects on *Alnus Cremastogyne* by Regulating Its Antioxidant and Osmotic Potential. *Sci. Rep.* **2018**, *8*, 5644. [CrossRef] [PubMed]
6. Hultine, K.R.; Bush, S.E.; West, A.G.; Ehleringer, J.R. Population Structure, Physiology and Ecohydrological Impacts of Dioecious Riparian Tree Species of Western North America. *Oecologia* **2007**, *154*, 85–93. [CrossRef]
7. Masson-Delmotte, V.; Zhai, P.; Pirani, A.; Connors, S.L.; Péan, C.; Berger, S.; Caud, N.; Chen, Y.; Goldfarb, L.; Gomis, M.I.; et al. IPCC Summary for Policymakers. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: Cambridge, UK, 2021; p. 41.
8. Kominoski, J.S.; Shah, J.J.F.; Canhoto, C.; Fischer, D.G.; Giling, D.P.; González, E.; Griffiths, N.A.; Larrañaga, A.; LeRoy, C.J.; Mineau, M.M.; et al. Forecasting Functional Implications of Global Changes in Riparian Plant Communities. *Fron. Ecol. Environ.* **2013**, *11*, 423–432. [CrossRef]
9. Marques, I.G.; Campelo, F.; Rivaes, R.; Albuquerque, A.; Ferreira, M.T.; Rodríguez-González, P.M. Tree Rings Reveal Long-Term Changes in Growth Resilience in Southern European Riparian Forests. *Dendrochronologia* **2018**, *52*, 167–176. [CrossRef]
10. Pezeshki, S.R.; Hinckley, T.M. Water Relations Characteristics of *Alnus rubra* and *Populus trichocarpa*: Responses to Field Drought. *Can. J. Forest Res.* **1988**, *18*, 1159–1166. [CrossRef]
11. Hacke, U.; Sauter, J.J. Drought-Induced Xylem Dysfunction in Petioles, Branches, and Roots of Populus *Balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. *Plant Physiol.* **1996**, *111*, 413–417. [CrossRef]
12. Rennenberg, H.; Loreto, F.; Polle, A.; Brilli, F.; Fares, S.; Beniwal, R.S.; Gessler, A. Physiological Responses of Forest Trees to Heat and Drought. *Plant Biol.* **2006**, *8*, 556–571. [CrossRef]
13. McDowell, N.G.; Beerling, D.J.; Breshears, D.D.; Fisher, R.A.; Raffa, K.F.; Stitt, M. The Interdependence of Mechanisms Underlying Climate-Driven Vegetation Mortality. *Trends Ecol. Evol.* **2011**, *26*, 523–532. [CrossRef]
14. Brunner, I.; Herzog, C.; Dawes, M.A.; Arend, M.; Sperisen, C. How Tree Roots Respond to Drought. *Front. Plant Sci.* **2015**, *6*, 547. [CrossRef]
15. Allen, C.D.; Macalady, A.K.; Chenchouni, H.; Bachelet, D.; McDowell, N.; Vennetier, M.; Kitzberger, T.; Rigling, A.; Breshears, D.D.; Hogg, E.T. A Global Overview of Drought and Heat-Induced Tree Mortality Reveals Emerging Climate Change Risks for Forests. *For. Ecol. Manag.* **2010**, *259*, 660–684. [CrossRef]
16. Duke, N.C.; Kovacs, J.M.; Griffiths, A.D.; Preece, L.; Hill, D.; Oosterzee, P.V.; Mackenzie, J.; Morning, H.S.; Burrows, D. Large-Scale Dieback of Mangroves in Australia's Gulf of Carpentaria: A Severe Ecosystem Response, Coincidental with an Unusually Extreme Weather Event. *Mar. Freshw. Res.* **2017**, *68*, 1816–1829. [CrossRef]
17. Restaino, C.; Young, D.J.N.; Estes, B.; Gross, S.; Wuenschel, A.; Meyer, M.; Safford, H. Forest Structure and Climate Mediate Drought-Induced Tree Mortality in Forests of the Sierra Nevada, USA. *Ecol. Appl.* **2019**, *29*, e01902. [CrossRef]
18. Stella, J.C.; Riddle, J.; Piégay, H.; Gagnage, M.; Trémélo, M.-L. Climate and Local Geomorphic Interactions Drive Patterns of Riparian Forest Decline along a Mediterranean Basin River. *Geomorphology* **2013**, *202*, 101–114. [CrossRef]
19. Kreuzwieser, J.; Rennenberg, H. Molecular and Physiological Responses of Trees to Waterlogging Stress. *Plant Cell Environ.* **2014**, *37*, 2245–2259. [CrossRef]
20. Chen, Y.; Chen, Y.; Zhou, H.; Hao, X.; Zhu, C.; Fu, A.; Yang, Y.; Li, W. Research Advances in Plant Physiology and Ecology of Desert Riparian Forests under Drought Stress. *Forests* **2022**, *13*, 619. [CrossRef]
21. Bastos, A.; Ciais, P.; Friedlingstein, P.; Sitch, S.; Pongratz, J.; Fan, L.; Wigneron, J.-P.; Weber, U.; Reichstein, M.; Fu, Z. Direct and Seasonal Legacy Effects of the 2018 Heat Wave and Drought on European Ecosystem Productivity. *Sci. Adv.* **2020**, *6*, eaba2724. [CrossRef]
22. Zscheischler, J.; Fischer, E.M. The Record-Breaking Compound Hot and Dry 2018 Growing Season in Germany. *Weather Clim. Extrem.* **2020**, *29*, 100270. [CrossRef]
23. Buras, A.; Rammig, A.; Zang, C.S. Quantifying Impacts of the Drought 2018 on European Ecosystems in Comparison to 2003. *Biogeosci. Discuss.* **2019**, *17*, 1655–1672. [CrossRef]
24. Peters, W.; Bastos, A.; Ciais, P.; Vermeulen, A. A Historical, Geographical and Ecological Perspective on the 2018 European Summer Drought. *Philos. Trans. R. Soc. Lond. Ser. B* **2020**, *375*, 20190505. [CrossRef]
25. Schuldt, B.; Buras, A.; Arend, M.; Vitasse, Y.; Beierkuhnlein, C.; Damm, A.; Gharun, M.; Grams, T.E.; Hauck, M.; Hajek, P. A First Assessment of the Impact of the Extreme 2018 Summer Drought on Central European Forests. *Basic Appl. Ecol.* **2020**, *45*, 86–103. [CrossRef]
26. Claessens, H.; Oosterbaan, A.; Savill, P.; Rondeux, J. A Review of the Characteristics of Black Alder (*Alnus glutinosa* (L.) Gaertn.) and Their Implications for Silvicultural Practices. *Forestry* **2010**, *83*, 163–175. [CrossRef]
27. Deptuła, M.; Piernik, A.; Nienartowicz, A.; Hulisz, P.; Kamiński, D. *Alnus glutinosa* L. Gaertn. as Potential Tree for Brackish and Saline Habitats. *Glob. Ecol. Conserv.* **2020**, *22*, e00977. [CrossRef]
28. Pogoda, P.; Ochał, W.; Orzeł, S. Modeling Diameter Distribution of Black Alder (*Alnus glutinosa* (L.) Gaertn.) Stands in Poland. *Forests* **2019**, *10*, 412. [CrossRef]
29. Diagne, N.; Arumugam, K.; Ngom, M.; Nambiar-Veetil, M.; Franche, C.; Narayanan, K.K.; Laplaze, L. Use of Frankia and Actinorhizal Plants for Degraded Lands Reclamation. *Biomed Res. Int.* **2013**, *2013*, 948258. [CrossRef]
30. Valor, T.; Camprodon, J.; Buscarini, S.; Casals, P. Drought-Induced Dieback of Riparian Black Alder as Revealed by Tree Rings and Oxygen Isotopes. *For. Ecol. Manag.* **2020**, *478*, 118500. [CrossRef]

31. Granier, A.; Breda, N. Modelling Canopy Conductance and Stand Transpiration of an Oak Forest from Sap Flow Measurements. *Ann. Sci. For.* **1996**, *53*, 537–546. [CrossRef]
32. Herbst, M.; Eschenbach, C.; Kappen, L. Water Use in Neighbouring Stands of Beech (Fagus *Sylvatica* L.) and Black Alder (*Alnus glutinosa* (L.) Gaertn.). *Ann. For. Sci.* **1999**, *56*, 107–120. [CrossRef]
33. Eschenbach, C.; Kappen, L. Leaf Water Relations of Black Alder [*Alnus glutinosa* (L.) Gaertn.] Growing at Neighbouring Sites with Different Water Regimes. *Trees* **1999**, *14*, 28–38. [CrossRef]
34. Chucholl, C.; Pfeiffer, M. First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with Orconectes limosus (Rafinesque, 1817). *Aquat. Invasions* **2010**, *5*, 405–412. [CrossRef]
35. Gasson, P.E.; Cutler, D.F. Tree Root Plate Morphology. *Arboricul. Assoc. J.* **1990**, *14*, 193–264. [CrossRef]
36. Jakubisová, M.; Jakubis, M.; Lukáčik, I. Simulation of Bank Protective Effect of Black Alder Root System (*Alnus glutinosa* (L.) Gaertner) According to RipRoot Model. *Thaiszia J. Bot.* **2014**, *24*, 111–124.
37. Du, B.; Kreuzwieser, J.; Winkler, J.B.; Ghirardo, A.; Schnitzler, J.-P.; Ache, P.; Alfarraj, S.; Hedrich, R.; White, P.; Rennenberg, H. Physiological Responses of Date Palm (*Phoenix dactylifera*) Seedlings to Acute Ozone Exposure at High Temperature. *Environ. Pollut.* **2018**, *242*, 905–913. [CrossRef] [PubMed]
38. Arab, L.; Kreuzwieser, J.; Kruse, J.; Zimmer, I.; Ache, P.; Alfarraj, S.; Al-Rasheid, K.A.; Schnitzler, J.-P.; Hedrich, R.; Rennenberg, H. Acclimation to Heat and Drought—Lessons to Learn from the Date Palm (*Phoenix dactylifera*). *Environ. Exp. Bot.* **2016**, *125*, 20–30. [CrossRef]
39. Polle, A.; Chakrabarti, K.; Schürmann, W.; Rennenberg, H. Composition and Properties of Hydrogen Peroxide Decomposing Systems in Extracellular and Total Extracts from Needles of Norway Spruce (*Picea abies* L., Karst.). *Plant Physiol.* **1990**, *94*, 312–319. [CrossRef]
40. Schupp, R.; Rennenberg, H. Diurnal Changes in the Glutathione Content of Spruce Needles (*Picea abies* L.). *Plant Sci.* **1988**, *57*, 113–117. [CrossRef]
41. Strohm, M.; Jouanin, L.; Kunert, K.J.; Pruvost, C.; Polle, A.; Foyer, C.H.; Rennenberg, H. Regulation of Glutathione Synthesis in Leaves of Transgenic Poplar (*Populus tremula* × *P. alba*) Overexpressing Glutathione Synthetase. *Plant J.* **1995**, *7*, 141–145. [CrossRef]
42. Samuilov, S.; Lang, F.; Djukic, M.; Djunisijevic-Bojovic, D.; Rennenberg, H. Lead Uptake Increases Drought Tolerance of Wild Type and Transgenic Poplar (*Populus tremula* × *P. alba*) Overexpressing Gsh 1. *Environ. Pollut.* **2016**, *216*, 773–785. [CrossRef]
43. Du, B.; Jansen, K.; Junker, L.V.; Eiblmeier, M.; Kreuzwieser, J.; Gessler, A.; Ensminger, I.; Rennenberg, H. Elevated Temperature Differently Affects Foliar Nitrogen Partitioning in Seedlings of Diverse Douglas Fir Provenances. *Tree Physiol.* **2014**, *34*, 1090–1101. [CrossRef]
44. Carroll, N.V.; Longley, R.W.; Roe, J.H. The determination of glycogen in liverand muscle by use of anthrone reagent. *J. Biol. Chem.* **1956**, *220*, 583–593. [CrossRef]
45. Peuke, A.D.; Gessler, A.; Rennenberg, H. The Effect of Drought on C and N Stable Isotopes in Different Fractions of Leaves, Stems and Roots of Sensitive and Tolerant Beech Ecotypes. *Plant Cell Environ.* **2006**, *29*, 823–835. [CrossRef]
46. Hummel, J.; Strehmel, N.; Selbig, J.; Walther, D.; Kopka, J. Decision Tree Supported Substructure Prediction of Metabolites from GC-MS Profiles. *Metabolomics* **2010**, *6*, 322–333. [CrossRef]
47. Chong, J.; Soufan, O.; Li, C.; Caraus, I.; Li, S.; Bourque, G.; Wishart, D.S.; Xia, J. MetaboAnalyst 4.0: Towards More Transparent and Integrative Metabolomics Analysis. *Nucleic Acids Res.* **2018**, *46*, W486–W494. [CrossRef]
48. Gupta, A.; Rico-Medina, A.; Caño-Delgado, A.I. The Physiology of Plant Responses to Drought. *Science* **2020**, *368*, 266–269. [CrossRef]
49. Sarker, U.; Oba, S. Catalase, Superoxide Dismutase and Ascorbate-Glutathione Cycle Enzymes Confer Drought Tolerance of Amaranthus Tricolor. *Sci. Rep.* **2018**, *8*, 16496. [CrossRef]
50. Laxa, M.; Liebthal, M.; Telman, W.; Chibani, K.; Dietz, K.-J. The Role of the Plant Antioxidant System in Drought Tolerance. *Antioxidants* **2019**, *8*, 94. [CrossRef]
51. Foyer, C.H.; Noctor, G. Ascorbate and Glutathione: The Heart of the Redox Hub. *Plant Physiol.* **2011**, *155*, 2–18. [CrossRef]
52. Smirnof, N. The Role of Active Oxygen in the Response of Plants to Water Deficit and Desiccation. *New Phytol.* **1993**, *125*, 27–58. [CrossRef]
53. Tausz, M.; Wonisch, A.; Peters, J.; Jiménez, M.S.; Morales, D.; Grill, D. Short-Term Changes in Free Radical Scavengers and Chloroplast Pigments in *Pinus canariensis* Needles as Affected by Mild Drought Stress. *J. Plant Physiol.* **2001**, *158*, 213–219. [CrossRef]
54. Tausz, M.; Šircelj, H.; Grill, D. The glutathione system as a stress marker in plant ecophysiology: Is a stress-response concept valid? *J. Exp. Bot.* **2004**, *55*, 1955–1962. [CrossRef] [PubMed]
55. García-Plazaola, J.I.; Becerril, J.M. Effects of drought on photoprotective mechanisms in European beech (*Fagus sylvatica* L.) seedlings from different provenances. *Trees* **2000**, *14*, 485–490. [CrossRef]
56. Dusart, N.; Gérard, J.; Le Thiec, D.; Collignon, C.; Jolivet, Y.; Vaultier, M.N. Integrated analysis of the detoxification responses of two Euramerican poplar genotypes exposed to ozone and water deficit: Focus on the ascorbate-glutathione cycle. *Sci. Total Environ.* **2019**, *651*, 2365–2379. [CrossRef] [PubMed]

57. Morell, S.; Follmann, H.; De Tullio, M.; Häberlein, I. Dehydroascorbate and Dehydroascorbate Reductase Are Phantom Indicators of Oxidative Stress in Plants. *FEBS Lett.* **1997**, *414*, 567–570. [CrossRef]
58. Eltayeb, A.E.; Kawano, N.; Badawi, G.H.; Kaminaka, H.; Sanekata, T.; Morishima, I.; Shibahara, T.; Inanaga, S.; Tanaka, K. Enhanced Tolerance to Ozone and Drought Stresses in Transgenic Tobacco Overexpressing Dehydroascorbate Reductase in Cytosol. *Physiol. Plant.* **2006**, *127*, 57–65. [CrossRef]
59. Sorkheh, K.; Shiran, B.; Rouhi, V.; Khodambashi, M.; Sofo, A. Regulation of the Ascorbate-Glutathione Cycle in Wild Almond during Drought Stress. *Russ. J. Plant Physiol.* **2011**, *58*, 76–84. [CrossRef]
60. Wang, W.; Peng, C.; Kneeshaw, D.D.; Larocque, G.R.; Luo, Z. Drought-Induced Tree Mortality: Ecological Consequences, Causes, and Modeling. *Environ. Rev.* **2012**, *20*, 109–121. [CrossRef]
61. Gao, D.; Gao, Q.; Xu, H.-Y.; Ma, F.; Zhao, C.-M.; Liu, J.-Q. Physiological Responses to Gradual Drought Stress in the Diploid Hybrid *Pinus densata* and Its Two Parental Species. *Trees* **2009**, *23*, 717–728. [CrossRef]
62. Horemans, N.; Foyer, C.H.; Asard, H. Transport and Action of Ascorbate at the Plant Plasma Membrane. *Trends Plant Sci.* **2000**, *5*, 263–267. [CrossRef] [PubMed]
63. Asada, K. The Water–Water Cycle as Alternative Photon and Electron Sinks. *Philos. Trans. R. Soc. Lond. Ser. B* **2000**, *355*, 1419–1431. [CrossRef]
64. Terai, Y.; Ueno, H.; Ogawa, T.; Sawa, Y.; Miyagi, A.; Kawai-Yamada, M.; Ishikawa, T.; Maruta, T. Dehydroascorbate Reductases and Glutathione Set a Threshold for High-Light–Induced Ascorbate Accumulation. *Plant Physiol.* **2020**, *183*, 112–122. [CrossRef] [PubMed]
65. Singh, M.; Kumar, J.; Singh, S.; Singh, V.P.; Prasad, S.M. Roles of Osmoprotectants in Improving Salinity and Drought Tolerance in Plants: A Review. *Rev. Environ. Sci. Bio/Technol.* **2015**, *14*, 407–426. [CrossRef]
66. Stagos, D. Antioxidant Activity of Polyphenolic Plant Extracts. *Antioxidants* **2020**, *9*, 19. [CrossRef] [PubMed]
67. Du, B.; Jansen, K.; Kleiber, A.; Eiblmeier, M.; Kammerer, B.; Ensminger, I.; Gessler, A.; Rennenberg, H.; Kreuzwieser, J. A Coastal and an Interior Douglas Fir Provenance Exhibit Different Metabolic Strategies to Deal with Drought Stress. *Tree Physiol.* **2016**, *36*, 148–163. [CrossRef]
68. Du, B.; Kruse, J.; Winkler, J.B.; Alfarraj, S.; Albasher, G.; Schnitzler, J.-P.; Ache, P.; Hedrich, R.; Rennenberg, H. Metabolic Responses of Date Palm (*Phoenix dactylifera* L.) Leaves to Drought Differ in Summer and Winter Climate. *Tree Physiol.* **2021**, *41*, 1685–1700. [CrossRef] [PubMed]
69. Merewitz, E. Chemical Priming-Induced Drought Stress Tolerance in Plants. In *Drought Stress Tolerance in Plants*; Springer International Publishing: Cham, Switzerland, 2016; pp. 77–103.
70. Duan, S.; Kwon, S.J.; Lim, Y.J.; Gil, C.S.; Jin, C.; Eom, S.H. L-3, 4-Dihydroxyphenylalanine Accumulation in Faba Bean (*Vicia faba* L.) Tissues during Different Growth Stages. *Agronomy* **2021**, *11*, 502. [CrossRef]
71. Nishizawa, A.; Yabuta, Y.; Shigeoka, S. Galactinol and Raffinose Constitute a Novel Function to Protect Plants from Oxidative Damage. *Plant Physiol.* **2008**, *147*, 1251–1263. [CrossRef]
72. Taji, T.; Ohsumi, C.; Iuchi, S.; Seki, M.; Kasuga, M.; Kobayashi, M.; Yamaguchi-Shinozaki, K.; Shinozaki, K. Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *Plant J.* **2002**, *29*, 417–426. [CrossRef]
73. Li, T.; Zhang, Y.; Liu, Y.; Li, X.; Hao, G.; Han, Q.; Dirk, L.M.A.; Downie, A.B.; Ruan, Y.-L.; Wang, J.; et al. Raffinose synthase enhances drought tolerance through raffinose synthesis or galactinol hydrolysis in maize and *Arabidopsis* plants. *J. Biol. Chem.* **2020**, *295*, 8064–8077. [CrossRef] [PubMed]
74. Riikonen, J.; Kontunen-Soppela, S.; Vapaavuori, E.; Tervahauta, A.; Tuomainen, M.; Oksanen, E. Carbohydrate concentrations and freezing stress resistance of silver birch buds grown under elevated temperature and ozone. *Tree Physiol.* **2013**, *33*, 311–319. [CrossRef] [PubMed]
75. Valluru, R.; Van den Ende, W. Myo-Inositol and beyond–Emerging Networks under Stress. *Plant Sci.* **2011**, *181*, 387–400. [CrossRef] [PubMed]
76. Návarová, H.; Bernsdorff, F.; Döring, A.-C.; Zeier, J. Pipecolic Acid, an Endogenous Mediator of Defense Amplification and Priming, Is a Critical Regulator of Inducible Plant Immunity. *Plant Cell* **2012**, *24*, 5123–5141. [CrossRef] [PubMed]
77. Aranda, I.; Sánchez-Gómez, D.; de Miguel, M.; Mancha, J.A.; Guevara, M.A.; Cadahía, E.; de Simón, M.B.F. *Fagus sylvatica* L. provenances maintain different leaf metabolic profiles and functional response. *Acta Oecol.* **2017**, *82*, 1–9. [CrossRef]
78. Sebastiana, M.; Duarte, B.; Monteiro, F.; Malhó, R.; Caçador, I.; Matos, A.R. The leaf lipid composition of ectomycorrhizal oak plants shows a drought-tolerance signature. *Plant Physiol. Biochem.* **2019**, *144*, 157–165. [CrossRef] [PubMed]
79. Kolb, R.M.; Rawyler, A.; Brändle, R. Parameters affecting the early seedling development of four neotropical trees under oxygen deprivation stress. *Ann. Bot.* **2002**, *89*, 551–558. [CrossRef] [PubMed]
80. Nowakowska, J.A.; Stocki, M.; Stocka, N.; Ślusarski, S.; Tkaczyk, M.; Caetano, J.M.; Tulik, M.; Hsiang, T.; Oszako, T. Interactions between Phytophthora cactorum, Armillaria gallica and Betula pendula Roth. Seedlings Subjected to Defoliation. *Forests* **2020**, *11*, 1107. [CrossRef]
81. Okazaki, Y.; Saito, K. Roles of Lipids as Signaling Molecules and Mitigators during Stress Response in Plants. *Plant J.* **2014**, *79*, 584–596. [CrossRef]

82. Malcheska, F.; Ahmad, A.; Batool, S.; Müller, H.M.; Ludwigmüller, J.; Kreuzwieser, J.; Randewig, D.; Hänsch, R.; Mendel, R.R.; Hell, R. Drought Enhanced Xylem Sap Sulfate Closes Stomata by Affecting ALMT12 and Guard Cell ABA Synthesis. *Plant Physiol.* **2017**, *174*, 798. [CrossRef]
83. Batool, S.; Uslu, V.V.; Rajab, H.; Ahmad, N.; Waadt, R.; Geiger, D.; Malagoli, M.; Xiang, C.-B.; Hedrich, R.; Rennenberg, H. Sulfate Is Incorporated into Cysteine to Trigger ABA Production and Stomatal Closure. *Plant Cell* **2018**, *30*, 2973–2987. [CrossRef] [PubMed]
84. Ernst, L.; Goodger, J.Q.; Alvarez, S.; Marsh, E.L.; Berla, B.; Lockhart, E.; Jung, J.; Li, P.; Bohnert, H.J.; Schachtman, D.P. Sulphate as a Xylem-Borne Chemical Signal Precedes the Expression of ABA Biosynthetic Genes in Maize Roots. *J. Exp. Bot.* **2010**, *61*, 3395–3405. [CrossRef] [PubMed]
85. Chan, K.X.; Wirtz, M.; Phua, S.Y.; Estavillo, G.M.; Pogson, B.J. Balancing Metabolites in Drought: The Sulfur Assimilation Conundrum. *Trends Plant Sci.* **2013**, *18*, 18–29. [CrossRef] [PubMed]
86. McDowell, N.G. Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. *Plant Physiol.* **2011**, *155*, 1051–1059. [CrossRef]