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ORIGINAL ARTICLE

Significance of current weather conditions for foliar traits of old-growth sessile oak (*Quercus petraea* Liebl) trees

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Abstract

Key message **Sessile oak leaves showed a high degree of plasticity to atmospheric and pedospheric conditions.**

Abstract The aim of the present study was to elucidate the significance of current weather conditions for foliar traits of adult sessile oak (*Quercus petraea*), one of the most valuable forest tree species in Central Europe. For this purpose, structural and functional traits were analysed in fully expanded, sun exposed leaves collected in south-west Germany from five old-growth forest stands, representing the meteorological and pedospheric conditions in the growing region, but differing in aridity during the 12 days before harvest in two consecutive years. Across the forest stands, most foliar traits differed significantly between wet and dry weather conditions before harvest as indicated by partial least square discriminant analysis (PLS-DA). These traits included fresh weight/dry weight ratio, leaf hydration, leaf-C content, leaf-C/N ratio, structural N, soluble protein-N, total amino acid-N, cell wall composition, numerous specific amino acids as well as soluble sugar content. Structural biomass, $\delta^{13}C$ signature, total N and total C as well as H_2O_2 contents were not affected by the weather before harvest. These results indicate a high plasticity of the foliar metabolism of drought-tolerant sessile oak to current weather conditions. They also suggest that sessile oak is characterized by a high potential to cope with the growth conditions expected as a consequence of future climate change.

Keywords Amino acids · Antioxidants · Ascorbate · Carbohydrate · Cellulose · Climate · Glutathione · Lignin · Protein · Soluble sugars · Structural N · Weather

Introduction

Besides beech (*Fagus sylvatica* L.), oak species (*Quercus spec.*) are the most common deciduous forest tree species in Central Europe (Thomas and Gausling 2000; Hanewinkel et al. 2013). The genus *Quercus* comprises ~ 400 deciduous and evergreen trees and shrubs occupying a wide variety of habitats in temperate, Mediterranean, subtropical and tropical areas (Nixon 2006). Although oaks are considered to be more tolerant to water shortage than most other deciduous trees in Central Europe, low water availability can limit their growth and development (Bruschi 2010; Jensen and Hansen 2010). In addition, different oak species vary considerably in susceptibility to water shortage. In a macrocosm experiment under controlled conditions, Hu et al. (2013a, b, 2015) investigated stress responses to drought and increasing temperature in leaves of *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., and *Quercus pubescens* Willd., three widely distributed oak species in Central Europe. The authors found

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that saplings of all three oak species contained higher foliar antioxidant and free amino acid levels in response to drought and air warming. However, these effects were species-specific with highest levels for drought-tolerant *Q. pubescens*. In addition, effects were more pronounced in ecotypes originating from drier habitats across the species studied (Hu et al. 2013a). Elevated levels of free amino acids in response to drought and increasing temperature were achieved in oak leaves at the expense of structural nitrogen (N), indicating high partitioning flexibility of leaf N contents under stress conditions (Hu et al. 2013b, 2015). Antioxidative defence and osmotic stress adjustment by amino acid accumulation in oak leaves were more pronounced on calcareous soil with low water holding capacity compared to acidic soil with higher water holding capacity (Hu et al. 2013b, 2015). These results clearly show that functional traits such as ROS (reactive oxygen species) scavenging capacity by antioxidants, and partitioning flexibility of N influence the tolerance of oak leaves to drought and increasing temperature as well as the plasticity of N metabolism. Because osmotic adjustment by amino acid accumulation was achieved at the expense of structural N that is largely determined by lignin contents, it may be assumed that the change in cellular N partitioning goes hand-in-hand with general changes in cell wall structure and composition. Therefore, measuring amino acids, protein and lignin precursors as well as compatible solutes such as carbohydrates involved in stress defence will provide valuable information on responses of oak trees to changing environmental conditions.

Previous studies indicated that plants of the same species grown in different geographical locations with different environmental conditions contain different levels of carbohydrates depending on water availability and photosynthesis (Scossa et al. 2016; Zanetti et al. 2016). However, the effects of aridity on carbohydrate composition and contents are complex, depending on intensity and duration of drought on one side and the tree species, size, age and tissue on the other side (Hartmann et al. 2015). This is due to the functional diversity of carbohydrates, acting as compatible solutes, precursors of structural polymers as well as mobile and dynamic storage compounds. In addition, soil water availability often shows a strong positive relationship to the $\delta^{13}C$ signature as long-term average indicator of stomatal conductance as previously reported also for oak trees (*Q. robur* and *Q. petrea*, Ponton et al. 2002). Ramírez-Valiente et al. (2010) associated differences in $\delta^{13}C$ signature of *Q. robur* with rainfall and temperature at the sites of origin, suggesting local adaptation in response to diverging weather conditions.

To prevent oxidative stress upon water shortage, not only reduced ROS generation, but also enhanced ROS scavenging is required for ROS homeostasis. Scavenging of ROS is achieved in plant cells by chemical reactions with antioxidants such as ascorbate (Asc) and glutathione (GSH), but largely depends on the enzymatic regeneration of these antioxidants in the Foyer–Halliwell–Asada pathway that uses these metabolites as co-substrates (Munné-Bosch et al. 2014; Noctor et al. 2012). Hence, the antioxidative capacity of plant cells depends on the activity of antioxidative enzymes that control the flux of electrons through the Foyer–Halliwell–Asada pathway in different cellular compartments, i.e. dehydroascorbate reductase and glutathione reductase activity. In addition, it depends on the levels of the antioxidants GSH and Asc in the cellular compartment(s) of ROS production (Wrzaczek et al. 2013; Gill and Tuteja 2010). In a study with different oak species, Hu et al. (2013b) found that drought-sensitive *Q. robur* reinforced its oxidative stress defence systems by enhancing leaf Asc and thiol levels. By contrast, the more drought-tolerant species *Q. petraea* and *Q. pubescens* were able to sufficiently scavenge the ROS produced upon oxidative stress at constitutive foliar Asc and thiol levels. Apparently, differences in stress tolerance between oak species seem to be a consequence of differences in ROS scavenging rather than ROS production.

The present study was carried out during summer 2014 and 2015 in five old-growth stands of sessile oak (*Quercus petraea)* in south-west Germany, one of the economically most important distribution area of this species in Central Europe. The stands chosen represent the meteorological and pedospheric conditions in the growing region, but differed in aridity during the 12 days before harvest in the two consecutive years studied. The aim of this study was to identify responses of foliar structural and physiological traits to current weather conditions before harvest using the De Martonne aridity index based on temperature and precipitation (De Martonne 1926). Investigations were performed with special emphasis on cell wall composition, ROS scavenging as well as nitrogen and carbon partitioning. In this context, we tested the hypothesis that current weather conditions determine structural as well as physiological traits in leaves of adult *Quercus petraea* trees.

Materials and methods

Forest stand characteristics

In the present study, leaves of old-growth sessile oak trees from Rhineland Palentine in midwest Germany, one of the most extended growing region of this species, were investigated. For this purpose, five forest stands were selected that largely cover old-growth sessil oak origin and age as well as pedospheric and meteorological conditions of sessile oak stands in the region (Table 1; Fig. 1). Stand origins included natural regeneration, seed plantation and coppice (Table 1). Tree age varied between 94 and 172 years; the

Table 1 Field site characteristics of the *Quercus petraea* stands

Name of stands		Bäckertälchen	Schindhübel	Heidenkopf	Stalwen	Mockenhalde
Stand characteristics						
Latitude N		49° 22′ 7″	49° 19′ 27″	49° 55′ 15″	49° 49′ 20″	49° 47′ 24″
Longitude E		7° 54′ 41″	7° 52′ 18″	7° 40′ 12″	7° 41′ 27‴	7° 53′ 33″
Altitude (m above sealevel)		380–420	350–400	430–465	250–330	220–240
Soils (FAO classification)		Podzoluvisol	Podzoluvisol	Luvisol	Planosol-Luvisol	Ranker-Luvisol
Stand origin		Natural regeneration	Natural regeneration	Seeds	Natural regeneration	Coppice
Stand age		146	172	94	167	130
Admixed tree species		Beech, Hornbeam, Scots pine, Larch, Spruce, Eastern white pine	Scots pine, Beech, Sweet chestnut	Beech, Spruce, Alder, Birch, Dougls fir	Beech, Hornbeam	Scots pine, Wild cherry, Birch, Spruce
Oak share (%)		26	45	44	54	63
Stand volume of oak (m^3 ha^{-1})		327	182	228	195	30
Mean diameter at breast height (cm)		40–60	40–60	40–60	> 60	< 40
Top height (m)		30.4	24.9	25.6	26.7	16.2
Leave area index of oak (m^2 m^{-2})$^+$		4.7	4.2	4.1	3.1	2.1
Whole year weather mean values						
Precipitation (l * m^2)	2014	793	812	684	666	639
	2015	607	626	504	462	414
Mean temperature (°C)	2014	10.8	11.0	10.1	11.1	11.5
	2015	10.5	10.7	9.7	10.8	11.2
Weather conditions 12 days before harvest						
12 day precipitation (L m^{-2})	2014	0.0026	0.0025	0.0039	0.0032	0.0032
	2015	8.6	8.7	13.2	7.3	7.2
12 day mean temperature (°C)	2014	14.3	14.5	15.5	17.8	17.8
	2015	13.4	13.6	11.2	13.7	14.2
Vegetation period aridity index (L m^{-2} $°C^{-1}$)	2014	0.07	0.06	0.09	0.07	0.06
	2015	234	230	432	194	187

Information on soils, stand origin, and stand age were provided by the Forest Services of the State of Rhineland-Palatinate. Oak share was calculated on a surface area basis. Leave area index (LAI) was estimated from measurements in a regional oak stand (Bréda et al. 1995).Weather conditions were calculated as mean day-aridities for the 12 days before leaf harvest from precipitation and temperatures as indicated in "Materials and methods"

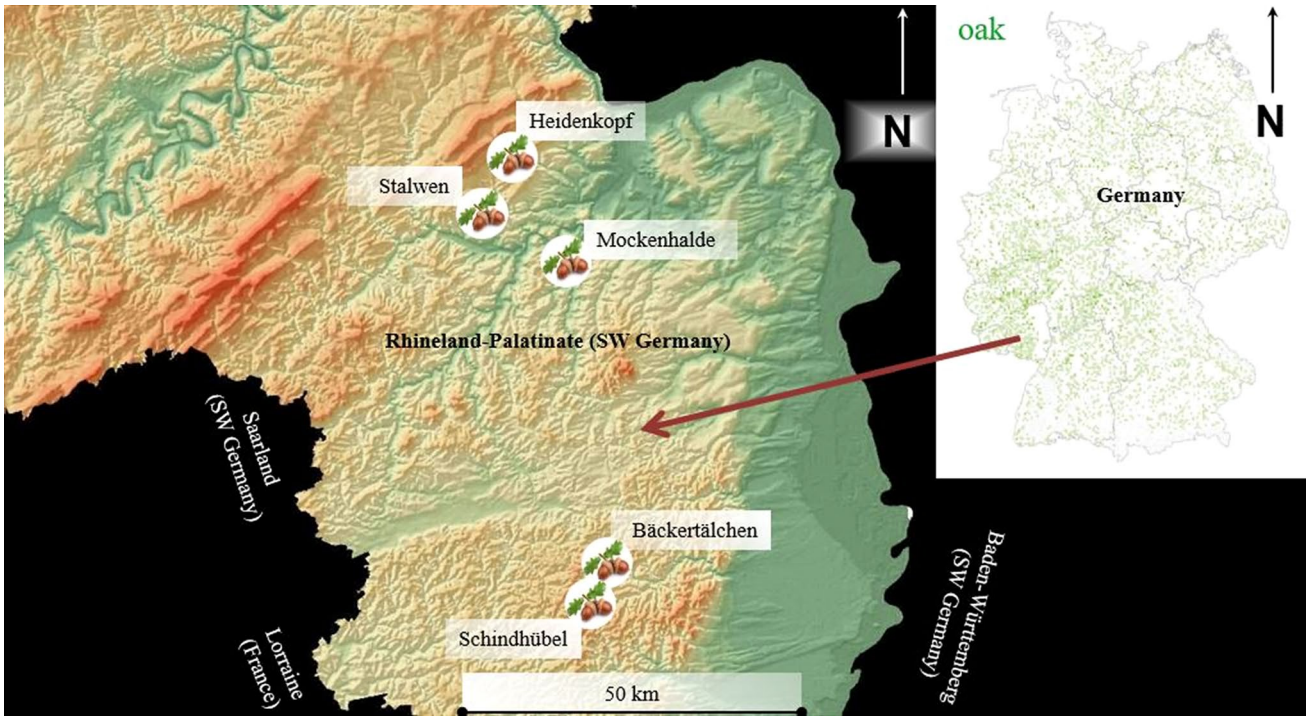

Fig. 1 Location of sample provenances

Table 2 Soil C and N contents at different depths (A: 0–10 cm and B:10–30 cm) from five stands with different aridities

	Bäckertälchen	Schindhübel	Heidenkopf	Stalwen	Mockenhalde
Soil A, C (% g^{-1} DW)	4.11 ± 1.29 b	17.3 ± 8.66 a	9.68 ± 3.6 b	9.57 ± 3.07 b	20.64 ± 7.00 a
Soil B, C (% g^{-1} DW)	1.9 ± 0.83 b	3.7 ± 0.69 b	2.30 ± 1.07 b	2.60 ± 0.20 b	7.10 ± 2.20 a
Soil A, N (% g^{-1} DW)	0.24 ± 0.07 c	0.63 ± 0.30 b	0.56 ± 0.20 bc	0.58 ± 0.15 bc	1.29 ± 0.43 a
Soil B, N (% g^{-1} DW)	0.10 ± 0.05 b	0.10 ± 0.03 b	0.20 ± 0.05 b	0.20 ± 0.01 b	0.50 ± 0.09 a
Soil A, C/N ratio	16.96 ± 0.64 b	26.99 ± 1.60 a	13.66 ± 0.04 b	16.19 ± 1.08 b	15.92 ± 1.15 b
Soil B, C/N ratio	18.70 ± 1.46 b	25.8 ± 3.50 a	11.30 ± 2.13 c	13.40 ± 0.41 b	15.30 ± 1.82 b

Data shown are means ± S.D. ($n = 8$)

Different letters indicate significant differences between stands. Stands are presented from low to high aridity

oak share between 26 and 63%, all with a predominating oak crown layer, but different admixed species; stand volume of oak trees between 30 and 327 m^3 ha^{-1}; and leaf area index of oak trees between 2.1 and 4.7 m^2 m^{-2} (Table 1). The soils of all stands investigated constitute Luvisols to Podzoluvisols (FAO classification) derived from pleistocene periglacial solifluction layers with poor to middle nutrient supply (Tables 1, 2).

We used the aridity index (De Martonne 1926) as an integrative variable to characterize weather dryness at the sites of growth. This indicator served previously to identify, locate or delimit origins that are prone to suffer from a deficit of water availability (Baltas 2007; Paparrizos 2018). The index was calculated by the following equation:

$$\text{Aridity index} = \frac{P}{T+10},$$

where P is the mean precipitation (mm) and T (°C) the mean air temperature. The De Martonne aridity index decreases with increasing aridity. Current weather conditions were calculated as the 12 day period that preceded leaf harvest for each stand (Table 1) (see supplementary material) (Seegmueller 2012). Foliar traits were compared between the relatively arid weather conditions before harvest in 2014 and the cooler and wetter weather conditions before harvest in 2015 (Table 1).

Leaf harvest and sample preparation

Sun exposed leaves were harvested in mid June 2014 and 2015 at the full expansion of the first flush from same sessile oak trees (*Quercus petreae* Liebl.) evaluated as undamaged and healthy by visual inspection. Bud break was during May and leaves were fully differentiated at leaf harvest. Within each stand, eight predominant or dominant oak trees as determined by Krafft tree classes were harvested (Kramer 1988). Leaves were taken from light-exposed crown branches harvested by the slingshot technique (Fig S1). Leaves samples were taken from trees of 16–30 m height (Table 1). Ten leaves were collected from every branch. Each of the eight trees was assessed in three replicate branches. All leaf samples were immediately frozen in liquid N_2 and stored at − 80 °C until further analyses.

Soil sampling

Soil samples were taken in the outer areas of the crown projection from the assessed oak trees summing up to eight replicates per stand in 2015. Samples were collected with a soil auger from 0 to 30 cm soil depths (A: 0–10 cm and B:10–30 cm), representing the main fine root zone under local conditions (Kern et al. 1961). The soil samples were frozen at − 80 °C until further preparation. For soil preparation, samples were sieved (< 2 mm) to remove roots and coarse material and dried at 60 °C for 48 h to determine total C and N contents.

Dry mass and leaf hydration determination

Leaf samples were homogenized in liquid N_2 to a fine powder and aliquots of 100 mg were dried at 60 °C for at least 48 h to weight constancy for dry mass determination. Leaf hydration (H; g H_2O g^{-1} DW) was determined as (FW–DW)/DW, where FW is the fresh mass and DW is the dry mass (Contin et al. 2014).

Biochemical analyses

Determination of $\delta^{13}C$ signature, total C and N contents in leaf and soil material

$\delta^{13}C$ signature as well as total C and N contents in leaf material dried at 60 °C for at least 48 h to weight constancy were determined in aliquots (0.8–1 mg) weighed into tin capsules (IVA Analysentechnik, Meerbusch, Germany). Samples were analyzed using a C/N elemental analyzer (NA 2500, CE Instruments, Milan, Italy) coupled via a Conflo II interface (Finnigan MAT GmbH, Bremen, Germany) to an isotope ratio mass spectrometer (Delta plus, Thermo Finnigan MAT GmbH, Bremen, Germany) as previously described in Geßler et al. (2005). For analysis of soil samples according to Liu et al. (2015a), 4 mg soil from the A (0–10 cm), and 20 mg from the B (10–30 cm) horizon were weighed into tin capsules and analysed using a Costech elemental analyzer (Costech International S.p.A., Milan, Italy) fitted with a Zero Blank autosampler, and coupled via a ConFlo-III interface to a Thermo Finnigan Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Waltham, MA, USA). Glutamic acid was used as the working standard and calibrated against the international standard USGS-40 (glutamic acid, $\delta^{13}C_{PDB} = -26.39‰$) for $\delta^{13}C$ analyses. Working standards were analyzed after every tenth sample to detect any potential instrument drift over time.

Determination of cell wall composition

Structural biomass was determined after removal of soluble cellular constituents (Arab et al. 2019). Lignin contents were analyzed by the thioglycolate method (Brinkmann et al. 2002), cellulose contents with the anthrone reagent (Updegraff 1969) using a UV-DU650 spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA) as previously described (Arab et al. 2019).

Determination of glutathione (GSH) and ascorbate (Asc), hydrogen peroxide contents, glutathione reductase (GR) and dehydroascorbate reductase (DHAR) activities

GSH and Asc were extracted from 50 mg frozen and powdered leaf material using 0.1 N HCl in the presence of 50 mg of polyvinylpolypyrrolidone and 10 µL 1 mM EDTA (Schwanz et al. 1996). From this extract, ascorbate was separated isocratically on a HP 1090 series II HPLC (Agilent, Waldbronn, Germany) at 40 °C with 10% methanol (MeOH), pH 2.0 and determined at $\lambda = 248$ nm (Schwanz et al. 1996). GSH was determined in 200 µL of the above extract after reduction of GSSG (oxidized glutathione) and derivatisation with monobromobimane (mBBr) (Schupp and Rennenberg 1988, Strohm et al. 1995). The GSH derivative was separated on a HP 1050 HPLC (Agilent, Waldbronn, Germany) in a H_2O: methanol (MeOH) gradient (10–20% MeOH, pH 4.3–4.1 for 30 min) at RT. The fluorescence of the GSH derivative was quantified with a HP 1046 A Fld detector (Agilent, Waldbronn, Germany) by excitation at $\lambda = 380$ nm and emission at 480 nm (Schupp et al. 1991). Hydrogen peroxide contents were quantified with the potassium iodide assay using a UV-DU650 spectrophotometer at $\lambda = 390$ nm (Beckman Coulter Inc., Fullerton, CA, USA) as previously reported (Loreto and Velikova 2001).

GR activity was measured kinetically from the NADPH consumption for GSSG reduction with a spectrophotometer at $\lambda = 340$ nm (Polle et al. 1990). DHAR activity was assayed directly by following the increase in absorbance at

265 nm, resulting from GSH-dependent production of Asc from DHA (dehydroascorbate) (Polle et al. 1990).

Determination of soluble protein contents and total amino acid

Total soluble protein in leaf samples was quantified as reported by Dannenmann et al. (2009). For determination of total amino acids, aliquots of 50 mg frozen and powdered leaf material were extracted according to Winter et al. (1992) in 200 µL 20 mM HEPES buffer (pH 7.0), containing 5 mM ethylene glycol tetraacetic acid (EGTA), 10 mM NaF and 1 mL methanol/chloroform (3.5:1.5 v/v). Total amino acids in the extracts were quantified after derivatization with ninhydrin using a UV-DU650 spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA) at $\lambda = 570$ nm. Specific amino compounds were analyzed using an ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, USA) as described by Luo et al. (2009).

Structural N calculation

Structural N content in plant material was calculated by subtracting the N fractions in total amino acids and soluble protein from total N. Since N assimilation takes place in the roots of sessile oak trees and, therefore, foliar nitrate contents are minute, nitrate was not considered in this calculation (Hu et al. 2013a).

Carbohydrate analysis

Carbohydrates were extracted from homogenized leaves samples and derivatized by a modification of the method by Kreuzwieser et al. (2009). For each sample, approximately 50 mg frozen leaf powder was weighed into a pre-frozen 2 mL round-bottom Eppendorf tube and 500 µL cold 95% (v/v) methanol (Sigma-Aldrich GmbH) were added as extraction medium. 30 µL of a solution of 2 mg mL^{-1} ribitol was used as internal standard. Tubes were rapidly heated to 70 °C and shaken at 1400*g* for 15 min. After brief vortexing, 200 µL chloroform and 400 µL distilled water were added, the samples were shaken at 1400*g* for 5 min, and centrifuged at 14,000*g* for 5 min. 10 µL aliquots of the supernatant were freeze-dried, methoxinated by adding 20 µL of a 20 mg mL^{-1} solution of methoxyamine hydrochloride in anhydrous pyridine (Sigma-Aldrich GmbH), and incubated at 30 °C for 90 min under shaking at 1400*g*. For trimethylsilylation, 35 µL N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA; Sigma-Aldrich GmbH) were added to each tube. Tubes were incubated at 37 °C for 30 min with 1400*g* shaking. Subsequently, 5 µL of an alkane standard solution (C8-C20, Sigma-Aldrich GmbH) were added to each sample and 50 µL supernatant were transferred to GC–MS vials (Agilent Technologies, Palo Alto, CA, USA) for GC–MS analysis. The derivatized metabolite samples were analyzed on an Agilent GC/MSD system consisting of an Agilent GC 6890 N gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with an autosampler (GC-PAL, CTC Analytics, Zwingen, Switzerland) and connected to a 5975C Inert XL EI MSD quadrupole MS detector (Agilent Technologies). The separation was achieved on a capillary column (HP-5MS 5% Phenyl Methyl Silox, length: 30 m, diameter: 0.25 mm, film thickness: 0.25 µm; Agilent Technologies) at a helium flow of 1 mL min^{-1}; GC–MS run conditions were set according to Kreuzwieser et al. (2009). The raw data files were processed with the Mass Hunter (version B.07.00/Build 7.0.457.0) workstation software for GC–MS (Agilent Technologies). Mass spectra were searched against the Golm Metabolome Database (Kopka et al. 2005) and carbohydrates were identified based on internal standards, spectrum similarity match, and retention time.

Statistical analysis

All analytical measurements were performed with leaves of eight replicate trees of five forest sites each. This includes determination of water content, FW/DW ratios, $\delta^{13}C$, total C, total N, SBM, lignin, cellulose, hydrogen peroxide, glutathione and ascorbate contents, total protein-N, total amino acid-N, specific amino acids, carbohydrate compounds as well as enzyme activities of glutathione reductase (GR) and dehydroascorbate reductase (DHAR). Data were positively tested for normal distribution and homoscedasticity. Significant differences between pooled data from all stands of each year were assessed using the unpaired Student's t test. Moreover, to characterize differences between the years, datasets were subjected to partial least square discriminant analysis (PLS-DA) using MetaboAnalyst 3.0 (Xia et al. 2015).

Results

Weather conditions before harvest were similar for all stands and relatively dry in 2014, whereas in 2015, weather conditions before harvest were cooler and wetter compared to 2014, as indicated by a higher aridity index (Table 1). Thus, water availability before harvest was higher in 2015 compared to 2014 across all stands. Weather conditions before harvest strongly affected the foliar traits analysed in the present study. PLS-DA analysis across stands and parameters measured revealed a clear separation between the 2014 and 2015 (Fig. 2A). To elucidate the foliar traits

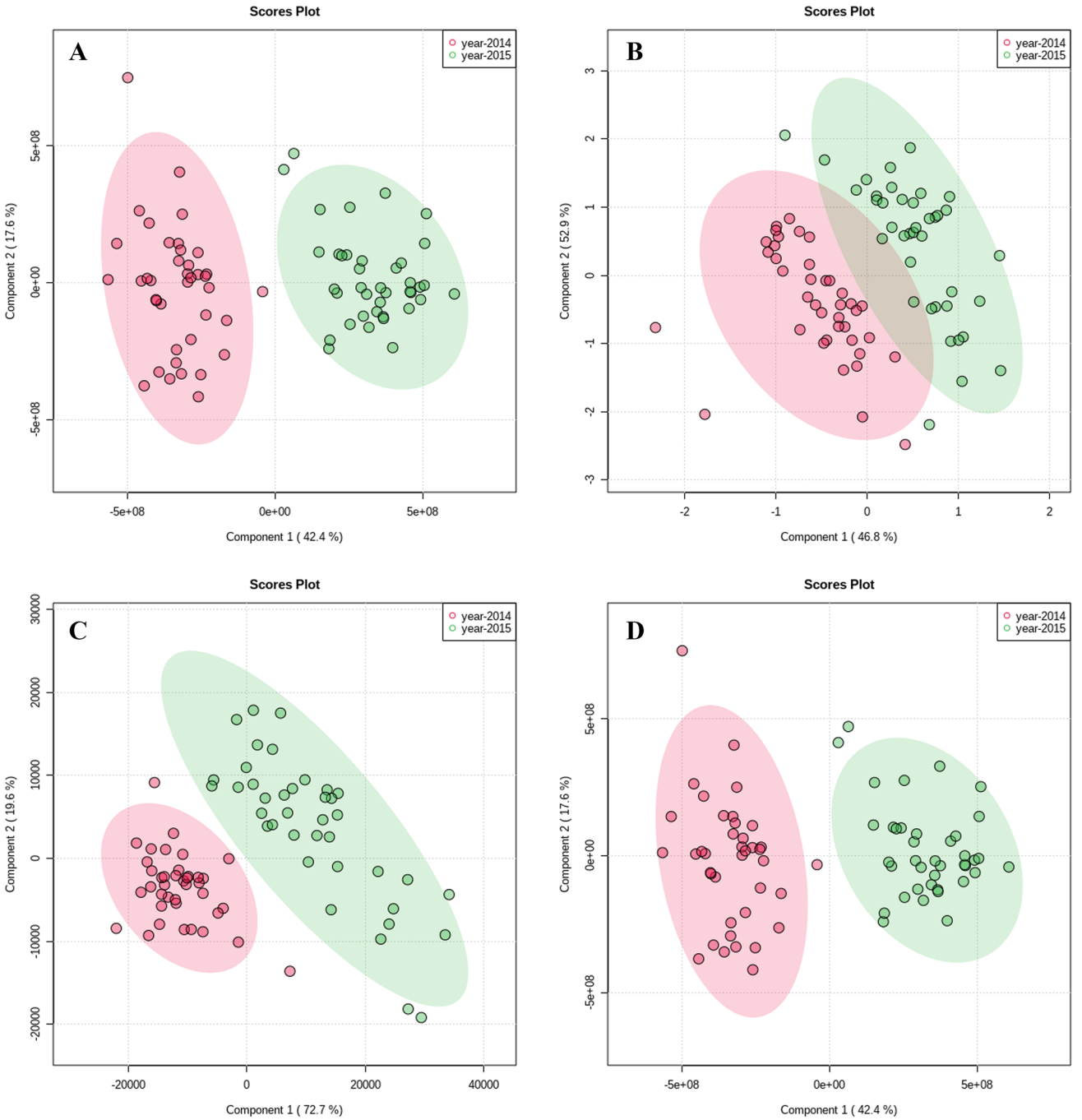

Fig. 2 **A** PLS-DA analysis of all parameter investigated, **B** PLS-DA analysis of water relations including FW/DW, leaf hydration (g H_2O g^{-1} DW), **C** PLS-DA analysis of stractural biomass and its carbon composition including SBM (mg g^{-1} DW), Leaf-C (mg g^{-1} DW), lignin (mg g^{-1} DW), lignin (mg g^{-1} SBM), Cellulose (mg g^{-1} DW), cellulose (mg g^{-1} SBM), Cellulose/lignin ratio in DW, sucrose (mg g^{-1} DW), Glucose (mg g^{-1} DW), glucose/sucrose ratio in DW, fructose (mg g^{-1} DW), G-6-P (mg g^{-1} DW), galactose (mg g^{-1} DW), **D** PLS-DA analysis of dry matter carbon and its composition including SBM (mg g^{-1} DW), Leaf-C (mg g^{-1} DW), lignin (mg g^{-1} DW), Lignin (mg g^{-1} SBM), cellulose (mg g^{-1} DW), cellulose (mg g^{-1} SBM), cellulose/lignin ratio in DW, sucrose (mg g^{-1} DW), glucose (mg g^{-1} DW), glucose/sucrose ratio in DW, fructose (mg g^{-1} DW), G-6-P (mg g^{-1} DW), galactose (mg g^{-1} DW)

that determine this separation and, hence, the response to different weather conditions before harvest, PLS-DA analyses were performed with subsets of the foliar parameters studied across the five oak stands.

For the parameters characterizing foliar water relations, only partial separation was observed by PLS-DA analysis between relatively dry 2014 and relatively wet 2015 (Fig. 2B). The FW/DW ratio of the leaves was slightly,

Table 3 Foliar traits of *Quercus petraea* across five stands with different aridities at arid (Σ 2014) and wet (Σ 2015) weather conditions before harvest

	Σ 2014 (dry weather conditions)	Σ 2015 (wet weather conditions)
Water relations		
Leaf hydration (g H_2O g^{-1} DW)	0.69 ± 0.11 B	1.39 ± 0.18 A
Dry matter carbon and its composition		
Leaf-C (mg g^{-1} DW)	435.40 ± 2.38 B	473.20 ± 1.89 A
Lignin (mg g^{-1} DW)	40.22 ± 8.32 B	46.10 ± 10.59 A
Lignin (mg g^{-1} SBM)	64.28 ± 10.59 B	73.87 ± 21.69 A
Cellulose (mg g^{-1} DW)	207.36 ± 35.12 A	144.25 ± 38.27 B
Cellulose (mg g^{-1} SBM)	329.41 ± 43.18 A	233.52 ± 62.13 B
Cellulose/lignin ratio in DW	5.10 ± 1.20 B	3.13 ± 0.50 A
Sucrose (mg g^{-1} DW)	22.35 ± 5.11 B	38.54 ± 4.02 A
Glucose (mg g^{-1} DW)	13.42 ± 6.25 A	9.02 ± 4.08 B
Glucose/sucrose ratio in DW	0.59 ± 0.23 A	0.25 ± 0.12 B
Fructose (mg g^{-1} DW)	13.54 ± 6.25 B	28.47 ± 15.34 A
G-6-P (mg g^{-1} DW)	2.22 ± 0.09 A	0.61 ± 0.21 B
ROS and antioxidative metabolism		
Ascorbate (µmol g^{-1} DW)	21.11 ± 6.41 B	30.79 ± 6.95 A
Glutathione (nmol g^{-1} DW)	1029 ± 645 B	1475 ± 748 A
GR activity (µmol g^{-1} DW)	3.03 ± 1.14 B	6.03 ± 1.69 A
DHAR activity (µmol g^{-1} DW)	1.68 ± 0.8 B	2.25 ± 1.14 A
N content and its partitioning		
Soluble protein-N (mg g^{-1} DW)	2.25 ± 0.74 B	3.58 ± 0.64 A
Structural N (mg g^{-1} DW)	21.37 ± 2.19 A	18.98 ± 2.59 B
Total amino acid-N (mg g^{-1} DW)	0.71 ± 0.42 A	0.47 ± 0.12 B
3-Phospho-glycerate derived amino acids (mg g^{-1} DW)	2.10 ± 1.22 A	1.22 ± 0.51 B
Pyruvate derived amino acids (mg g^{-1} DW)	4.90 ± 2.42 A	4.01 ± 1.2 B
Oxalo acetate drived amino acids (mg g^{-1} DW)	7.21 ± 1.05 A	4.01 ± 1.5 B

Data shown are means ± S.D. (n = 8)

G-6-P glucose-6-phosphate, *ROS* reactive oxygen species, *GR* glutathione reductase, *DHAR* dehydroascorbate reductase

Different big letters indicate significant differences between pooled data from all stands

but not significantly lower at more arid weather conditions, although the foliar water content was significantly decreased. Still the $\delta^{13}C$ signature as an integrative measure of stomatal conductance was not affected (Table S1).

Full separation was observed for structural parameters investigated (Fig. 2C), although foliar SBM was similar in leaves harvested after arid and wet weather conditions before harvest across the sessile oak stands. However, total foliar C content was significantly reduced at the more arid weather conditions. This reduction was reflected by reduced lignin, sucrose and fructose contents of the leaves. Since cellulose and glucose contents increased, also the cellulose/lignin and glucose/sucrose ratios were increased at more arid conditions before harvest (Table 3). In addition, the foliar contents of various other soluble carbohydrates including lyxose, sorbose, myo-inositol, and glucose-6-phosphate were enhanced at more arid conditions before harvest (Table 4). These increases resulted in a full separation of the foliar carbohydrate composition between arid and wet weather conditions before harvest across the five oak stands (Fig. 2D).

Table 4 Leaf carbohydrate contents of *Quercus petraea* across five stands with different aridities at arid (Σ 2014) and wet (Σ 2015) weather conditions before harvest

Carbohydrate composition		Σ 2014 (dry weather conditions)	Σ 2015 (wet weather conditions)
Monosacarides	RT (min)	Normalized Peak area	Normalized Peak area
Lyxose	24.29	2.93 ± 1.14 A	0.67 ± 0.29 B
Fructose	27.90	213.42 ± 115.92 B	280.34 ± 53.68 A
Glucose	28.13	390.14 ± 176.67 A	281.35 ± 55.78 B
Glucopyranose	29.47	331.74 ± 20.83 A	280.36 ± 135.27 A
Galactose	31.94	21.24 ± 13.94 A	18.35 ± 7.64 A
Sorbose	27.90	501.23 ± 144.67 A	316.47 ± 54.13 B
Glucose-6-phosphat	34.64	6.39 ± 3.19 A	1.37 ± 0.70 B
Myo-inositol	31.11	244.32 ± 176.67 A	66.20 ± 22.39 B
Di-trisaccharides			
Cellobiose	36.19	52.13 ± 25.86 A	27.83 ± 11.07 B
Sucrose	38.83	826.54 ± 111.87 B	1369.68 ± 148.16 A
Cellobiitol	40.30	131.47 ± 50.34 A	65.10 ± 26.48 B
Raffinose	47.15	12.98 ± 10.14 A	2.95 ± 1.32 B
Mannitol	28.78	280.25 ± 130.78 A	194.05 ± 90 B
Sugar acid			
Gluconic acid	29.79	17.12 ± 13.39 A	5.06 ± 2.31 B
Galactonic acid	29.79	15.92 ± 3.78 B	23.98 ± 4.2 A
Saccharic acid	30.00	15.92 ± 3.78 B	23.82 ± 3.3 A

Data shown are means ± S.D. (n = 8)

Different big letters indicate significant differences between pooled data

Surprisingly, such a separation was not observed for the parameters of the antioxidative metabolism studied (Fig. 3A). The H_2O_2 content of the leaves as an indicator of ROS accumulation was not significantly affected by more arid weather conditions before harvest (Table S1). The antioxidants ascorbate and glutathione as well as the activity of the antioxidative enzymes GR and DHAR as rate limiting components of the Foyer–Haliwell–Asada cycle of ROS scavenging were even reduced at more arid weather conditions (Table 3). These results indicate that enhanced ROS production at the arid weather conditions before harvest could be scavenged by the constitutive antioxidative metabolism of the leaves.

Similar to the antioxidatve metabolism of oak leaves, also N metabolism did not show a clear separation between wet and more arid weather conditions before harvest, neither of total N and its partitioning, nor for the specific amino acid composition (Fig. 3B, C). Whereas the foliar total N content was not affected by the weather conditions before harvest across the stands studied, soluble protein-N declined, structural N and amino acid-N increased at more arid weather conditions before harvest. The increase in amino acid-N was achieved by increased amounts of 3-phospho-glycerate derived, pyruvate derived and oxalo acetate derived amino acids (Table 3, S1). At the level of individual amino acids, this increase was achieved by significantly enhanced foliar glycine, isoleucine, glutamine, proline, aspartate, threonine, phenylalanine and tyrosine contents, whereas other amino acids were not significantly affected by the weather conditions before harvest (Table 5).

Discussion

Impacts of current weather conditions on water relations and structural traits of sessile oak leaves

In the present study, foliar FW/DW ratio and leaf hydration were higher across stands in 2015 at wet conditions before harvest compared to 2014 at dry conditions before harvest. This result indicates modulation of foliar water relations by current water availability, as also observed in previous studies (Contin et al. 2014; Baslam et al. 2014; Suguiyama et al. 2016). The carbon isotope composition (i.e. $\delta^{13}C$ signature) is widely considered a sensitive indicator of long-term water availability to plants, provided photosynthetic processes are not impaired (Warren et al. 2001). In the present study, leaf $\delta^{13}C$ signatures did not differ between 2014 and 2015, indicating a limited responsiveness of this parameter to short-term aridity. Aridity did not affect the share of foliar SBM on dry weight in all stands in 2014, but reduced the lignin content of SBM. Apparently, oak stands respond to short-term aridity by changing the foliar cell wall composition, as also found in other species subjected to drought (Joly and Zaerr 1987; Donaldson 2002; Zwiazek 1991).

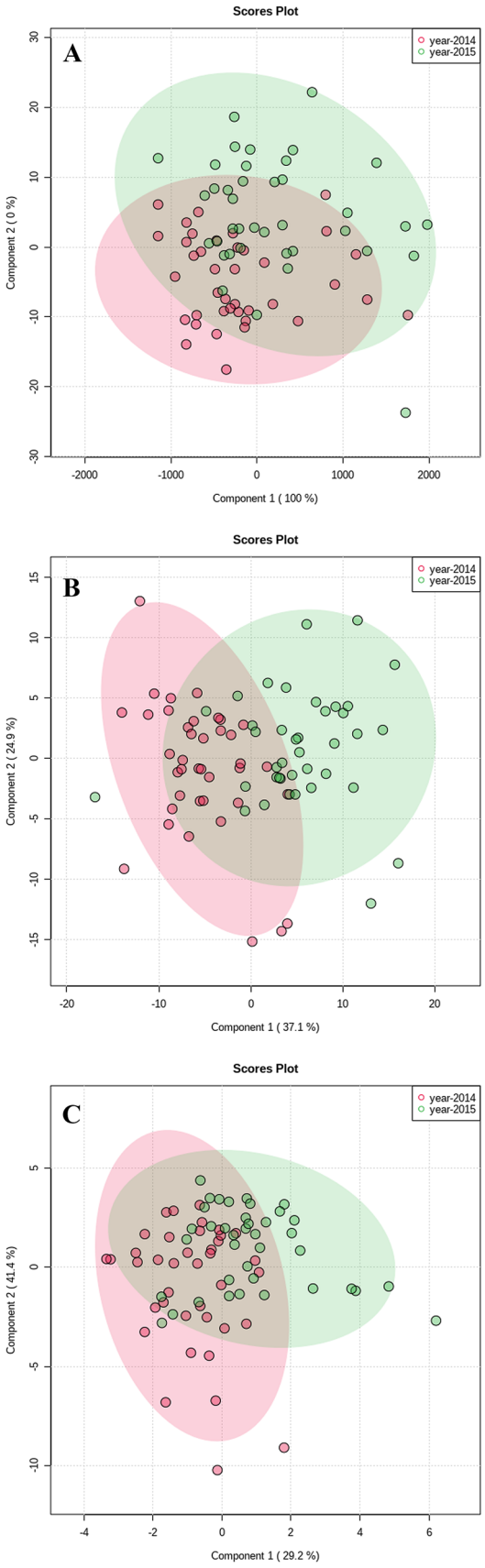

Fig. 3 **A** PLS-DA analysis of ROS and parameters of the antioxidative metabolism including H_2O_2 (µmol g-1 DW), ascorbate (µmol g^{-1} DW), glutathione (nmol g^{-1} DW), GR activity (µmol g^{-1} DW), DHAR activity (µmol g^{-1} DW), **B** PLS-DA analysis of N content and its partitioning including total N (mg g^{-1} DW), soluble protein-N (mg g^{-1} DW), structural N (mg g^{-1} DW), total amino acid-N (mg g^{-1} DW), 3-phospho-glycerate derived amino acids (mg g^{-1} DW), pyruvate derived amino acids (mg g^{-1} DW), 2-ketoglutarat drived amino acids (mg g^{-1} DW), Oxalo acetate drived amino acids (mg g^{-1} DW), aromatic amino acids (mg g^{-1} DW), other amino compounds (mg g-1 DW). **C** PLS-DA analysis of specific amino acids concentrations (mg^{-1} g DW) including histidine, cysteine, serine, glycine, alanine, valine, leucine, isoleucine, glutamate, glutamine, proline, argenine, aspartate, asparagine, methionine, threonine, tryptophan, phenylalanine, tyrosine, ornithine, GABA, AABA

Impacts of current weather conditions on oxidative stress and its compensation in leaves

Arid weather conditions can stimulate the formation of reactive oxygen species (ROS), particularly at high irradiation (Munné-Bosch and Peñuelas 2004). Non-enzymatic antioxidants such as Asc and GSH and their regeneration in the Foyer–Haliwell–Asada cycle play a key role in the detoxification of ROS (Hossain et al. 2013). In the present study, the contents of both antioxidants were lower in 2014 at drier weather conditions before harvest compared to 2015 across stands. Since this was also observed for GR and DHAR activity that are involved in the regeneration of Asc and GSH, it appears that part of the antioxidative metabolism of sessile oak leaves is even down-regulated at dry weather conditions. This result is surprising, because also ROS levels such as H_2O_2 contents that are thought to be kept under control mainly by the components of the Foyer–Haliwell–Asada cycle (Noctor et al. 2012; Schnaubelt et al. 2015) remained unchanged at drier weather conditions before harvest in 2014. Therefore, it may be concluded that in sessile oak leaves other antioxidative compounds such as secondary metabolites get enhanced significance for ROS scavenging upon dry weather conditions. This view is supported by a recent report from a free-air cross-exchange experiment with sessile oak saplings indicating a high significance of secondary metabolites in sessile oak leaves for ROS scavenging (Arab et al. 2020).

Impacts of current weather conditions on N and C contents and partitioning in leaves

Drought stress was found to increase the foliar N concentration in *Malus domestica* (Jie et al. 2010) and in *Q. Robur* (Picon et al. 1997). However, Dichio et al. (2007) reported that leaf N concentration decreased in drought-treated plants of *Prunus persica*. In the present study, neither differences in soil N contents nor in aridity before harvest changed the foliar total N contents significantly, supporting

Table 5 Specific amino acid concentrations (mg^{-1} g DW) of *Quercus petraea* leaves across five stands with different aridities at arid (Σ 2014) and wet (Σ 2015) weather conditions before harvest

		Σ 2014 (dry weather conditions)	Σ 2015 (wet weather conditions)
Ribose 5-phosphate derived	Histidine	0.18 ± 0.09 A	0.07 ± 0.03 A
3-Phospho-glycerate derived	Cysteine	0.34 ± 0.18 A	0.26 ± 0.10 A
	Serine	0.99 ± 0.56 A	0.59 ± 0.25 A
	Glycine	0.84 ± 0.71 B	0.52 ± 0.28 A
Pyruvate derived	Alanine	1.45 ± 1.05 A	1.15 ± 0.57 A
	Valine	2.79 ± 1.05 A	2.59 ± 0.82 A
	Leucine	0.08 ± 0.06 A	0.056 ± 0.03 A
	Isoleucine	0.72 ± 0.04 B	0.54 ± 0.23 A
2-Ketoglutarate	Glutamate	6.53 ± 3.31 A	7.32 ± 3.38 A
	Glutamine	0.84 ± 0.71 B	0.57 ± 0.35 A
	Proline	2.41 ± 1.22 B	1.57 ± 0.68 A
	Argenine	0.67 ± 0.52 A	0.33 ± 0.28 A
Oxaloacetate-derived	Aspartate	2.3 ± 1.42 B	1.67 ± 0.98 A
	Asparagine	1.8 ± 0.97 A	1.43 ± 0.90 A
	Methionine	0.26 ± 0.23 A	0.37 ± 0.26 A
	Threonine	0.95 ± 0.34 B	0.79 ± 0.30 A
Aromatics	Tryptophan	0.40 ± 0.11 A	0.45 ± 0.19 A
	Phenylalanine	0.30 ± 0.16 B	0.23 ± 0.07 A
	Tyrosine	0.27 ± 0.20 B	0.19 ± 0.05 A
Others	Ornithine	0.31 ± 0.18 A	0.28 ± 0.21 A
	GABA	0.20 ± 0.16 A	0.16 ± 0.07 A
	AABA	0.34 ± 0.30 A	0.23 ± 0.26 A

Data shown are means ± S.D. (n = 8)

Different big letters indicate significant differences between pooled data

the view that foliar N content is not subjected to short-term changes. In line with our results, Li et al. (2013) found that air warming and drought did not affect leaf N contents in different *Quercus* species/ecotypes. However, differences to short-term aridity before harvest modulated N partitioning in sessile oak leaves in the present study. We observed higher protein contents at lower N availability in the soil and decreasing soluble protein-N at the benefit of both total amino acid-N and structural N at dry weather conditions before harvest. Similar results were previously obtained for *Quercus robur* and *Quercus petraea*, where ecotypes from drier origins showed lower soluble protein but higher structural N compared with ecotypes from wetter areas (Hu et al. 2013a). Moreover, Kimura et al. (1998) and Uemura et al. (2000) showed that leaves of *Fagus japonica* tree were well adjusted to the current-year light environment both anatomically and physiologically as mass-based N and Chl concentrations and N rearrangements within a leaf substantially adjusted to the current-year light environment. The significance of winter bud traits on foliar traits of fully expanded leaves of mature trees also depends on the overall storage capacity of the trees that usually overroles bud traits at the end of leaf development. In addition, among the traits studied mostly soluble sugars and total nitrogen contents may be affected by bud traits. Since soluble sugars were largely higher in a wet year after a dry year and total N contents were not different, a significant influence of bud traits developed in the dry year on leaf traits in the subsequent wet year is unlikely. Still, we cannot exclude that bud traits developed in the previous year may have contributed to the differences in foliar traits observed. The authors assumed that this N partitioning pattern might be related to differences in leaf structural characteristics, but also indicates a tight control of N acquisition by the roots at sufficient N supply to the trees.

Amino acids, amines, organic acids, sugars and sugar alcohols constitute major solutes that are responsive to drought stress. These primary metabolites perform important functions in plant adaptation to drought, because they can serve as energy reserves, osmolytes to maintain cell turgor, antioxidants, and/or as signal transduction molecules (Bartels and Sunkars 2005; Seki et al. 2007; Shulaev et al. 2008; Silvente et al. 2012). In the present study, drier weather conditions before harvest caused an obvious increase of foliar amino acid-N across stands in 2014 compared to 2015, mostly at the expense of soluble protein-N. In

addition, a coordinated increase in the foliar concentration of amino acids derived from 3-phospho-glycerate, pyruvate and oxaloacetate was observed across all stands at drier weather conditions before harvest in 2014. Apparently, drought strengthens the use of N for osmotic adjustment, as also observed in a recent study in dwarf bamboo under short-term drought stress (Liu et al. 2015b). These results support the view that accumulation of amino acids can aid stress tolerance in plants through osmotic adjustment, detoxification of reactive oxygen species, and by intracellular pH regulation (Silvente et al. 2012; Muttucumaru, et al. 2015). Also for other species, weather conditions were reported to cause a high variation in the concentrations of individual amino acids from year to year, although their relative proportions on total free amino acids remained constant (Ortega-Heras et al. 2014; Bouzas-Cid et al. 2018).

In the present study, the significant accumulation of foliar lyxose, glucose, sorbose, glucose-6-phosphate, myo-inositol, cellobiose, cellobitol, raffinose and mannitol contents across stands in 2014 under dry weather conditions before harvest suggest a close association between these metabolites and drought tolerance of sessile oak. Lu et al. (2009) also found a significant increase of total soluble sugar and sucrose in hybrid Bermudagrass under drought stress. Apparently, accumulation of soluble sugars in water-stressed tissues as osmoprotectants is a more general phenomenon in herbaceous and woody plants (Skirycz et al. 2010; Munnik and Vermeer 2010; Perera et al. 2008).

Yoshimura et al. (2016) reported that the stored carbohydrate is quickly reformed between soluble sugar and starch within sapwood during several days, due to water stress. However, our results show that foliar carbohydrate contents were mostly lower in the dry compared to the wet year indicating that a compensation of reduced foliar carbohydrate accumulation under dry conditions by the mobilisation of carbohydrates from sapwood was not effective. Furthermore, Viklund et al (2008) showed that the contents of free amino acids and sugars in potatoes can vary significantly between years and that these variations can be explained by differences in precipitation and temperature. Therefore, it can be assumed that the accumulation of carbohydrates in the leaves could contribute to an increased osmotic potential at reduced cell hydration, and may facilitate the maintenance of cell turgor, thereby mitigating injurious effects of short-term drought to sessile oak leaves.

Conclusions

The present investigation shows that structural and physiological traits in leaves of adult sessile oak trees are largely determined by the weather conditions before harvest. Thus, the central hypothesis of this study was validated for foliar traits such as lignin, cellulose and some soluble sugar (sucrose, glucose, fructose, and glucose-6-phosphate) contents. Our results also show that only few physiological traits of leaves from adult sessile oak trees are independent of current weather conditions across the five forest stands investigated, indicating a high degree of plasticity of sessile oak leaves to atmospheric and pedospheric conditions. However, a combined analysis of foliar and sapwood traits over an extended period of time with multiple sampling would provide more detailed information of plasticity of sessile oak leaves to claimate change in future.

Author contribution statement LA: performed most of the measurements, analyzed data and wrote the manuscript with input from all authors. SS: conceived and planned the experiments, analyzed the antioxidants and edited the manuscript. JK: helped with GC–MS measurements and edited the manuscript. ME: helped with UPLC measurements. MD: helped with soil measurements and edited the manuscript. HR: designed the project, supervised the study and edited the manuscript. All authors contributed to the final version of the manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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