Differences in isoprenoid-mediated energy dissipation pathways between coastal and interior Douglas-fir seedlings in response to drought

Laura Verena Junker-Frohn^{1,2,3}, Anita Kleiber⁴, Kirstin Jansen^{5,6}, Arthur Gessler^{5,7,8},
 Jürgen Kreuzwieser⁴, Ingo Ensminger^{1,2}

¹Department of Biology, Graduate Programs in Cell & Systems Biology and Ecology &
 Evolutionary Biology, University of Toronto, 3359 Mississauga Road, Mississauga, ON,
 Canada.

²Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg, Wonnhaldestr. 4, 79100
 Freiburg, Germany.

³Present address: Institute of Bio and Geosciences IBG-2, Plant Sciences, Forschungszentrum
 Jülich GmbH, Jülich, Germany.

⁴Chair of Tree Physiology, Institute of Forest Sciences, Albert-Ludwigs-Universität Freiburg,
 Georges-Köhler-Allee 53, 79110 Freiburg, Germany.

⁵Institute for Landscape Biogeochemistry, Leibniz Centre for Agricultural Landscape Research
 (ZALF), Eberswalder Str. 84, 15374 Müncheberg, Germany.

⁶Present address: Institute of Ecology, Leuphana University of Lüneburg, Universitätsstr. 1,
 21335 Lüneburg, Germany.

⁷Institute of Terrestrial Ecosystems, ETH Zurich, 8092, Zürich, Switzerland.

⁸Swiss Federal Research Institute WSL, Zürcherstr. 111, 8903 Birmensdorf, Switzerland.

Corresponding author: Laura Junker, +1-905-569-4988, l.junker@fz-juelich.de

Key words: *Pseudotsuga menziesii*, intraspecific variation, drought, photosynthesis, non-
 photochemical quenching, xanthophyll cycle

ABSTRACT

Plants have evolved energy dissipation pathways to reduce photooxidative damage under drought, when photosynthesis is hampered. Non-volatile and volatile isoprenoids are involved in non-photochemical quenching of excess light energy and scavenging of reactive oxygen species. A better understanding of trees' ability to cope with and withstand drought stress will contribute to mitigate the negative effects of prolonged drought periods expected under future climate conditions. Therefore we investigated, if Douglas-fir (*Pseudotsuga menziesii*) provenances from habitats with contrasting water availability reveal intraspecific variation in isoprenoid-mediated energy dissipation pathways. In a controlled drought experiment with one-year-old seedlings of an interior and a coastal Douglas-fir provenance, we assessed the photosynthetic capacity, pool sizes of non-volatile isoprenoids associated with the photosynthetic apparatus as well as pool sizes and emission of volatile isoprenoids. We observed variation in the amount and composition of non-volatile and volatile isoprenoids among provenances, which could be linked to variation in photosynthetic capacity under drought. The coastal provenance exhibited an enhanced biosynthesis and emission of volatile isoprenoids, which is likely sustained by generally higher assimilation rates under drought. In contrast, the interior provenance showed an enhanced photoprotection of the photosynthetic apparatus by generally higher amounts of non-volatile isoprenoids and increased amounts of xanthophyll cycle pigments under drought. Our results demonstrate that there is intraspecific variation in isoprenoid-mediated energy dissipation pathways among Douglas-fir provenances, which may be important traits to select provenances suitable to grow under future climate conditions.

INTRODUCTION

Tree species show inter- and intraspecific variation in response to limited water availability (Bolte et al. 2009; George et al. 2015; Hess et al. 2016; Niinemets 2016). In context of global warming, trees will be exposed to longer and more frequent drought events (Montwé et al. 2015). Therefore, a better understanding of the mechanisms contributing to trees' drought stress tolerance is needed (Montwé et al. 2015; St Clair and Howe 2007). The North American conifer species Douglas-fir is an economically highly relevant forest tree that allows to study drought tolerance mechanisms, as it thrives under different climatic conditions. A coastal subspecies (*var. menziesii*) occurs along the west coast under mainly moist maritime climatic conditions, and an interior subspecies (*var. glauca*) inhabits mountainous regions with a rather dry continental climate (Aas 2008; Ferrell and Woodard 1966; Lavender and Hermann 2014). Variation in climatic conditions within the coastal and interior habitats lead to local adaptation of provenances to moist or dry conditions also within the coastal and interior subspecies, respectively (Aitken et al., 1995; Anekonda et al., 2002; Montwé et al., 2015). Provenances originating from dry habitats are generally considered to be better adapted to drought, but typically exhibit a lower productivity (Bansal et al. 2015; Montwé et al. 2015; Sergent et al. 2014). A common garden experiment with well-watered Douglas-fir seedlings revealed considerable variation in photosynthetic carbon assimilation between provenances of both subspecies (Zhang and Marshall 1995). Intraspecific variation in the regulation of photosynthetic gas exchange under drought is indicated by intraspecific variation in carbon discrimination (Aitken et al. 1995; Jansen et al. 2013).

Generally, plants respond to drought by reduced stomatal conductance, which minimizes water loss by transpiration, but hampers photosynthetic carbon assimilation (Flexas et al. 2004). Reduced photochemical quenching of absorbed light energy increases the demand for dissipation of excess light energy. Excess light energy otherwise promotes the formation of reactive oxygen species (ROS), which lead to photooxidative damage of proteins, pigments and lipids, especially of the photosystems (Chaves et al. 2009). Plants have evolved diverse energy dissipation pathways to minimize the energetic imbalance between absorbed and utilized light energy (Wilhelm and Selmar 2011). As a first line of defense, plants quench excess light energy by non-photochemical quenching (NPQ). As a second protective mechanism, triplet chlorophylls and generated ROS are detoxified to minimize photooxidative damage (Baroli and Niyogi 2000; Munekage et al. 2002).

Isoprenoids are secondary metabolites involved in diverse energy dissipation pathways. Non-volatile isoprenoids, which are often referred to as essential isoprenoids, include the carotenoids of the photosynthetic apparatus, which are involved in NPQ and scavenging of ROS (de Bianchi

et al. 2010). The xanthophyll cycle provides an instantaneous mechanism to facilitate NPQ by the conversion of violaxanthin via antheraxanthin to zeaxanthin, which safely dissipate excess energy in terms of heat (Demmig-Adams and Adams 2006). Other carotenoids of the photosynthetic apparatus also have an antioxidant function and scavenge ROS (Esteban et al. 2015; Junker and Ensminger 2016). Although the composition of the major plant carotenoids, lutein, neoxanthin, the xanthophyll cycle pigments, and β -carotene, is remarkably consistent among species (Pogson et al. 1998), adjustments of the carotenoid composition of the photosynthetic apparatus contribute to the acclimation of plants to different environmental conditions (Esteban et al. 2015; Junker et al. 2017). Under drought, an increased carotenoid-to-chlorophyll ratio has been observed in many species, e.g. in Mediterranean tree species, and in Chinese spruce *Picea asperata* (Duan et al. 2005; Faria et al. 1998). Moreover, increased pool sizes of xanthophyll cycle pigments enhance the photoprotective capacity of the xanthophyll cycle and have been observed in response to drought in many species (Wujeska et al. 2013). In field-grown mature Douglas-fir, increased xanthophyll cycle pigments have been observed in response to increased light intensities, but not to decreased soil water availability (Junker et al. 2017), which is not consistent with previous studies (Esteban et al. 2015).

Many plant species including Douglas-fir also produce volatile isoprenoids (*i.e.* monoterpenes, sesquiterpenes). It has been demonstrated that plants react to biotic and abiotic stresses with altered contents, emission rates and compositions to cope with the stress (Blande et al. 2014; Peñuelas and Munné-Bosch 2005; Possell and Loreto 2013). The often observed enhanced emission of volatile isoprenoids in response to stresses suggests their importance to mediate stress, despite the implicated loss of previously fixed carbon (Behnke et al. 2007; Loreto et al. 1998; Niinemets 2016; Vickers et al. 2009). For example, the adverse effects of tropospheric ozone on plants can be ameliorated by the emission of volatile isoprenoids most likely as they prevent the accumulation of ROS (Loreto et al. 2004). A similar ROS quenching mechanism by volatile isoprenoids has been proposed for plants under drought stress (Beckett et al. 2012; Ryan et al. 2014; Velikova et al. 2016). Moreover, volatile isoprenoids seem also to mediate enhanced thermotolerance and protect the photosynthetic apparatus from heat stress by stabilizing thylakoid membranes (Velikova et al. 2012; Velikova et al. 2011). Furthermore, the dissipation of excess energy during biosynthesis of volatile isoprenoids is an assumed mechanism enhancing plant tolerance against oxidative stress (Peñuelas and Munné-Bosch 2005). In addition to their significance in plant response to abiotic stress, volatile isoprenoids play an important role in plant protection against herbivores and in multitrophic plant-herbivore-predator interactions (Baldwin 2010). Particularly conifers, including Douglas fir, possess large pools of volatile isoprenoids in nearly all tissues required for biotic stress defence (Giunta et al. 2016). Interior and coastal Douglas-fir provenances have been shown to vary in pool sizes and composition of

stored monoterpenes (Junker et al. 2017; Kleiber et al. 2017). In addition, Douglas-fir has been shown to increase mono- and sesquiterpene emissions in response to heat and biotic stress (Joó et al. 2011). In contrast, Junker et al. (2017) observed increased monoterpene emissions of field-grown mature Douglas-fir in response to high light conditions, but not temperature.

The suite of photoprotective processes that are employed in response to drought have been shown to vary between species (Peguero-Pina et al. 2009; Wujeska et al. 2013), and there is evidence that photoprotective processes contribute to local adaptation of tree provenances to environmental conditions (Ramírez-Valiente et al. 2015). A *Picea asperata* population from a dry habitat showed greater capacity for dissipation of excitation energy as heat compared to a population from a moist habitat (Duan et al. 2005). Provenances of *Quercus coccifera* L. also revealed some intraspecific variation in energy dissipation (Balaguer et al. 2001). In previous work, we have demonstrated that there is intraspecific variation in the non-volatile isoprenoids associated with the photosynthetic apparatus as well as α -tocopherol and volatile isoprenoids among Douglas-fir provenances (Du et al. 2016; Junker et al. 2017; Kleiber et al. 2017).

The aim of this study was to assess if two Douglas-fir provenances that originate from contrasting habitats show differences in non-volatile and volatile isoprenoids, which are involved in different energy dissipation pathways in response to drought. Photosynthetic gas exchange, non-volatile isoprenoids as well as volatile isoprenoid pools and emission were studied in seedlings of an interior and a coastal provenance exposed to reduced watering for six weeks, followed by a two week rewatering phase in comparison to well-watered control seedlings. Seedlings from both provenances are expected to employ isoprenoid-mediated energy dissipation mechanisms to mitigate photooxidative stress when photosynthetic gas exchange is reduced in response to drought. The coastal provenance has been shown to maintain higher assimilation rates under drought compared to the interior provenance (Kleiber et al. 2017). Therefore, we hypothesized that the interior provenance has higher amounts of non-volatile isoprenoids compared to the coastal provenance, which contribute to enhance NPQ of excess light energy to compensate for lower photochemical quenching of light energy under drought. We furthermore hypothesized that provenances differ in energy dissipation mediated by volatile isoprenoids, due to differences in the demand for scavenging of ROS. By focusing on non-volatile and volatile isoprenoids, we expected to reveal different strategies to dissipate excess energy under drought. This will contribute to identify new drought and water stress related traits, which will be important for the identification and selection of provenances that are most suitable for future drier climatic conditions.

MATERIALS AND METHODS

Plant material

One-year-old seedlings of an interior (*var. glauca*) and a coastal (*var. menziesii*) Douglas-fir provenance were obtained from nurseries. Seedlings of the interior provenance Fehr Lake (INT) (seedlot: FDI 39841, N50.71, W120.86) were obtained from BC Timber Sales (Vernon, Canada). INT originates from a dry habitat (800 m above sea level, 5.8 °C mean annual temperature) in British Columbia with an annual precipitation of 333 mm, and 162 mm precipitation during the growing season (May to September). Seedlings of the coastal provenance Snoqualmie (COA) (seedlot: pme 07(797) 412-10) were obtained from Forestry Commission, Wykeham Nursery (Sawdon, England). COA originates from a humid habitat (457-610 m above sea level, 7.9 °C mean annual temperature) with an annual precipitation of 2134 mm, and 365 mm precipitation during the growing season. Upon arrival in March/ April, seedlings were planted in 3-liter pots with a height of 23 cm and a width of 14 cm, which allowed for adequate root development during the course of the experiment. We used a medium-fibrous peat soil (Container substrate 1 medium + GreenFibre basic, pH = 5.3; Klasmann-Deilmann GmbH, Geeste, Germany) and fertilized seedlings with NPK fertilizer (N170 + P200 + K230 + Mg100 + S150).

Growth conditions

The experiment was carried out at the Leibniz Centre for Agricultural Landscape Research in Müncheberg, Germany, from June to September of 2011 in two walk-in environmental chambers (VB 8018, Vötsch Industrietechnik GmbH, Germany) equipped with metal halide lamps (Powerstar HQI-BT 400 W/D PRO Daylight, Osram GmbH, Munich, Germany). Temperature was maintained at 21 °C during day and night, with a relative humidity of 70 %. Light intensity at canopy height was 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 16 h per day. Seedlings were randomly distributed among both chambers and daily and watered to field capacity. After more than three months of acclimation to growth conditions, the drought treatment for half of the seedlings per chamber started on July 20th after the second flush had occurred, which was mainly observed in COA.

Control and drought treatment

Control seedlings were watered daily from July 20th (day 0) onwards with 200 ml water, to maintain a target soil water content of about 35 %. Watering of the seedlings of the drought treatment was reduced to only 65 ml until August 10th (day 21), to achieve decreasing soil water content. From August 11th to 31st, watering was entirely withheld. On the evening of August 31

(day 42), seedlings of the drought treatment were rewatered to increase the target soil water content to the level of control plants. All seedlings were grown for another two weeks with daily supply of 200 ml water. Daily soil moisture measurements with ECH2O sensors (EC5, Decagon Devices, Inc., Pullman, USA) in the upper 10 cm of substrate were used as guidance for consistent soil water content of control and drought seedlings, which was calculated according to Schindler et al. (2010). As control and drought COA seedlings consistently had a higher water use compared to INT, they received about 10% additional watering to keep soil water content at similar levels in both provenances. Pre-dawn twig water potential was determined on day 7, 17, 26, 36, 42, 43 and 52 by sampling of N=3 seedlings each using a pressure chamber (Model 3015G4, Soil moisture Equipment Corp., Santa Barbara, CA, USA) according to Scholander *et al.* (1965). All water-potential measurements were performed on seedlings that were not used for other sampling and gas exchange measurements.

Sampling

Five seedlings per provenance and treatment were randomly selected for sampling prior to the drought treatment on June 28th (shown as day 0), and on days 28, 41 (drought), 56 (rewatered). First, samples of current-year needles (excluding second flush) were taken prior to the drought treatment for the determination of non-volatile isoprenoids and stored volatile isoprenoids. Needles were cut from the twigs, weighed and immediately frozen in liquid nitrogen. Samples were stored at -80°C and ground immersed in liquid nitrogen using mortar and pestle. Afterwards, each seedlings was separated in fractions of current-year needles, last-year-needles, wood, bark, and roots that were individually weighed for determination of fresh mass. Plant material was oven-dried for 48 hours at 105°C to estimate dry mass. Total seedling biomass was determined by adding dry mass of all plant parts including estimated dry mass of frozen samples.

Photosynthesis measurements

Photosynthesis measurements were conducted on June 28th (shown as day 0), day 20, 41, 43 and 56. Gas exchange was measured on N=5 seedlings per provenance and treatment in current-year needles of the uppermost whirl using a LI-COR 6400 XT portable gas exchange system (LI-COR Biosciences, Lincoln, NE, USA). About 10-15 needles on an intact twig were placed into the cuvette to form a flat area. Measurement conditions in the closed cuvette were set to a 400 ml min⁻¹ flow rate, 25 °C block temperature, 40 % relative humidity, and a CO₂ concentration of 400 ppm. Prior to starting the gas exchange measurements, needles were dark-adapted for 25 minutes. Measurements of the steady state of photosynthetic CO₂ gas exchange were taken at

0, 400, 500, 1000, 1500 and 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity after a minimum of 5 min acclimation time at each light intensity. Altogether, measurements took usually 35 min per plant, and needles were allowed to acclimate to the conditions in the Licor chamber for at least 20 min prior to the data at 1000 μmol reported in Fig. 3. Light exposed needle surface area was determined using WinSeedle software and scanner (Regents Instruments Inc., Québec, Canada). The rate of photosynthetic gas exchange was expressed per projected needle area exposed to the light. Light response curves were modelled using the non-rectangular function $A = ((\Phi I + A_{\max} - ((\Phi I + A_{\max})^2 - 4 \Theta \Phi I A_{\max})^{0.5}) / 2 \Theta) - R$ with Φ = maximum quantum yield, I = light intensity, A = net assimilation rate, $A_{\max}$ = maximum net assimilation rate, Θ = coefficient of curvature, and R = rate of respiration (Chartier and Prioul 1977). Parameter estimation was performed using the excel spreadsheet described by Lobo et al (2013), which uses the solver function to minimize the sum of squared errors and also yields light curve parameters including the light compensation point (I_{comp}) and light intensity at half-maximum rate of photosynthesis ($I_{\text{sat}(50)}$).

Analysis of non-volatile isoprenoids

The non-volatile isoprenoids, chlorophylls and carotenoids, directly associated with the photosynthetic apparatus, were extracted using 98 % methanol buffered with 0.5 M ammonium acetate and analysed by HPLC-DAD following the protocol described in Junker and Ensminger (2016). A high-performance liquid chromatography (HPLC) system (model 1260, Agilent Technologies, Böblingen, Germany) with a quaternary pump (model 1260), autosampler (model 1260, set to 4 °C), column oven (model 1260, set to 25 °C) and photodiode array detector (model 1290, recording absorption at 450 nm and 656 nm wavelength) was used for reverse-phase chromatography using a C₃₀-column (5 μm , 250*4.6 mm; YMC Inc., Wilmington, NC, USA). Three solvents (A: 100 % methanol, B: 100% methyl-tert-butyl-ether, C: water buffered with 20 mM ammonium acetate) were used to run a gradient starting with 92 % A, 5 % B, and 3 % C. After 3 minutes, Solvent A was gradually replaced by solvent B, while solvent C remained constantly at 3 %. The amount of B increased to 33.6 % at 17 minutes, 81.3 % B at 22 minutes, and reached a maximum of 81.3 % B from 23 to 27 minutes. Afterwards, the initial solvent concentration was re-established within one minute, and the column reconditioned for seven minutes prior to the next run. Peaks were quantified using standards for chlorophyll a, chlorophyll b and β -carotene from Sigma Aldrich (Oakville, ON, Canada) and standards for antheraxanthin, α -carotene, lutein, neoxanthin, violaxanthin and zeaxanthin from DHI Lab products (Hørsholm, Denmark). ChemStation B.04.03 software (Agilent Technologies, Böblingen, Germany) was used for peak integration.

Extraction of stored volatile isoprenoids

Volatile isoprenoids stored in sampled needles were extracted using 500 µl 100 % methanol per 25 mg fresh weight. After 20 min of stirring at 30 °C followed by centrifugation, extracted isoprenoids were bound by stirring at 1400 rpm with pre-conditioned polydimethylsiloxane (PDMS) coated Twisters[®] (10 mm length, 1 mm PDMS coat; Gerstel, Germany) for 60 min at 30 °C. A control sample (100 % methanol instead of the extract) was run with every set of samples to control for background contamination. Twisters[®] were dried with a lint free paper tissue and placed into glass cartridges for immediate analysis with GC-MS.

Sampling of emitted volatile isoprenoids

Volatile emissions of control- and drought seedlings were collected on day 21, 25 and 53 independent from other measurements. Customized one liter glass enclosures consisting of a lower half with a wide opening to insert seedlings and a tightly closing upper half were used to enclose the whole seedling without physical contact. To avoid any effects from young second-flush needles, second-flush twigs of five seedlings per provenance and treatment were removed two days prior to the start of volatile isoprenoid sampling (day 19). Additionally, all seedlings were placed in one environmental chamber to minimize influences of potential differences in chamber atmospheres on volatile emission measurements. One day prior to the measurements, seedlings were carefully inserted into the lower parts of the enclosures to avoid contamination of measurements by needle injuries. The opening around the stem was sealed using sealing tape (Terostat VII, Henkel Teroson GmbH, Heidelberg, Germany). Before the actual measurement, the airtight enclosure was closed and supplied with a mixture of synthetic air (Air Liquide, Ludwigshafen, Germany) and 400 ppm CO₂. Temperature in the cuvette was maintained at 24.5 ± 1.5 °C, with an illumination of 690 ± 50 µmol m⁻² s⁻¹. After 5 min acclimation time, air was drawn from the outlet of the cuvettes with a flow rate of 200 ml min⁻¹ using an air sampling pump (Analyt-MTC, Müllheim, Germany). Emitted volatiles were trapped using air sampling tubes packed with adsorbent beds of 20 mg Tenax TA 60/80 and 30 mg Carbotrap B 20/40 (Supelco, Bellafonte, PA, USA) between glass wool. After 40 min, air sampling tubes were disconnected and stored in airtight glass vials at 4 °C until GC-MS analysis. To determine the needle mass in the enclosure, needles were sampled after the end of the experiment to determine their dry mass. Monoterpene emission rates were calculated per dry mass and over time. Zero references using an empty cuvette were measured once per day to correct for background emission.

295 *Volatile isoprenoid analysis*

296 Emitted and stored volatile isoprenoids were analysed using a gas chromatograph (GC, model
 297 7890A, Agilent, Germany), coupled to a mass-selective detector (MS, 5975C, Agilent, Germany)
 298 and equipped with a thermodesorption/cold injection system (TDU-CIS; Gerstel, Germany)
 299 according to Kleiber et al. (2017). Air sampling tubes/ Twisters® were desorbed with the TDU
 300 at 240°C, cryofocused at -100°C and heated to 240°C in the CIS prior to injection into the GC-
 301 MS: Separation on a DB-624 column (Agilent, Germany) occurred during an oven temperature
 302 program beginning at 40 °C, increasing at a rate of 6 °C min⁻¹ for 3 min to 100 °C, when the
 303 temperature ramp speed up to 16 °C min⁻¹ until the column reached 230 °C. Volatile isoprenoids
 304 were identified by comparison of peaks and de-convoluted fragmentation spectra to external
 305 standards and to the NIST database using the AMDIS software (National Institute of Standards
 306 and Technology (NIST), Gaithersburg, MD, USA). Monoterpenes include L- α -bornyl acetate,
 307 camphene, (+)-carene, 3-carene, α -citral, β -citronellal, β -citronellol, citronellyl acetate,
 308 eucalyptol, (-)-isopulegol, trans-geraniol, geraniol acetate, limonene, linalool, β -myrcene,
 309 ocimene, β -phellandrene, α -pinene, β -pinene, sabinene, α -terpinene, α -terpineol, L-4-terpineol,
 310 γ -terpinene, and tricyclene. Sesquiterpenes include γ -cadinene, α -caryophyllene,
 311 β -caryophyllene, α -cubebene, β -elemene, δ -elemene, (+)-longifolene, and nerolidol. Conversion
 312 from fresh to dry mass was determined by drying approximately 10 g of frozen needles for 4 h at
 313 200 °C.

315 *Statistics*

316 All statistical tests were performed using R 3.0.3 (R Core Team 2014). A linear mixed-effect
 317 model using *Provenance* and *Treatment* as fixed factors and *sampling day* as random factor was
 318 used to evaluate if provenance or treatment specific differences occurred under the drought
 319 treatment (function *lmer*, package *lme4*, Bates et al. 2013). Models using *Provenance*,
 320 *Treatment*, *Provenance + Treatment* and *Provenance x Treatment* were compared to a null
 321 model considering only an intercept and *sampling day* as random factor. Based on lowest Akaike
 322 Information Criterion (AIC; Akaike 1973) the best-fit model was chosen. Consequently, table 1
 323 only shows p-values for factors, which improve a model, and NA was used to denote factors not
 324 relevant to describe a parameter's response. Significance of the fixed factors was assessed by a
 325 pairwise comparison of the best-fit model to the best-fit model minus one of each of its fixed
 326 effects (function *anova*, Zuur 2009). For *Provenance x Treatment* models, the interaction effect
 327 was tested comparing it to the *Provenance + Treatment* model using the *anova* function. It must
 328 be noted that gas exchange and volatile emission parameters were repeatedly assessed from the
 329 same set of plants, which may have led to an overestimation of provenance-specific effects.

Differences between provenances and treatments on each sampling day were determined using the parametric Kruskal-Wallis-rank-sum-Test (function *kruskal.test*) followed by a Tukey-type pairwise comparison using the function *npaircomp* (package *npaircomp*). Due to only minor variation in the composition of stored and emitted volatile isoprenoids, amounts were averaged per provenance and treatment and summarized in Table 2.

Differences in responses between provenances as shown in Figures 8 and S1 were determined comparing linear models (function *lm*) describing the response of a provenance to a parameter as *Provenance* x *Parameter* by an Tukey-type comparison using the function *lsmeans* (package *lsmeans*).

RESULTS

Water availability and plant growth

Control seedlings were grown at a volumetric soil water content of 37 ± 5 %, which corresponds to 37% volumetric soil water content (Fig. 1a). For drought seedlings, volumetric soil water content decreased to a minimum of 2 % in both provenances by day 42. The predawn twig water potential of drought seedlings of both provenances decreased only after day 36, with lowest twig water potential of -2.4 MPa in INT and -2.7 MPa in COA on day 42 (Fig. 1b). When seedlings were rewatered on the evening of day 42, soil water content rapidly increased and twig water potential recovered to control levels within one day. The twig water potential of both provenances was thus upheld as soil water content decreased to values as low as 5 % under the drought treatment (Fig. S1a).

Carbon allocation was considerably affected under drought conditions. INT seedlings had an initially higher total biomass of 18.6 ± 0.5 g dry mass (DM) compared to COA with only 6.0 ± 0.9 g DM (Fig. 2a). Under the control treatment, seedlings of COA and INT gained comparable biomass of 16.5 g and 13.0 g, respectively, resulting in a biomass of 31.6 g DM for INT and 22.5 g DM for COA (Fig. 2a). Growth of both provenances was reduced by the drought treatment and still delayed after rewatering, but to a stronger extent in INT. While INT gained only 3.7 g DM during the experiment, COA gained with 8.1 g DM more than twice as much, resulting in a total biomass of 22.3 g in INT drought seedlings and 14.1 g in COA drought seedlings. Needles of INT seedlings were thicker and more rigid compared to needles of COA, which is expressed in a leaf mass per area (LMA) of 15.1 mg cm^{-2} compared to 13.3 mg cm^{-2} (Fig. 2b). LMA showed an increase of about 25 % in control seedlings of both provenances and was significantly lower by about 12 % under the drought treatment (Fig. 2b, Table 1), but were comparable to control seedlings two weeks after rewatering.

Photosynthetic gas exchange

COA showed generally higher gas exchange rates compared to INT, but both provenances showed similar decreases under the drought treatment. COA control seedlings showed an initial stomatal conductance (g_s) of $0.18 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, which decreased over the course of the experiment to $0.11 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. g_s of INT control seedlings was consistently lower and ranged between 0.10 to $0.15 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Fig. 3a). Under the drought treatment, g_s decreased to a minimum of $0.03 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in COA drought seedlings and $0.01 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in INT drought seedlings. Consequently, g_s in both provenances was downregulated before twig water potential dropped at the end of the drought treatment (Fig. S1b). Upon rewatering, g_s increased to $0.04 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in both provenances within one day and reached g_s comparable to control seedlings within two weeks after rewatering. COA thus showed a significantly higher g_s compared to INT, and both provenances were equally impacted by the drought treatment (Table 1). Assimilation rates (A) were tightly linked to g_s , and showed essentially the same, but nonsignificant, provenance-specific difference and decrease under drought (Fig. 3b; Table 1). Control seedlings of both provenances showed an A between 8 and $12 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, while drought seedlings exhibited minimum values of $3.5 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in COA and $1.1 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in INT at the end of the drought treatment. A correlated well with g_s and revealed only minor variation of the intrinsic water use efficiency ($WUE = A/g_s$, indicated by similar slopes of the linear regression lines for COA and INT in Fig. S1c). The maximum assimilation rate (A_{max}), calculated from light response curve measurements, revealed a non-significantly higher photosynthetic capacity of control and drought seedlings from COA compared to INT on day 0 and 41 (Fig. 4, Table S1). After recovery (day 56), A_{max} of control and previously drought exposed seedlings of both provenances were comparable (Fig. 4c, Table S1). Reduced A_{max} under the drought treatment (day 41) went along with an increased half-saturation light intensity and light compensation point in both provenances (Table S1).

Non-volatile isoprenoids associated with the photosynthetic apparatus

Chlorophylls and carotenoids, which are derived from the non-volatile isoprenoid biosynthetic pathway, revealed provenance- and treatment-specific variation. The chlorophyll content decreased over the course of the experiment in both provenances. COA showed a significantly higher chlorophyll content with initial values of $6.7 \text{ } \mu\text{mol g}^{-1} \text{ DM}$ compared to $4.9 \text{ } \mu\text{mol g}^{-1} \text{ DM}$ in INT (Fig. 5a, Table 1). Both provenances showed an increased chlorophyll content under the drought treatment (Fig. 5a). Although we could not observe a significant effect for individual sampling dates (Fig. 5a), the observed non-significant variation contributed to an overall significant *Treatment* effect (Table 1). After rewatering, the chlorophyll content of drought

seedlings recovered to the level of control seedlings (Fig. 5a). The chlorophyll a/b ratio was stable in control seedlings of both provenances, with higher chlorophyll a/b ratio in INT compared to COA (Fig. 5b). Under the drought treatment, the chlorophyll a/b ratio decreased in both provenances, but to a greater extent in INT compared to COA (Fig. 5b). This is also indicated by *Provenance* x *Treatment* as best describing model, although the interaction effect was not significant (Table 1). The carotenoid/ chlorophyll ratio in both provenances increased as chlorophylls decreased in control seedlings over the course of the experiment, and was significantly higher in INT compared to COA (Fig. 5c; Table 1). Although we did not observe a significant *Treatment* effect, we observed a nonsignificant increase of the carotenoid/ chlorophyll ratio in INT seedlings at the end of the drought treatment, which recovered by the end of the experiment.

Despite variation in total carotenoids, COA and INT showed comparable levels of the pigments of the xanthophyll cycle, which showed an increase from 55 to 100 $\mu\text{mol mol}^{-1}$ Chl in both provenances during the first month of the experiment (Fig. 6a). During the whole experiment, xanthophyll content of COA seedlings of the drought treatment was equal to control seedlings, with a decrease to initial levels during the last two weeks of the experiment. In contrast, INT seedlings showed a nonsignificantly increased xanthophyll content at the end of the drought treatment compared to control seedlings, revealing a *Provenance* x *Treatment* interaction (Table 1). The de-epoxidation state of the xanthophyll cycle (DEPS) also showed a general increase during the first month of the experiment (Fig. 6b). Both provenances showed a significant treatment effect, with increased DEPS at the end of the drought treatment, which was more pronounced for INT (Fig. 6b, Table 1).

The other major carotenoids showed constitutive differences between provenances, but did not exhibit significant variation in response to the drought treatment. β -carotene and neoxanthin levels were relatively constant throughout the experiment in both provenances, but revealed significantly higher content in INT compared to COA (Fig. 6c,d, Table 1). Neoxanthin was additionally slightly enhanced under the drought treatment, indicated by lowest AIC of the *Provenance* + *Treatment* model, but the effect of *Treatment* was non-significant. Lutein increased in both provenances over the course of the experiment and showed significantly higher levels in INT compared to COA, but was not affected by the drought treatment (Fig. 6e; Table 1). α -carotene decreased over the course of the experiment, but did not vary between provenances or in response to drought (Fig. 6f, Table 1).

Volatile isoprenoid pools and emission

Both provenances maintained rather constant volatile isoprenoid contents and emission rates over the course of the experiment, but revealed constitutive provenance-specific differences. Monoterpene content per dry mass of INT were about $190 \mu\text{mol g}^{-1}$, and significantly higher compared to only about $140 \mu\text{mol g}^{-1}$ monoterpene content per dry mass in COA (Fig. 7a, Table 1). Sesquiterpene content per dry mass of control seedlings of both provenances were relatively stable around $1.5 \mu\text{mol g}^{-1}$ over the course of the experiment (Fig. 7b). While sesquiterpene contents in INT seedlings of the drought treatment did not deviate from control seedlings, COA seedlings of the drought treatment showed increased sesquiterpene levels with a maximum of $3.5 \mu\text{mol g}^{-1}$, displaying a significant *Provenance* x *Treatment* interaction (Table 1). Emission rates of monoterpenes were quite stable and did not vary among treatments, but showed a high tree-by-tree variation (data not shown). Monoterpene emissions of COA were significantly higher compared to INT (Fig. 7c; Table 1).

The composition of volatile isoprenoid pools and emission showed little variation between treatments, but varied between provenances (Table 2). COA revealed higher amounts of stored β -pinene and 3-carene, but lower amounts of α -pinene and camphene compared to INT. Sesquiterpene pools were much more similar between provenances, but COA exhibited slightly higher amounts of β -caryophyllene and lower amounts of (+)-longifolene compared to INT (Table 2). The composition of monoterpene emissions exhibited high variation between individual seedlings, but did not significantly differ between provenances (Table 2).

Regulation of the non-volatile and volatile isoprenoid metabolism in response to reduced assimilation rates under drought

INT and COA seedlings employed different isoprenoid-mediated energy dissipation mechanisms, when assimilation rates were decreased in response to drought. Fig. 8 shows non-volatile and volatile isoprenoids involved in the dissipation of excess energy, which showed significant *Treatment* effects or *Provenance* x *Treatment* effects. The chlorophyll a/b ratio in both provenances decreased in response to decreased *A*, with a slightly stronger response in INT compared to COA (Fig. 8a, Table 1). The xanthophyll cycle pool sizes in both provenances were increased when *A* was decreased, but revealed a higher plasticity in INT compared to COA (Fig. 8b). Both provenances showed a marked increase of DEPS in response to decreasing *A*, confirming the significance of DEPS in response to *Treatment* (Fig. 8c, Table 1). Sesquiterpene levels of both provenances increased with decreasing assimilation rate (Fig. 8d, Table 1). COA showed higher sesquiterpene pool sizes and stronger induction as *A* decreased, confirming the

significant *Provenance* x *Treatment* interaction in adjustments of sesquiterpenes in response to drought (Table 1).

DISCUSSION

Provenance-specific differences in photosynthetic gas exchange affect water use and biomass accumulation

Our results revealed differences in photosynthetic gas exchange and dissipation of excess energy by non-volatile and volatile isoprenoids between the two Douglas-fir provenances. Drought seedlings of both provenances downregulated stomatal conductance (g_s) prior to a change in twig water potential (Fig. 3a, Fig. S1b), which is typical for the isohydric regulation of stomatal conductance in conifers (McCulloh et al. 2014; Warren et al. 2003, but see for a general criticism of the isohydry/anisohydry concept Martínez-Vilalta and García-Forner, 2016). Thereby, drought seedlings of both provenances maintained a high and nearly unchanged twig water potential despite an decreasing soil water content (Fig. 1, S1a). Reduced g_s limited assimilation rates (A) (Fig. 3b, S1c; see Flexas et al. 2004). Reduced A under drought impaired plant growth and led to reduced levels of nonstructural carbohydrates in needles, indicated by a reduced leaf mass per area (Fig. 2) (Bansal et al. 2013; Poorter et al. 2009).

Generally higher g_s and consequently A in the coastal provenance (COA) compared to the interior provenance (INT) under control and drought conditions contributed to a faster growth of this provenance (Fig. 2a, 3b, see Zhang et al. 2004). Nevertheless, higher g_s of COA also led to a higher water loss by transpiration (data not shown) and exhaustion of soil water content as monitored by ECH2O probes. Although this did not negatively affect COA during the short-term drought period applied here, it increases the susceptibility of COA to prolonged drought periods (McDowell 2011). In contrast, the generally lower g_s of INT indicates a rather conservative water use, which is thought to increase survival and allow for a faster recovery after longer drought (Attia et al. 2015). Indeed, in our study, INT showed a faster increase of A and g_s after rewatering (Fig. 3).

Light response curves revealed that photosynthesis under drought is not light limited, but light energy is available in excess and cannot be used in photochemical reactions (Fig. 4, Table S1). Reduced photochemical quenching of absorbed light energy consequently enhances the need for alternative energy dissipation pathways to quench excess light energy non-photochemically and minimize photooxidative damage. An efficient protection of the photosynthetic apparatus is also indicated by the fast recovery of photosynthetic assimilation rate in both provenances upon rewatering (Fig. 3b).

Increased amounts of non-volatile isoprenoids in the interior provenance under drought

Provenances revealed significant differences in the composition of non-volatile isoprenoids associated with the photosynthetic apparatus. INT showed significantly lower chlorophyll content, higher chlorophyll a/b ratio and higher carotenoid/chlorophyll content compared to COA (Fig. 5, Table 1). These characteristics are typical for acclimation to high irradiance (Esteban et al. 2015). Lower chlorophyll content lowers the uptake of light energy (Lei et al. 1996; Murchie and Horton 1997). A high chlorophyll a/b ratio indicates reduced antennae complex size, which limits light absorbance (Bailey et al. 2001). A higher carotenoid-chlorophyll ratio as observed in INT, especially at the end of the drought treatment, suggests increased need for non-photochemical quenching (NPQ), quenching of triplet chlorophylls and scavenging of reactive oxygen species (ROS) (Baquedano and Castillo 2006). Besides these constitutive differences between provenances, a more pronounced decrease of the chlorophyll a/b ratio in response to drought in INT compared to COA indicated by a significant *Provenance x Treatment* effect suggests a provenance-specific regulation of the chlorophyll metabolism (Fig. 8a, Table 1). The physiological function of a decreased chlorophyll a/b ratio under drought is unknown, although it has been previously observed in different tree species including Aleppo pine (*Pinus halapensis* Miller), Phoenician juniper (*Juniperus phoenicea* L.) and beech (*Fagus sylvatica* L.) (Baquedano and Castillo 2006; Garcia-Plazaola and Becerril 2000). The overall decrease in chlorophyll content in all seedlings was likely related to decreasing nitrogen content of the soil over the course of the experiment, as seedlings were last fertilized before the drought treatment to avoid tampering of soil moisture measurements (see Ripullone et al. 2003).

Despite generally higher carotenoid levels in INT compared to COA, both provenances showed equal amounts of xanthophyll cycle pigments (Fig. 6a), which indicated the importance of the xanthophyll cycle for protection of the photosynthetic apparatus from excess light energy (Demmig-Adams et al. 2014; Niyogi 2000). In accordance with our hypothesis of an enhanced demand for photoprotection to compensate for lower photochemical quenching of light energy, INT showed increased xanthophyll cycle pool sizes under drought, while pool sizes remained unchanged in COA (Fig. 6a). This suggested a higher potential for energy dissipation by NPQ, when *A* was reduced (Fig. 8b, see Murchie and Niyogi, 2011). The enhanced demand for NPQ in drought seedlings of both provenances was indicated by an increased de-epoxidation state of the xanthophyll cycle pigments (DEPS; Fig. 6b, Table 1). Although the increase of DEPS was more pronounced in INT, the induction of DEPS in response to reduced assimilation rates under drought was similar in both provenances (Fig. 8c), which indicated a conserved regulation of the xanthophyll cycle.

In accordance with generally higher carotenoid levels in INT compared to COA, we also observed higher amounts of the major isoprenoids β -carotene, neoxanthin and lutein (Fig. 6c-e), which are involved in protection of the photosystem reaction centers from oxidative damage (Nayak et al., 2002; Telfer, 2005), detoxification of superoxide anions (Dall'Osto et al. 2007) and deactivation of triplet chlorophyll (Dall'Osto et al. 2006), respectively. Earlier experiments with the same provenances used in this experiment revealed enhanced amounts of the ROS scavenger α -tocopherol, an non-volatile C₂₀-isoprenoid, in INT compared to COA, which emphasized the enhanced potential for ROS scavenging by non-volatile isoprenoids in INT (Du et al. 2016). The non-significant increase of neoxanthin under drought (Fig. 6d, Table 1) might be a side effect of an enhanced biosynthesis of the xanthophyll cycle pigments, which are also the immediate precursors for the biosynthesis of neoxanthin (Ruiz-Sola and Rodríguez-Concepción 2012). The observed decrease of α -carotene in seedlings of both provenances (Fig. 6f) can be attributed to seasonal variation, as was previously observed in spruce, *Picea abies* (Siefermann-Harms 1994) and is considered an adjustment to high light (Matsubara et al. 2009; Telfer 2005).

The coastal provenance shows enhanced dissipation of energy by the biosynthesis and emission of volatile isoprenoids

Both provenances exhibited substantial variation in amount and composition of stored and emitted mono- and sesquiterpenes (Fig. 7), which are assumed to have antioxidant properties and contribute to the mediation of oxidative stress under abiotic stress (Blande et al. 2014; Loreto and Schnitzler 2010; Possell and Loreto 2013; Vickers et al. 2009). Intraspecific variation in amount and composition of stored monoterpenes was previously observed within species of the genus *Quercus* and *Pinus pinaster*, but could not be related to abiotic stress tolerance (de Simón et al. 2017; Staudt et al. 2004). In the present study, COA showed lower monoterpene content, but much higher monoterpene emissions compared to INT (Fig. 7a,c). The monoterpene contents and emission rates observed in the present work (ca. 10-80 pmol g⁻¹ DW min⁻¹ i.e. ca. 40-320 ng g⁻¹ FW h⁻¹) were well within the range of published data. For example, in a recent study with an interior Douglas fir provenance, Giunta et al. (2016) observed emission rates of 910±430 ng g⁻¹ FW h⁻¹ and contents of which were close to the ones found in the present work (ca. 13 µg g⁻¹ DW). Other studies reported emission rates of 100-5,000 ng g⁻¹ h⁻¹ (Constable et al. 1999; Helmig et al. 2013; Joó et al. 2011; Pressley et al. 2004). Different temperatures during the experiments and genotypic variation most likely contributed to the broad range in emission rates found. Emitted monoterpenes either originate from stored pools or are synthesized *de novo* (Ghirardo et al. 2011; Taipale et al. 2011). In conifer species, namely *Pinus sylvestris* and *Picea abies*, a third to half of emitted monoterpenes were synthesized *de novo* (Ghirardo et al. 2010).

We observed discrepancies between the composition of stored and emitted monoterpenes in both provenances under control and drought conditions, which suggest that substantial amounts of emitted monoterpenes originate from *de novo* biosynthesis (Table 2).

The effects of drought stress on content and composition of volatile isoprenoids is controversially discussed (Niinemets 2016); moderate drought seems to stimulate biosynthesis of these compounds most likely to inhibit accumulation of ROS (Beckett et al. 2012; Sancho-Knapik et al. 2017; Velikova et al. 2016) whereas biosynthesis is rather hampered under severe drought stress (Nogués et al. 2015; Tattini et al. 2015). We did not observe a depletion of monoterpene pool sizes, but rather stable monoterpene emission rates also under drought (Fig. 7a,c). Higher monoterpene emission rates of COA compared to INT thus indicated an enhanced biosynthesis of monoterpenes to substitute emitted monoterpenes. This is in accordance with an earlier study of both provenances, which revealed enhanced biosynthesis of volatile isoprenoids stored in roots of COA, but not INT (Kleiber et al. 2017). In addition, we observed strongly induced sesquiterpene pool sizes in COA compared to only a minor induction in INT (Fig. 7b, Fig. 8d). The strong *Provenance* x *Treatment* interaction effect indicated the involvement of sesquiterpenes in the drought responses of coastal Douglas-fir, but not interior Douglas-fir (Table 1). This indicates provenance-specific variation in the regulation of the cytoplasmic mevalonate pathway that yields sesquiterpenes, which is independent from the plastidal non-mevalonate pathway that yields monoterpenes, tocopherols, and carotenoids (Laule et al. 2003). Although the exact function of sesquiterpenes is still unknown (Palmer-Young et al. 2015), an increased emission of sesquiterpenes in coastal Douglas-fir has been previously observed also in response to heat (Joó et al. 2011).

COA and INT employ different isoprenoid-mediated strategies to cope with drought

INT exhibited lower photochemical quenching of light energy, and in accordance with our hypothesis consequently met an increased demand for NPQ by enhanced pool sizes of non-volatile isoprenoids and especially an increased biosynthesis of xanthophyll cycle pigments under drought (Fig. 5c, Fig. 8b). In contrast, COA showed an enhanced biosynthesis and emission of volatile isoprenoids compared to INT, and especially enhanced biosynthesis of sesquiterpenes under drought (Fig. 7, Fig. 8d). The provenance-specific variation in the employed suit of isoprenoid-mediated energy dissipation pathways implied, that the biosynthesis of non-volatile and volatile isoprenoids was regulated independently, thus revealing the importance of volatile isoprenoids to prevent photooxidative damage as a second line of energy dissipation besides NPQ (Saunier et al. 2017; Wilhelm and Selmar 2011). This is especially interesting, as the emission of volatile isoprenoids implies a loss of previously fixed carbon and

might result in reduced growth (Ryan et al. 2014; Šimpraga et al. 2011). However, we observed even faster growth under drought in COA compared to INT (Fig. 2a) suggesting that higher assimilation rates maintained by COA under drought and the dissipation of excess energy by the biosynthesis and emission of volatile isoprenoids may have entailed each other in this provenance.

Conclusions

Variation in the amount and composition of non-volatile and volatile isoprenoids between Douglas-fir seedlings of an interior and a coastal provenance indicated provenance-specific differences in the employed suit of isoprenoid-mediated energy dissipation pathways. When photochemical quenching of light energy was reduced in response to drought, the interior provenances had increased pool sizes of xanthophyll cycle pigments, while the coastal provenances exhibited strongly induced sesquiterpene pool sizes. We conclude that Douglas-fir provenances employ different isoprenoid-mediated energy dissipation pathways, which might be the result of local adaptation to habitats with contrasting water availability.

ACKNOWLEDGEMENTS

The study was financially supported by the German Science Foundation (DFG, grants EN829/5-1, KR 2010/4-1, GE1090/7-1), the Forest Research Institute of the German State Baden-Württemberg (FVA), and the National Science and Engineering Research Council of Canada (NSERC).

REFERENCES

- Aas, G. 2008. Die Douglasie (*Pseudotsuga menziesii*) in Nordamerika: Verbreitung, Variabilität und Ökologie. LWF Wissen. 59:7-12.
- Aitken, S.N., K.L. Kavanagh and B.J. Yoder. 1995. Genetic variation in seedling water-use efficiency as estimated by carbon isotope ratios and its relationship to sapling growth in Douglas-fir. Forest Genetics. 2:199-206.
- Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. In Second international symposium on information theory Eds. B.N. Petrov and F. Csaki. Akademiai Kiado, Budapest, Hungary, pp 267-281.
- Attia, Z., J.-C. Domec, R. Oren, D.A. Way and M. Moshelion. 2015. Growth and physiological responses of isohydric and anisohydric poplars to drought. Journal of Experimental Botany

- Balaguer, L., E. Martínez-Ferri, F. Valladares, M.E. Pérez-Corona, F.J. Baquedano, F.J. Castillo and E. Manrique. 2001. Population Divergence in the Plasticity of the Response of *Quercus coccifera* to the Light Environment. *Functional Ecology*. 15:124-135.
- Baldwin, I.T. 2010. Plant volatiles. *Current Biology*. 20:392-397.
- Bansal, S., G. Hallsby, M.O. Löfvenius and M.-C. Nilsson. 2013. Synergistic, additive and antagonistic impacts of drought and herbivory on *Pinus sylvestris*: leaf, tissue and whole-plant responses and recovery. *Tree Physiology*. 33:451-463.
- Bansal, S., C.A. Harrington, P.J. Gould and J.B. St.Clair. 2015. Climate-related genetic variation in drought-resistance of Douglas-fir (*Pseudotsuga menziesii*). *Global Change Biology*. 21:947-958.
- Baquedano, F.J. and F.J. Castillo. 2006. Comparative ecophysiological effects of drought on seedlings of the Mediterranean water-saver *Pinus halepensis* and water-spenders *Quercus coccifera* and *Quercus ilex*. *Trees*. 20:689-700.
- Baroli, I. and K.K. Niyogi. 2000. Molecular genetics of xanthophyll-dependent photoprotection in green algae and plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*. 355:1385-1394.
- Beckett, M., F. Loreto, V. Velikova, C. Brunetti, M. Di Ferdinando, M. Tattini, C. Calfapietra and J.M. Farrant. 2012. Photosynthetic limitations and volatile and non-volatile isoprenoids in the poikilochlorophyllous resurrection plant *Xerophyta humilis* during dehydration and rehydration. *Plant, Cell & Environment*. 35:2061-2074.
- Behnke, K., B. Ehlting, M. Teuber, M. Bauerfeind, S. Louis, R. Hänsch, A. Polle, J. Bohlmann and J.-P. Schnitzler. 2007. Transgenic, non-isoprene emitting poplars don't like it hot. *The Plant Journal*. 51:485-499.
- Blande, J.D., J.K. Holopainen and Ü. Niinemets. 2014. Plant volatiles in polluted atmospheres: stress responses and signal degradation. *Plant, Cell & Environment*. 37:1892-1904.
- Bolte, A., C. Ammer, M. Lof, P. Madsen, G.-J. Nabuurs, P. Schall, P. Spathelf and J. Rock. 2009. Adaptive forest management in central Europe: Climate change impacts, strategies and integrative concept. *Scandinavian Journal of Forest Research*. 24:473-482.
- Chartier, P. and J.L. Prioul. 1977. Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO₂ fixation: A critical analysis of the methods used. *Annals of Botany*. 41:789-800.
- Chaves, M.M., J. Flexas and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*. 103:551-560.
- Constable, J.V.H., M.E. Litvak, J.P. Greenberg and R.K. Monson. 1999. Monoterpene emission from coniferous trees in response to elevated CO₂ concentration and climate warming. *Global Change Biology*. 5:252-267.
- Dall'Osto, L., S. Cazzaniga, H. North, A. Marion-Poll and R. Bassi. 2007. The Arabidopsis aba4-1 mutant reveals a specific function for neoxanthin in protection against photooxidative stress. *The Plant Cell Online*. 19:1048-1064.
- Dall'Osto, L., C. Lico, J. Alric, G. Giuliano, M. Havaux and R. Bassi. 2006. Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection in vivo under strong light. *BMC Plant Biology*. 6:32.
- de Simón, B.F., M. Sanz, M.T. Cervera, E. Pinto, I. Aranda and E. Cadahía. 2017. Leaf metabolic response to water deficit in *Pinus pinaster* Ait. relies upon ontogeny and genotype. *Environmental and Experimental Botany*. 140:41-55.

- de Bianchi, S., M. Ballottari, L. Dall'Osto and R. Bassi. 2010. Regulation of plant light harvesting by thermal dissipation of excess energy. *Biochemical Society Transactions*. 38:651-660.
- Demmig-Adams, B. and W.W. Adams. 2006. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytologist*. 172:11-21.
- Demmig-Adams, B., S.-C. Koh, C.M. Cohu, O. Muller, J.J. Stewart and W.W. Adams. 2014. Non-Photochemical Fluorescence Quenching in Contrasting Plant Species and Environments. *In* Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria Eds. B. Demmig-Adams, G. Garab, W. Adams Iii and Govindjee. Springer Netherlands, Dordrecht, pp 531-552.
- Du, B., K. Jansen, A. Kleiber, M. Eiblmeier, B. Kammerer, I. Ensminger, A. Gessler, H. Rennenberg and J. Kreuzwieser. 2016. A coastal and an interior Douglas fir provenance exhibit different metabolic strategies to deal with drought stress. *Tree Physiology*. 36:148-63.
- Duan, B., Y. Lu, C. Yin, O. Junttila and C. Li. 2005. Physiological responses to drought and shade in two contrasting *Picea asperata* populations. *Physiologia Plantarum*. 124:476-484.
- Esteban, R., O. Barrutia, U. Artetxe, B. Fernández-Marín, A. Hernández and J.I. García-Plazaola. 2015. Internal and external factors affecting photosynthetic pigment composition in plants: a meta-analytical approach. *New Phytologist*. 206:268-280.
- Faria, T., D. Silvério, E. Breia, R. Cabral, A. Abadia, J. Abadia, J.S. Pereira and M.M. Chaves. 1998. Differences in the response of carbon assimilation to summer stress (water deficits, high light and temperature) in four Mediterranean tree species. *Physiologia Plantarum*. 102:419-428.
- Ferrell, W.K. and E.S. Woodard. 1966. Effects of Seed Origin on Drought Resistance of Douglas-Fir (*Pseudotsuga Menziesii*) (Mirb.) Franco. *Ecology*. 47:499-503.
- Flexas, J., J. Bota, J. Cifre, J. Mariano Escalona, J. Galmés, J. Gulías, E.-K. Lefi, S. Florinda Martínez-Cañellas, M. Teresa Moreno, M. Ribas-Carbó, D. Riera, B. Sampol and H. Medrano. 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. *Annals of Applied Biology*. 144:273-283.
- García-Plazaola, J.I. and J.M. Becerril. 2000. Effects of drought on photoprotective mechanisms in European beech (*Fagus sylvatica* L.) seedlings from different provenances. *Trees-Structure and Function*. 14:485-490.
- George, J.-P., S. Schueler, S. Karanitsch-Ackerl, K. Mayer, R.T. Klumpp and M. Grabner. 2015. Inter- and intra-specific variation in drought sensitivity in *Abies* spec. and its relation to wood density and growth traits. *Agricultural and Forest Meteorology*. 214-215:430-443.
- Ghirardo, A., J. Gutknecht, I. Zimmer, N. Brüggemann and J.P. Schnitzler. 2011. Biogenic volatile organic compound and respiratory CO₂ emissions after ¹³C-labeling: online tracing of C translocation dynamics in poplar plants. *PLoS One*. 6:e17393.
- Ghirardo, A., K. Koch, R. Taipale, I.N.A. Zimmer, J.-P. Schnitzler and J. Rinne. 2010. Determination of de novo and pool emissions of terpenes from four common boreal/alpine trees by ¹³CO₂ labelling and PTR-MS analysis. *Plant, Cell & Environment*. 33:781-792.

- Giunta, A.D., J.B. Runyon, M.J. Jenkins and M. Teich. 2016. Volatile and within-needle terpene changes to Douglas-fir trees associated with Douglas-fir beetle (*Coleoptera: Curculionidae*) attack. *Environmental Entomology*. 45:920-929.
- Helmig, D., R.W. Daly, J. Milford and A. Guenther. 2013. Seasonal trends of biogenic terpene emissions. *Chemosphere*. 93:35-46.
- Hess, M., H. Wildhagen, L.V. Junker and I. Ensminger. 2016. Transcriptome responses to temperature, water availability and photoperiod are conserved among mature trees of two divergent Douglas-fir provenances from a coastal and an interior habitat. *BMC Genomics*. 17:682.
- Jansen, K., J. Sohr, U. Kohnle, I. Ensminger and A. Gessler. 2013. Tree ring isotopic composition, radial increment and height growth reveal provenance-specific reactions of Douglas-fir towards environmental parameters. *Trees - Structure and Function*. 27:37-52.
- Joó, É., J. Dewulf, C. Amelynck, N. Schoon, O. Pokorska, M. Šimpraga, K. Steppe, M. Aubinet and H. Van Langenhove. 2011. Constitutive versus heat and biotic stress induced BVOC emissions in *Pseudotsuga menziesii*. *Atmospheric Environment*. 45:3655-3662.
- Junker, L.V. and I. Ensminger. 2016. Fast detection of leaf pigments and isoprenoids for ecophysiological studies, plant phenotyping and validating remote-sensing of vegetation. *Physiologia Plantarum*. 158:369-381.
- Junker, L.V., A. Kleiber, K. Jansen, H. Wildhagen, M. Hess, Z. Kayler, B. Kammerer, J.-P. Schnitzler, J. Kreuzwieser, A. Gessler and I. Ensminger. 2017. Variation in short-term and long-term responses of photosynthesis and isoprenoid-mediated photoprotection to soil water availability in four Douglas-fir provenances. 7:40145.
- Kleiber, A., Q. Duan, K. Jansen, L. Verena Junker, B. Kammerer, H. Rennenberg, I. Ensminger, A. Gessler and J. Kreuzwieser. 2017. Drought effects on root and needle terpenoid content of a coastal and an interior Douglas fir provenance. *Tree Physiology*. 37:1648-1658.
- Laule, O., A. Fürholz, H.-S. Chang, T. Zhu, X. Wang, P.B. Heifetz, W. Gruissem and M. Lange. 2003. Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*. 100:6866-6871.
- Lavender, D.P. and R.K. Hermann. 2014. Douglas-Fir: The Genus *Pseudotsuga*. OSU College of Forestry, Corvallis, OR.
- Lei, T.T., R. Tabuchi, M. Kitao and T. Koike. 1996. Functional relationship between chlorophyll content and leaf reflectance, and light-capturing efficiency of Japanese forest species. *Physiologia Plantarum*. 96:411-418.
- Lobo, F.d.A., M.P. de Barros, H.J. Dalmagro, Â.C. Dalmolin, W.E. Pereira, É.C. de Souza, G.L. Vourlitis and C.E. Rodríguez Ortiz. 2013. Fitting net photosynthetic light-response curves with Microsoft Excel — a critical look at the models. *Photosynthetica*. 51:445-456.
- Loreto, F., A. Förster, M. Dürr, O. Csiky and G. Seufert. 1998. On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant, Cell & Environment*. 21:101-107.
- Loreto, F., P. Pinelli, F. Manes and H. Kollist. 2004. Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves. *Tree Physiol*. 24:361-7.

- Loreto, F. and J.-P. Schnitzler. 2010. Abiotic stresses and induced BVOCs. *Trends in Plant Science*. 15:154-166.
- Martínez-Vilalta, J. and N. García-Forner. 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. *Plant, Cell & Environment*. 40:962-976.
- Matsubara, S., G.H. Krause, J. Aranda, A. Virgo, K.G. Beisel, P. Jahns and K. Winter. 2009. Sun-shade patterns of leaf carotenoid composition in 86 species of neotropical forest plants. *Functional Plant Biology*. 36:20-36.
- McCulloh, K.A., D.M. Johnson, F.C. Meinzer and D.R. Woodruff. 2014. The dynamic pipeline: hydraulic capacitance and xylem hydraulic safety in four tall conifer species. *Plant, Cell & Environment*. 37:1171-1183.
- McDowell, N.G. 2011. Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. *Plant Physiology*. 155:1051-1059.
- Montwé, D., H. Spiecker and A. Hamann. 2015. Five decades of growth in a genetic field trial of Douglas-fir reveal trade-offs between productivity and drought tolerance. *Tree Genetics & Genomes*. 11:1-11.
- Munekage, Y., M. Hojo, J. Meurer, T. Endo, M. Tasaka and T. Shikanai. 2002. PGR5 Is Involved in Cyclic Electron Flow around Photosystem I and Is Essential for Photoprotection in Arabidopsis. *Cell*. 110:361-371.
- Murchie, E.H. and P. Horton. 1997. Acclimation of photosynthesis to irradiance and spectral quality in British plant species: chlorophyll content, photosynthetic capacity and habitat preference. *Plant, Cell & Environment*. 20:438-448.
- Murchie, E.H. and K.K. Niyogi. 2011. Manipulation of Photoprotection to Improve Plant Photosynthesis. *Plant Physiology*. 155:86-92.
- Niinemets, Ü. 2016. Uncovering the hidden facets of drought stress: secondary metabolites make the difference. *Tree Physiology*. 36:129-132.
- Niyogi, K.K. 2000. Safety valves for photosynthesis. *Current Opinion in Plant Biology*. 3:455-460.
- Nogués, I., M. Medori and C. Calfapietra. 2015. Limitations of monoterpene emissions and their antioxidant role in *Cistus* sp. under mild and severe treatments of drought and warming. *Environmental and Experimental Botany*. 119:76-86.
- Palmer-Young, E.C., D. Veit, J. Gershenzon and M.C. Schuman. 2015. The Sesquiterpenes(E)- β -Farnesene and (E)- α -Bergamotene Quench Ozone but Fail to Protect the Wild Tobacco *Nicotiana attenuata* from Ozone, UVB, and Drought Stresses. *PLoS One*. 10:e0127296.
- Peguero-Pina, J.J., D. Sancho-Knapik, F. Morales, J. Flexas and E. Gil-Pelegrin. 2009. Differential photosynthetic performance and photoprotection mechanisms of three Mediterranean evergreen oaks under severe drought stress. *Functional Plant Biology*. 36:453-462.
- Peñuelas, J. and S. Munné-Bosch. 2005. Isoprenoids: an evolutionary pool for photoprotection. *Trends in Plant Science*. 10:166-169.
- Pogson, B.J., K.K. Niyogi, O. Björkman and D. DellaPenna. 1998. Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in Arabidopsis mutants. *Proceedings of the National Academy of Sciences of the United States of America*. 95:13324-13329.

- Poorter, H., Ü. Niinemets, L. Poorter, I.J. Wright and R. Villar. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*. 182:565-588.
- Possell, M. and F. Loreto. 2013. The Role of Volatile Organic Compounds in Plant Resistance to Abiotic Stresses: Responses and Mechanisms. *In* *Biology, Controls and Models of Tree Volatile Organic Compound Emissions* Eds. Ü. Niinemets and R.K. Monson. Springer Netherlands, pp 209-235.
- Pressley, S., B. Lamb, H. Westberg, A. Guenther, J. Chen and E. Allwine. 2004. Monoterpene emissions from a Pacific Northwest Old-Growth Forest and impact on regional biogenic VOC emission estimates. *Atmospheric Environment*. 38:3089-3098.
- R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ramírez-Valiente, J.A., K. Koehler and J. Cavender-Bares. 2015. Climatic origins predict variation in photoprotective leaf pigments in response to drought and low temperatures in live oaks (*Quercus* series *Virentes*). *Tree Physiology*. 35:521-534.
- Ripullone, F., G. Grassi, M. Lauteri and M. Borghetti. 2003. Photosynthesis–nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus × euroamericana* in a mini-stand experiment. *Tree Physiology*. 23:137-144.
- Ruiz-Sola, M.Á. and M. Rodríguez-Concepción. 2012. Carotenoid Biosynthesis in *Arabidopsis*: A Colorful Pathway. *The Arabidopsis Book*. 10:e0158.
- Ryan, A.C., C.N. Hewitt, M. Possell, C.E. Vickers, A. Purnell, P.M. Mullineaux, W.J. Davies and I.C. Dodd. 2014. Isoprene emission protects photosynthesis but reduces plant productivity during drought in transgenic tobacco (*Nicotiana tabacum*) plants. *New Phytologist*. 201:205-216.
- Sancho-Knapik, D., M.Á. Sanz, J.J. Peguero-Pina, Ü. Niinemets and E. Gil-Pelegrín. 2017. Changes of secondary metabolites in *Pinus sylvestris* L. needles under increasing soil water deficit. *Annals of Forest Science*. 74:24.
- Saunier, A., E. Ormeño, H. Wortham, B. Temime-Roussel, C. Lecareux, C. Boissard and C. Fernandez. 2017. Chronic Drought Decreases Anabolic and Catabolic BVOC Emissions of *Quercus pubescens* in a Mediterranean Forest. *Frontiers in Plant Science*. 8
- Schindler, U., W. Durner, G. von Unold, L. Mueller and R. Wieland. 2010. The evaporation method: Extending the measurement range of soil hydraulic properties using the air-entry pressure of the ceramic cup. *Journal of Plant Nutrition and Soil Science*. 173:563-572.
- Scholander, P.F., H.T. Hammel, E.D. Bradstreet and E.A. Hemmingsen. 1965. Sap Pressure in Vascular Plants. *Science*. 148:339-346.
- Sergent, A.-S., N. Bréda, L. Sanchez, J.-C. Bastein and P. Rozenberg. 2014. Coastal and interior Douglas-fir provenances differ in growth performance and response to drought episodes at adult age. *Annals of Forest Science*. 71:709-720.
- Sieffermann-Harms, D. 1994. Light and temperature control of season-dependent changes in the α - and β -carotene content of spruce needles. *Journal of Plant Physiology*. 143:488-494.
- Šimpraga, M., H. Verbeeck, M. Demarcke, É. Joó, O. Pokorska, C. Amelynck, N. Schoon, J. Dewulf, H. Van Langenhove, B. Heinesch, M. Aubinet, Q. Laffineur, J.F. Müller and K. Steppe. 2011. Clear link between drought stress, photosynthesis and biogenic volatile organic compounds in *Fagus sylvatica* L. *Atmospheric Environment*. 45:5254-5259.
- St Clair, B.J. and G.T. Howe. 2007. Genetic maladaptation of coastal Douglas-fir seedlings to future climates. *Global Change Biology*. 13:1441-1454.

- Staudt, M., C. Mir, R. Joffre, S. Rambal, A. Bonin, D. Landais and R. Lumaret. 2004. Isoprenoid emissions of *Quercus* spp. (*Q. suber* and *Q. ilex*) in mixed stands contrasting in interspecific genetic introgression. *New Phytologist*. 163:573-584.
- Taipale, R., M.K. Kajos, J. Patokoski, P. Rantala, T.M. Ruuskanen and J. Rinne. 2011. Role of de novo biosynthesis in ecosystem scale monoterpene emissions from a boreal Scots pine forest. *Biogeosciences*. 8:2247-2255.
- Tattini, M., F. Loreto, A. Fini, L. Guidi, C. Brunetti, V. Velikova, A. Gori and F. Ferrini. 2015. Isoprenoids and phenylpropanoids are part of the antioxidant defense orchestrated daily by drought-stressed *Platanus × acerifolia* plants during Mediterranean summers. *New Phytologist*. 207:613-626.
- Telfer, A. 2005. Too much light? How b-carotene protects the photosystem II reaction centre. *Photochemical & Photobiological Sciences*. 4:950-956.
- Velikova, V., C. Brunetti, M. Tattini, D. Doneva, M. Ahrar, T. Tsonev, M. Stefanova, T. Ganeva, A. Gori, F. Ferrini, C. Varotto and F. Loreto. 2016. Physiological significance of isoprenoids and phenylpropanoids in drought response of Arundinoideae species with contrasting habitats and metabolism. *Plant, Cell & Environment*. 39:2185-2197.
- Velikova, V., T.D. Sharkey and F. Loreto. 2012. Stabilization of thylakoid membranes in isoprene-emitting plants reduces formation of reactive oxygen species. *Plant signaling & behavior*. 7:139-141.
- Velikova, V., Z. Várkonyi, M. Szabó, L. Maslenkova, I. Nogues, L. Kovács, V. Peeva, M. Busheva, G. Garab, T.D. Sharkey and F. Loreto. 2011. Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. *Plant Physiology*. 157:905-916.
- Vickers, C.E., J. Gershenzon, M.T. Lerdaun and F. Loreto. 2009. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biology*. 5:283-291.
- Warren, C.R., N.J. Livingston and D.H. Turpin. 2003. Responses of gas exchange to reversible changes in whole-plant transpiration rate in two conifer species. *Tree Physiology*. 23:793-803.
- Wilhelm, C. and D. Selmar. 2011. Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. *Journal of Plant Physiology*. 168:79-87.
- Wujeska, A., G. Bossinger and M. Tausz. 2013. Responses of foliar antioxidative and photoprotective defence systems of trees to drought: a meta-analysis. *Tree Physiology*.
- Zhang, J.W. and J.D. Marshall. 1995. Variation in Carbon Isotope Discrimination and Photosynthetic Gas Exchange Among Populations of *Pseudotsuga menziesii* and *Pinus ponderosa* in Different Environments. *Functional Ecology*. 9:402-412.
- Zhang, X., R. Zang and C. Li. 2004. Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. *Plant Science*. 166:791-797.
- Zuur, A.F. 2009. Mixed effects models and extensions in ecology with R. Statistics for biology and health. Springer, New York [etc.].

FIGURE LEGENDS

Fig. 1: Soil water content and drought stress of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Volumetric soil water content and B) predawn twig water potential (Ψ). Data show mean of $n = 3$ measurements ($\pm$ SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) between provenances and treatments are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

Fig. 1: Soil water content and predawn twig water potential

Fig. 2: Growth and needle morphology of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Biomass, B) leaf mass per area (LMA). Data show mean of $n = 5$ measurements ($\pm$ SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) between provenances and treatments are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

Fig. 2: Carbon allocation

Fig. 3: Gas exchange parameters of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Stomatal conductance (g_s), and B) assimilation rate (A). Data show mean of $n = 5$ measurements ($\pm$ SE) taken at a light intensity of $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) between provenances and treatments are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

Fig. 3: Gas exchange parameters

935 **Fig. 4:** Light response curve of interior (INT) and coastal (COA) Douglas-fir seedlings under
936 control and drought conditions taken on A) day 0, B) day 41, and C) day 56. Data show mean of
937 $n = 3-5$ measurements ($\pm$ SE). Significant differences ($p < 0.05$ of the maximum assimilation rate
938 using Kruskal-Wallis-rank-sum-test) between provenances and treatments are indicated by
939 different letters.

Fig. 4: Light curves

Fig. 5: Chlorophylls and carotenoids of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Total chlorophylls (Chlorophyll content per dry mass), B) chlorophyll a to chlorophyll b ratio (Chl a Chl b^{-1}) and C) carotenoids per total chlorophyll ($\text{Carotenoids Chl}^{-1}$). Data show mean of $n = 5$ samples ($\pm \text{SE}$). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) between provenances and treatments are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

Fig. 5: Total chlorophylls and carotenoids

949 **Fig. 6:** Non-volatile isoprenoids of interior (INT) and coastal (COA) Douglas-fir under control
 950 and drought conditions. A) Xanthophyll cycle pigments per chlorophylls (VAZ Chl^{-1}), B) de-
 951 epoxidation state of the xanthophyll cycle (DEPS, calculated as $(0.5A+Z) (V+A+Z)^{-1}$), C)
 952 β -carotene per chlorophylls, D) neoxanthin per chlorophylls, E) lutein per chlorophylls and F)
 953 α -carotene per chlorophylls. Data show mean of $n = 5$ samples ($\pm$ SE). Significant differences
 954 ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) between provenances and treatments are indicated
 955 by different letters. Light grey background indicates period of reduced watering, dark grey
 956 background indicates period of withheld watering, followed by rewatering.

Fig. 6: Carotenoids per Chl

Fig 7: Volatile isoprenoids of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) stored volatile monoterpenes (monoterpene content per dry mass), B) stored volatile sesquiterpenes (sesquiterpene content per dry mass) and C) monoterpene emission. Data show mean of $n = 5$ samples ($\pm$ SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) between provenances and treatments are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

Fig. 7: Volatile isoprenoid pools and emissions

Fig. 8: Adjustments of isoprenoids involved in energy dissipation pathways in response to decreasing assimilation rate (A). A) Chlorophyll a/ b ratio (Chl a Chl b⁻¹) B) xanthophyll cycle pigments per total carotenoids (VAZ Chl⁻¹), C) de-epoxidation state of the xanthophyll cycle (DEPS), D) stored volatile sesquiterpenes in relation to assimilation rate (A). Each data point represents mean of $n = 5$ measurements ($\pm$ SE) taken per sampling day. Lines represent a linear regression fit per provenance across seedlings of the control (closed circles) and drought treatment (open circles). R^2 -values indicate the coefficient of determination per provenance, p -values indicate significant differences estimated by ANOVA comparison of linear models describing the independent variable interaction, n.s. denotes non-significant differences. A was measured at a light intensity of $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Fig. 8: Treatment-induced adjustments of isoprenoids

Table 1: The effect of provenance and treatment on biomass, photosynthetic gas exchange and isoprenoid metabolism. For each parameter, the best model including Provenance, Treatment and the interaction thereof was chosen according to the Akaike information criterion (AIC). The effects of Treatment and Provenance were assessed by pairwise comparison of models with and without each factor using a log-likelihood ratio test. Significance levels are given as p-values, which are bolded if $p < 0.05$.

Biomass = total above- and belowground dry mass of seedlings, LMA = leaf mass per area of current-year needles, g_s = stomatal conductance, A = assimilation rate. Chlorophylls a+b = total chlorophyll per dry mass, Carotenoids per Chl = total carotenoids per chlorophylls, VAZ per Chl = xanthophyll cycle pigments per chlorophylls, DEPS = de-epoxidation statue of the xanthophyll cycle pigments, β -carotene per Chl = β -carotene per chlorophylls, Neoxanthin per Chl = Neoxanthin per chlorophylls, Lutein per Chl = Lutein per total chlorophylls, α -carotene per Chl = α -carotene per chlorophylls, Stored monoterpenes = total stored monoterpene content per dry mass, stored sesquiterpenes = total stored sesquiterpene content per dry mass, Emission of monoterpenes = total monoterpene emissions.

	Parameter	Model best describing Response	p-values		
			Provenance	Treatment	Interaction
Morphology	Biomass	Provenance	0.0000	NA	NA
	LMA	Treatment	NA	0.0073	NA
Gas exchange	g_s	Provenance + Treatment	0.0075	0.0000	NA
	A	Provenance + Treatment	0.1179	0.0000	NA
Non-volatile isoprenoids	Chlorophyll a + b	Provenance + Treatment	0.0000	0.0138	NA
	Chlorophyll a/ b ratio	Provenance x Treatment	0.0000	0.0002	0.1528
	Carotenoids per Chl	Provenance	0.0000	NA	NA
	VAZ per Chl	Provenance x Treatment	0.0443	0.1244	0.0900
	DEPS per Chl	Treatment	NA	0.0088	NA
	β -carotene per Chl	Provenance	0.0001	NA	NA
	Neoxanthin per Chl	Provenance + Treatment	0.0000	0.1261	NA
	Lutein per Chl	Provenance	0.0000	NA	NA
	α -carotene per Chl	-	NA	NA	NA
	Stored monoterpenes	Provenance	0.0483	NA	NA
Volatile isoprenoids	Stored sesquiterpenes	Provenance x Treatment	0.0188	0.0022	0.0196
	Emission of monoterpenes	Provenance	0.0002	NA	NA

Table 2: Composition of volatile isoprenoid pools and emissions. The percentage of the five most abundant stored monoterpenes and sesquiterpenes as well as emitted monoterpenes per provenance are listed. For control plants data from all samplings was averaged (N=15, $\pm$ SE), while drought plants exclude samples taken after recovery (N=10, $\pm$ SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) are indicated by different letters.

Composition of volatile isoprenoid pools and emissions (%)					
		INT		COA	
		Control	Drought	Control	Drought
Pools					
Monoterpenes	α -pinene	23.3 $\pm$ 0.3 ^a	24.7 $\pm$ 0.6 ^a	18.8 $\pm$ 0.4 ^b	19.2 $\pm$ 0.7 ^b
	β -pinene	10.3 $\pm$ 1.7 ^a	12.1 $\pm$ 1.8 ^a	56.3 $\pm$ 2.5 ^b	58.9 $\pm$ 2.0 ^b
	Camphene	50.5 $\pm$ 1.7 ^a	46.5 $\pm$ 2.4 ^a	5.7 $\pm$ 2.1 ^b	2.4 $\pm$ 0.4 ^b
	3-carene	0.6 $\pm$ 0.1 ^a	1.2 $\pm$ 0.5 ^a	4.9 $\pm$ 0.7 ^b	4.6 $\pm$ 0.7 ^b
	Limonene	3.0 $\pm$ 0.2 ^a	2.9 $\pm$ 0.3 ^{ab}	2.1 $\pm$ 0.1 ^b	1.9 $\pm$ 0.2 ^{ab}
Sesquiterpenes	α -caryophyllene	31.7 $\pm$ 3.8	36.2 $\pm$ 4.0	32.2 $\pm$ 3.0	30.5 $\pm$ 2.3
	β -caryophyllene	8.6 $\pm$ 2.4 ^a	7.2 $\pm$ 2.1 ^{ab}	13.1 $\pm$ 1.4 ^{ab}	15.5 $\pm$ 1.6 ^b
	β -elemene	22.8 $\pm$ 3.9	25.5 $\pm$ 4.1	21.2 $\pm$ 2.4	23.2 $\pm$ 1.1
	δ -elemene	16.4 $\pm$ 2.3	16.4 $\pm$ 2.6	11.4 $\pm$ 2.0	18.5 $\pm$ 2.3
	(+)-longifolene	17.1 $\pm$ 7.5 ^a	7.3 $\pm$ 2.1 ^a	5.2 $\pm$ 2.1 ^{ab}	0.0 $\pm$ 0.0 ^b
Emissions					
Monoterpenes	α -pinene	28.2 $\pm$ 7.3	25.7 $\pm$ 5.5	26.5 $\pm$ 3.5	34.1 $\pm$ 9.6
	β -pinene	5.7 $\pm$ 2.5	17.9 $\pm$ 5.4	22.8 $\pm$ 5.1	21.6 $\pm$ 6.2
	Camphene	10.5 $\pm$ 4.7 ^{ab}	4.1 $\pm$ 5.2 ^a	3.4 $\pm$ 1.2 ^b	1.6 $\pm$ 0.6 ^b
	3-carene	9.4 $\pm$ 4.5	9.6 $\pm$ 11.7	13.1 $\pm$ 3.5	8.4 $\pm$ 4.4
	Limonene	8.9 $\pm$ 4.3	17.1 $\pm$ 31.4	13.4 $\pm$ 6.9	17.7 $\pm$ 10.5