(Demmig-Adams & Adams, 2006; Fréchette *et al.*, 2015). The deepoxidation of violaxanthin via antheraxanthin to zeaxanthin in response to excess light energy provides an instantaneous mechanism to facilitate NPQ, while the pool size of the xanthophyll cycle pigments is adjusted on the long-term and determines the photoprotective capacity (Niinemets *et al.*, 1998; Demmig-Adams & Adams, 2006). Lutein is important for correct folding of the antennae proteins, involved in light harvesting (Jahns & Holzwarth, 2012) and can protect plants from photooxidative stress by the deactivation of triplet chlorophyll (Dall'Osto *et al.*, 2006). Neoxanthin is another xanthophyll involved in protection of PSII from oxidation by detoxifying superoxide anions (O_2^-) (Dall'Osto *et al.*, 2007a). While the abovementioned xanthophylls are bound to the antenna complexes of the photosystems, β-carotene protects the reaction centers of the photosystems from oxidative damage (Nayak et al., 2002; Telfer, 2005). Some plant species additionally biosynthesize α-carotene which occurs especially in shade-tolerant species (Matsubara et al., 2009) and also varies seasonally (Ottander et al., 1995; Siefermann-Harms, 1994). Furthermore, carotenoids are important antioxidants for scavenging of ROS (Esteban *et al.*, 2015b).

Although the composition of the photosynthetic apparatus is remarkably consistent among species (Pogson *et al.*, 1998), adjustments of the photoprotective isoprenoids contribute to the acclimation of plants to different environmental conditions (Esteban *et al.*, 2015a). Under drought, when photochemical quenching of light energy is reduced and the demand for NPQ is enhanced, increased carotenoid-to-chlorophyll ratio have been observed in many species, e.g. in mediterranean tree species, and the spruce *Picea asperata* (Faria *et al.*, 1998; Duan *et al.*, 2005). Furthermore, increased pool sizes of xanthophyll cycle pigments have been observed in response to drought in many species, including many forest tree species (Wujeska *et al.*, 2013). Other plant species also exhibit increased amounts of lutein in response to drought (Esteban *et al.*, 2015a).

Many plant species also produce non-essential volatile isoprenoids, consisting of hemi-, mono- and sesquiterpenes, that are involved in mitigation of abiotic stress (Peñuelas & Munné-Bosch, 2005). While some volatile isoprenoids can be stored within specialized leaf structures, others are emitted immediately after their biosynthesis (Ghirardo *et al.*, 2010). Volatile isoprenoids are thought to enhance abiotic stress tolerance by the use of excess energy during biosynthesis, stabilization of membranes and their antioxidant function (Possell & Loreto, 2013; Palmer-

Young *et al.*, 2015). The emission of volatile isoprenoids occurs constitutively as well as induced by abiotic or biotic stress conditions (Loreto *et al.*, 1998; Peñuelas *et al.*, 2005). Emitted volatile isoprenoid emissions thus partly originate from pools of stored isoprenoids and partly originate from *de novo* synthesis (Ghirardo *et al.*, 2011).

Intraspecific differences in photoprotective mechanisms in response to drought might be involved in variation of drought responses among tree provenances originating from contrasting habitats. Nevertheless, evidence for intraspecific variation in photoprotective mechanisms is scarce. Intraspecific differences in chlorophylls and energy quenching have been observed in two *Picea asperata* populations from contrasting climates (Duan *et al.*, 2005). In contrast, provenances from *Quercus coccifera L.* varied in energy quenching, but did not differ in photosynthetic pigments (Balaguer *et al.*, 2001). Intraspecific variation in isoprenoid-mediated photoprotective mechanisms among Douglas-fir provenances was suggested by a study with field-grown mature Douglas-fir trees of four provenances (Junker et al., unpublished). Seedlings of the two provenances used in this experiment have been shown to differ in levels of antioxidants ascorbate and α-tocopherol induced by drought (Du *et al.*, 2016). Intraspecific variation in the regulation of stomatal conductance and photoprotective isoprenoids in response to drought might reveal different strategies to cope with drought stress among provenances and identify traits which contribute to drought and could thus facilitate the selection of provenances suitable to grow under future climate conditions.

The aim of this study was to assess if two Douglas-fir provenances, that originate from contrasting habitats, vary in isoprenoid.mediated photoprotective mechanisms and induction thereof in response to drought. Photosynthetic gas exchange, photosynthetic pigments as well as volatile isoprenoid pools and emission were studied in seedlings of an interior and a coastal provenance exposed to limited watering for six weeks, followed by a two week recovery phase in comparison to well-watered control seedlings. Seedlings from both provenances are expected to reduce photosynthetic gas exchange in response to limited soil water availability to avoid water loss. Consequently, we expect an induction of isoprenoid-mediated photoprotective mechanisms to mitigate photooxidative stress. We hypothesized that interior seedlings will show lower stomatal conductance to minimize water loss which is needed to withstand prolonged drought periods which are frequently occurring in their natural habitat. Consequently, we expect enhanced NPQ of excess energy by essential isoprenoids in the interior provenance, to combat

lower photochemical quenching of light energy. In contrast, the coastal provenance is thought to sustain higher assimilation rates at the expense of higher water loss, but a lower need for photoprotective mechanisms. We furthermore expect that provenances differ in the photoprotective mechanisms mediated by volatile isoprenoids, because they are under much less selective pressure compared to essential isoprenoids. Nevertheless, differences in capacity of the antioxidant machinery, as previously observed for the provenances studied in our experiment, may reduce the demand for enhanced photoprotection mediated by essential isoprenoids or enhanced accumulation and emission of non-essential volatile isoprenoids.

3.3 Materials and Methods

3.3.1 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. Seedlings of Snoqualmie were grown from seeds collected from a group of trees in seed zone Snoqualmie, Washington (Zone 412 of the Tree Seed Zone Map, 1973). Seedlings of Fehr Lake were grown from seed collected in a seed orchard, which was established using seeds collected in the respective seed zones. Upon arrival in March/ April, seedlings were planted in 3l pots with medium-fibrous peat soil (Container substrate 1 medium + GreenFibre basic, pH = 5.3; Klasmann-Deilmann GmbH, Geeste, Germany) and fertilized with NPK fertilizer (N170 + P200 + K230 + Mg100 + S150 mg l^{-1}).

3.3.2 Growth conditions

The experiment was carried out at the Leibniz Centre for Agricultural Landscape Research in Müncheberg, Germany from June to September of 2011. 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 µmol m^{-2} s^{-1} for 16 h per day. Seedlings were randomly distributed among both chambers and watered daily with 100 ml water (50 % tap water, 50 % distilled water). The drought treatment for half of the seedlings per chamber started after more than three months of acclimation under growth conditions as mentioned above on July 20th after the second flush in most seedlings of COA and some seedlings of INT had occurred.

3.3.3 Control and drought treatment

Control seedlings were watered daily from July 20th (day 0) onwards with 200 ml water, while watering of the seedlings of the drought treatment was only 65 ml until August 10th (day 21). From August 10 to August 31 watering was entirely withheld. On the evening of August 31 (day 42), seedlings of the drought treatment were rewatered generously. 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 availability of control and drought stress seedlings. Soil moisture tension was calculated according to Schindler et al. (2010). COA seedlings consistently required about ten percent more water in order to keep soil moisture comparable to interior seedlings.

Pre-dawn twig water potential was monitored throughout the experiment on days 7, 17, 26, 36, 42, 43 and 52 using twigs of N=3 seedlings. Twig water potential was determined using a pressure chamber (Model 3015G4, Soil moisture Equipment Corp., Santa Barbara, CA, USA) according to Scholander *et al*. (1965).

3.3.4 Sampling

Samples of current-year needles (excluding second flush) were taken prior to the drought treatment on June 28th (shown as day 0), and on days 28, 41 (drought), 56 (rewatered) for the determination of photosynthetic pigments and stored volatile isoprenoids. Needles were cut from the twigs and immediately frozen in liquid nitrogen. Samples were stored at -80°C and ground immersed in liquid nitrogen using mortar and pestle.

3.3.5 Photosynthesis measurement

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 CO_2 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 CO_2 gas exchange were taken at 0, 400, 500, 1000, 1500 and 2000 µmol photons m^{-2} s^{-1} light intensity after a minimum of 5 min acclimation time at each light intensity. Light response curves were modelled using a nonlinear least square fit (function *nls*; Chambers and Bates, 1992) of the function y=A*(1-exp(-b*L))+R with A = maximum net photosynthesis rate, b = coefficient of curvature, L = light intensity, and R = rate of respiration. Maximum photosynthetic rate (A_{max}) was calculated as A_{max}= A+R, light compensation point (I_0) as I_0=-1/b*ln((1+R)*A^{-1}) and light intensity at half-maximum rate of photosynthesis (I1/2) as I1/2=-1/b*ln(0.5+0.5 A*R^{-1}). Light exposed needle surface area was then 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. Intrinsic water-use efficiency was calculated as the ratio of net CO_2 assimilation rate to stomatal conductance (IWUE= A/g_s).

3.3.6 Analysis of photosynthetic pigments

Pigments 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 290 nm, 450 nm and 656 nm wavelength) was used for reverse-phase chromatography using a C_{30}-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). Standards for antheraxanthin, α-carotene, lutein, neoxanthin, violaxanthin and zeaxanthin were obtained from DHI Lab products (Hørsholm, Denmark). ChemStation B.04.03 software (Agilent Technologies, Böblingen, Germany) was used for peak integration.

3.3.7 Sampling of emitted volatile isoprenoids

Emitted volatile isoprenoids of current-year samples were collected on day 21, 25 (drought) and 53 (rewatered) independent from other measurements using customized one liter glass enclosure consisting of a lower half with a wide opening to insert seedlings and an upper half which was set atop enclosing the whole seedling without physical contact. To avoid any effects from young second-flush needles, second-flush twigs were removed two days prior to the start of volatile isoprenoid sampling (day 19). One day prior to the measurements, seedlings were carefully inserted into the lower parts of the enclosures to avoid a 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 CO_2. 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 analysis. To determine the needle mass in the enclosure, needles within the enclosure were sampled after the end of the experiment and their dry weight was determined. Monoterpene emission rates were calculated per dry weight and over time. Zero references using an empty cuvette were used to correct for background emission.

3.3.8 Extraction of stored volatile isoprenoids

Volatile isoprenoids stored in sampled needles were extracted using 500 µl 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 (pure 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 TDU-CIS and GC-EI/MS.

3.3.9 Volatile isoprenoid analysis

Emitted and stored volatile isoprenoids were analysed using gas chromatograph (GC, model 7890A, Agilent, Germany), coupled to a mass-selective detector (MS, 5975C, Agilent, Germany) and equipped with a thermodesorption/cold injection system (TDU-CIS; Gerstel, Germany) according to Müller et al. (2013). Cartridges/ Twisters ® were desorbed with the TDU at 240°C, cryofocused at -100°C and heated to 240°C in the CIS prior to injection into the GC-MS: Separation on a DB-624 column (Agilent, Germany) occurred during an oven temperature programme beginning at 40 °C, increasing at a rate of 6 °C min^{-1} for 3 min to 100 °C, when the

temperature ramp speed up to 16 °C min^{-1} until the column reached 230 °C. Volatile isoprenoids were identified by comparison of peaks and de-convoluted fragmentation spectra to external standards and to the NIST database using the AMDIS software (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA). Monoterpenes include camphene, (+)-carene, 3-carene, limonene, β-myrcene, ocimene, β-phellandrene, α-pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, and tricyclene. Sesquiterpenes include γ-cadinene, α-caryophyllene, β-caryophyllene, α-cubebene, β-elemene, δ-elemene, and (+)-longifolene. Conversion from fresh to dry weight was determined by drying approximately 10 g of frozen needles for 4 h at 200 °C.

3.3.10 Statistics

All statistical tests were performed using R 3.0.3 (R Development Core Team, 2010). A linear mixed-effect model using *Provenance* and *Treatment* as fixed factors and *sampling day* as random factor were used to evaluate if provenance or treatment specific differences occurred during the drought stress (function *lmer*, package *lme4*, Bates et al. 2013). Models using *Provenance*, *Treatment*, *Provenance* + *Treatment* and *Provenance* x *Treatment* were compared to a null model considering only an intercept and *sampling day* as random factor. Based on lowest Akaike Information Criterion (AIC; Akaike 1973) the best-fit model was chosen. Significance of the fixed factors was assessed by a pairwise comparison of the best-fit model to the best-fit model minus one of each of its fixed effects (function *anova*, Zuur 2009).

Differences between provenances and treatments on each sampling day were determined using the parametric Kruskal-Wallis-rank-rum-Test (function *kruskal.test*) followed by a Tukey-type pairwise comparison using the function *nparcomp* (package *nparcomp*). 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 3.3.

Differences in responses between provenances as shown in Figures 8 and 9 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*). It must be noted that gas exchange parameters were repeatedly assessed from the same set of plants. Therefore, provenance-specific effects may be overestimated. Nevertheless, we

consider our approach valid, since the only parameter which revealed provenance-specific differences in response to the gas exchange parameter assimilation, sesquiterpenes, revealed an obvious difference.

3.4 Results

3.4.1 Water availability and plant growth

For control seedlings, a target soil water tension (pF) of 2.4 log(-ψ(cm H_2O)) was maintained, corresponding to 37% volumetric soil water content (Fig. 3.1a). For drought-stressed seedlings, pF increased to a maximum of 7.7 log(-ψ(cm H_2O)) in both provenances by day 42, which corresponds to a volumetric water content as low as 2 %. Seedlings of the coastal provenance (COA) were watered with about 10 % more of the amount which seedlings of the interior provenance (INT) received, to keep soil water availability comparable between both provenances and be able to compare adjustments of photoprotective isoprenoids between provenances despite differences in water use. When seedlings were rewatered on the evening of day 42, soil water tension dropped to control levels within one day. Predawn twig water potential of drought stressed seedlings of both provenances decreased only after day 36, with lowest water potential of -2.4 MPa in INT and -2.7 MPa in COA on day 42 (Fig. 3.1b). After rewatering, twig water potential of drought stressed seedlings recovered to control plant levels within one day.

INT seedlings had an initially higher total biomass of 18.6±0.5 g dry weight (DW) compared to COA with only 6.0±0.9 g DW. During the control treatment, COA gained 16.5 g biomass and INT gained 13.0 g biomass (Fig. 3.2a). Biomass gain of both provenances was reduced under drought and still delayed after rewatering, resulting in a total biomass gain of 8.1 g in COA drought seedlings and 3.5 g in INT drought seedlings. Leaf mass per area (LMA) showed a slight increase in control seedlings of both provenances (Fig. 3.2b). Needles of INT seedlings were thicker and more rigid compared to needles of COA, which is expressed in a slightly higher LMA (Fig. 3.2b). LMA of both provenances were significantly lower during the drought treatment (Table 3.1), but were comparable to control seedlings two weeks after rewatering.

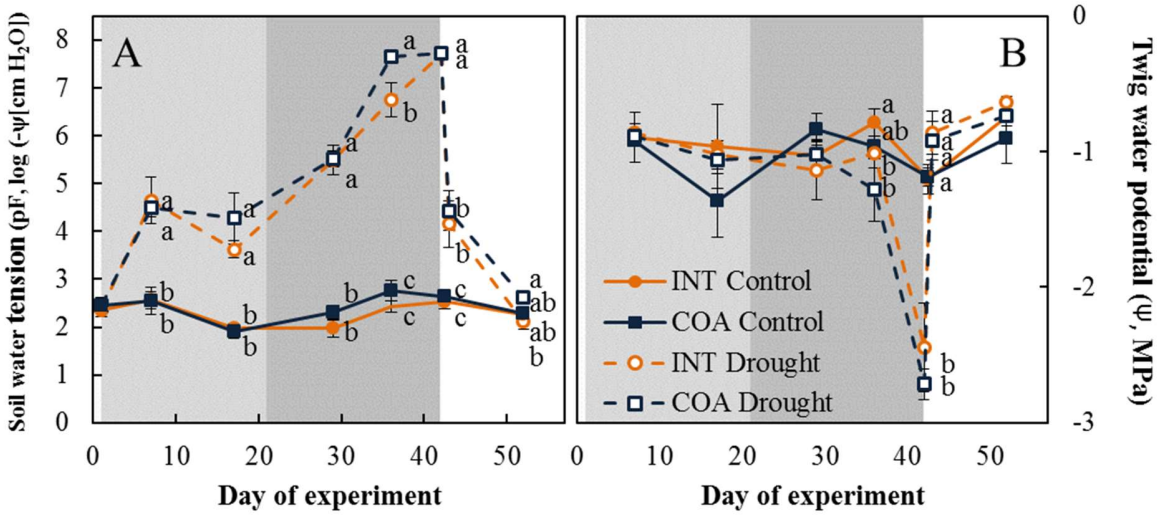

Fig. 3.1: Soil water availability and drought stress of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Soil water tension (pF) and B) pre-dawn twig water potential (Ψ). Data show mean of n = 3 measurements (±SE). Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

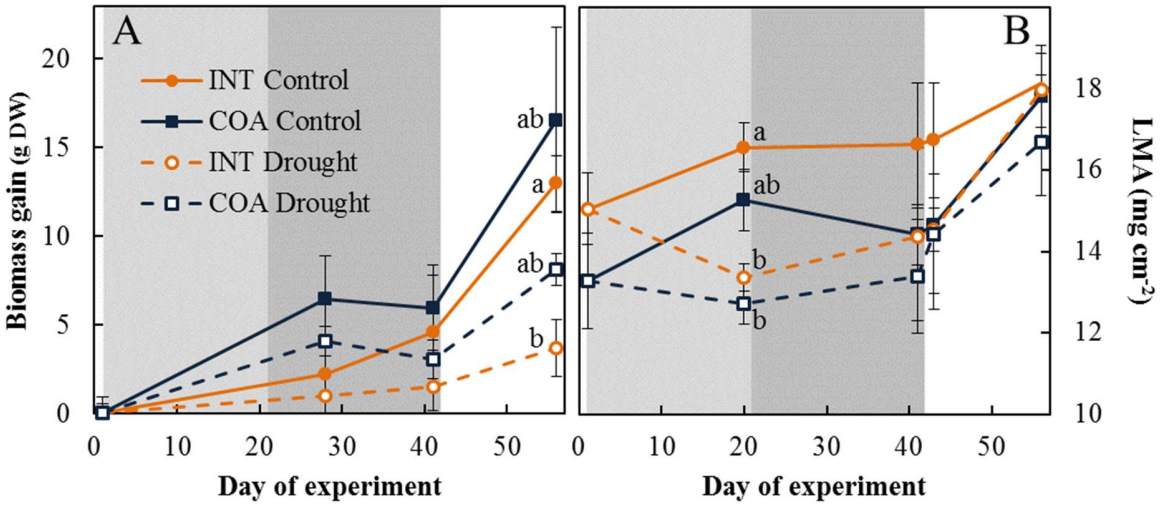

Fig. 3.2: Growth and needle morphology of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Biomass gain, B) leaf mass per area (LMA). Data show mean of n = 5 measurements (±SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

Table 3.1: 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. IWUE = Intrinsic water use efficiency, Chlorophylls a+b = total chlorophyll per fresh weight, Carotenoids = total carotenoids per total chlorophyll, VAZ = xanthophyll cycle pigments per total carotenoids, DEPS= de-epoxidation status of the xanthophyll cycle pigments, Lutein = Lutein per total carotenoids, Neoxanthin = Neoxanthin per total carotenoids, β-carotene = β-carotene per total carotenoids, α-carotene = α-carotene per total carotenoids, Stored monoterpenes = total stored monoterpenes per dry weight, stored sesquiterpenes = total stored sesquiterpenes per dry weight, Emission of monoterpenes = total monoterpene emissions.

	Parameter	Model best describing Response	p-values Treatment	Provenance	Interaction
Morphology	Biomass gain	Provenance	NA	0.1354	NA
	Leaf mass per area	Treatment	**0.0073**	NA	NA
Gas exchange	Stomatal conductance (g_s)	Treatment + Provenance	**0.0000**	**0.0075**	NA
	Transpiration rate (E)	Treatment + Provenance	**0.0000**	**0.0079**	NA
	Assimilation rate (A)	Treatment + Provenance	**0.0000**	0.1179	NA
	IWUE	Treatment + Provenance	**0.0003**	0.0582	NA
Pigments	Chlorophyll a + b	Provenance	NA	**0.0251**	NA
	Chlorophyll a/ b ratio	Provenance x Treatment	**0.0003**	**0.0000**	**0.0813**
	Carotenoids	Provenance	NA	**0.0001**	NA
	VAZ	-	NA	NA	NA
	DEPS	Treatment	**0.0086**	NA	NA
	β-carotene	Provenance	NA	**0.0278**	NA
	Neoxanthin	Provenance	NA	**0.0073**	NA
	α-carotene	-	NA	NA	NA
	Lutein	Provenance	NA	0.0960	NA
Volatile isoprenoids	Stored monoterpenes	Provenance	NA	0.1230	NA
	Stored sesquiterpenes	Treatment x Provenance	**0.0015**	**0.0000**	**0.0204**
	Emission of monoterpenes	Provenance	NA	**0.0006**	NA

3.4.2 Photosynthetic gas exchange

COA control seedlings showed an initial stomatal conductance (g_s) of 0.18 mmol H_2O m^{-2} s^{-1} which decreased over the course of the experiment to 0.11 mmol H_2O m^{-2} s^{-1}. g_s of INT control

seedlings was consistently lower and ranged between 0.1 to 0.15 mmol H_2O m^{-2} s^{-1} (Fig. 3.3a). g_s decreased to a minimum of 0.03 mmol H_2O m^{-2} s^{-1} in COA drought seedlings and 0.01 mmol H_2O m^{-2} s^{-1} in INT drought seedlings. Upon rewatering, g_s increased to 0.04 mmol H_2O m^{-2} 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 3.1).

Transpiration rates (*E*) exhibited a pattern similar to g_s, with generally higher values in COA compared to INT and reduced *E* during the drought treatment in both provenances (Fig. 3.3b, Table 3.1). COA control seedlings reached maximum *E* of 3.7 mol H_2O m^{-2} s^{-1} compared to 3.0 mol H_2O m^{-2} s^{-1} in INT control seedlings (Fig. 3.3b). *E* in both provenances decreased during the drought treatment to a minimum of 0.6 mol H_2O m^{-2} s^{-1} in COA which was twice as high compared to INT seedlings of the drought treatment with 0.3 mol H_2O m^{-2} s^{-1}. Therefore, *E* was significantly affected by *Treatment* as well as *Provenance* (Table 3.1).

Assimilation rates (*A*) were 10-12 µmol CO_2 m^{-2} s^{-1} for control seedlings of both provenances, with a drop to lower values of 8 µmol CO_2 m^{-2} s^{-1} at the end of the experiment (Fig. 3.3c). During the drought treatment, both provenances showed significantly lowered *A*, with minimum values of 3.5 µmol CO_2 m^{-2} s^{-1} in COA and 1.1 µmol CO_2 m^{-2} s^{-1} in INT at the end of the drought treatment (Fig. 3.3c, Table 3.1). One day after rewatering, *A* was increased by 3.2 ±0.9 µmol CO_2 m^{-2} s^{-1} in INT but only by 1.6 ±0.6 µmol CO_2 m^{-2} s^{-1} in COA. Within 14 days after rewatering, *A* of both provenances recovered to control plant levels.

The intrinsic water use efficiency (*IWUE*), calculated as the ratio between assimilation rate and stomatal conductance (A/g_s), did not vary in control seedlings of both provenances. INT control seedlings had an initially higher IWUE of 98 µmol CO_2 mol^{-1} H_2O compared to 75 µmol CO_2 mol^{-1} H_2O in COA control seedlings, but did not significantly vary over the course of the experiment (Fig. 3.3c, Table 3.1). Both provenances revealed significantly increased IWUE under drought, with maximum values of about 115 µmol CO_2 mol^{-1} H_2O in both provenances at the end of the drought treatment.

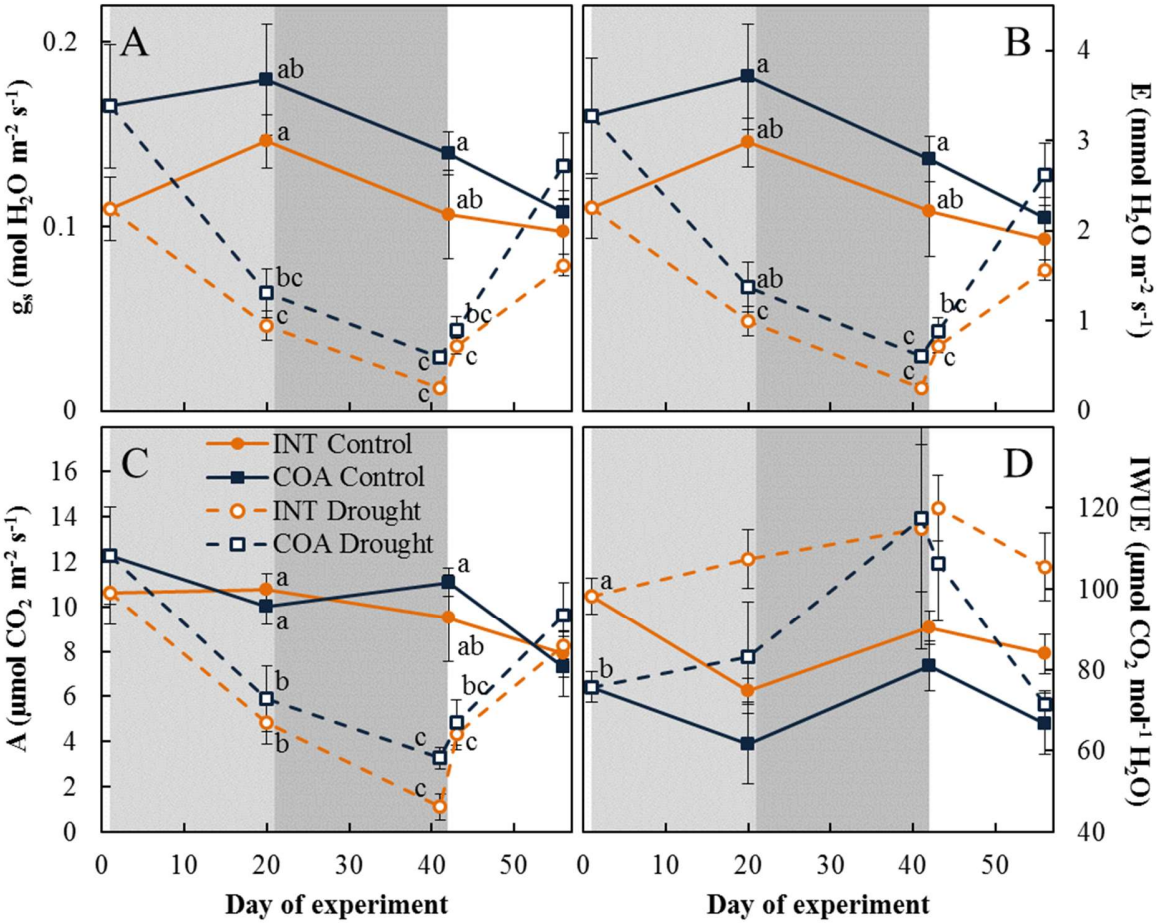

Fig. 3.3: Gas exchange parameters of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Stomatal conductance (g_s), B) transpiration rate (E), C) assimilation rate (A), and D) intrinsic water use efficiency (IWUE). Data show mean of n = 5 measurements (±SE) taken at a light intensity of 1000 µmol photons m^{-2} s^{-1}. Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

Measurements of light response curves allowed additionally to estimate the maximum assimilation rate (A_{max}), the half-saturation light intensity and the light compensation point (Fig. 3.4; Table 3.2). On day 0 and day 41, control seedlings of COA showed insignificantly higher A_{max} at light intensities exceeding 750 µmol photons m^{-2} s^{-1} compared to INT control seedlings (Fig. 3.4a,b, Table 3.2). At the end of the drought treatment (day 41), A_{max} was significantly reduced in both provenances (Fig. 3.4b; Table 3.2). After recovery (day 56), A_{max} of control and previously drought exposed seedlings of both provenances were comparable (Fig. 3.4c,

Table 3.2). Treatment-induced differences in A_{max} on day 42 also affected the half-saturation light intensity and the light compensation point which were increased in seedlings of the drought treatment compared to seedlings of the control treatment in both provenances (Table 3.2).

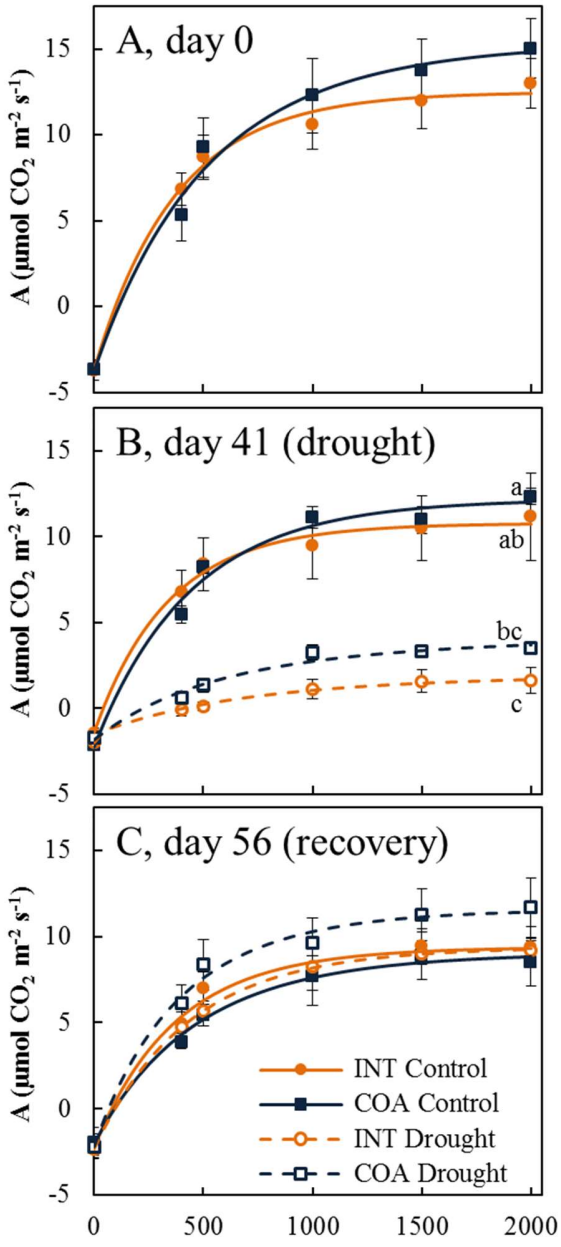

Fig. 3.4: Light response curve of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions taken on A) day 0, B) day 41, and C) day 56. Data show mean of n = 3-5 measurements (±SE). Significant differences ($p < 0.05$ of the maximum assimilation rate using Kruskal-Wallis-rank-sum-test) are indicated by different letters.

Table 3.2: Cardinal points of the light response curves of an interior and a coastal Douglas-fir provenance under control conditions, drought and recovery. Maximum assimilation rate, light intensity at half maximum assimilation rate and light compensation point were estimated from exponential curve-fitting of gas exchange measurements obtained from n=5 (±SE) seedlings per provenance and treatment. Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) are indicated by different letters.

	INT		COA	
	Control	Drought	Control	Drought
Maximum assimilation rate (µmol CO_2 m^{-2} s^{-1})				
day 0	12.5 ±2.6		15.5 ±3.1	
day 41	10.8 ±4.5^{ab}	2.0 ±1.8^{c}	12.2 ±1.4^{a}	4.4 ±1.3^{bc}
day 56	9.4 ±2.5	9.4 ±1.2	9.0 ±1.2	11.6 ±3.7
Half-saturation light intensity (µmol photons m^{-2} s^{-1})				
day 0	361 ±27^{a}		524 ±203^{b}	
day 41	273 ±33^{a}	934 ±770^{ab}	382 ±55^{a}	781 ±465^{b}
day 56	348 ±46	402 ±70	413 ±56	360 ±105
Light compensation point (µmol photons m^{-2} s^{-1})				
day 0	98 ±17		125 ±57	
day 41	44 ±13	439 ±348	75 ±20	221 ±116
day 56	80 ±20	98 ±32	83 ±57	74 ±53

3.4.3 Photosynthetic pigments

The chlorophyll content decreased over the course of the experiment in both provenances. COA showed a significantly higher chlorophyll content with initial values of 2.1 µmol g^{-1} FW compared to 1.6 µmol g^{-1} FW in INT (Fig. 3.5a, Table 3.1). Both provenances showed non-significant increased chlorophyll content during the drought treatment, but recovered to the level of control seedlings by day 56. 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. 3.5b). During the drought treatment, the chlorophyll a/b ratio decreased in both provenances, but to a stronger extent in INT compared to COA, thus exhibiting a significant *Provenance* x *Treatment* interaction (Table 3.1). 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. 3.5c;Table 3.1). 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 and significantly higher carotenoid/chlorophyll ratios in recovered INT seedlings compared to recovered COA seedlings.

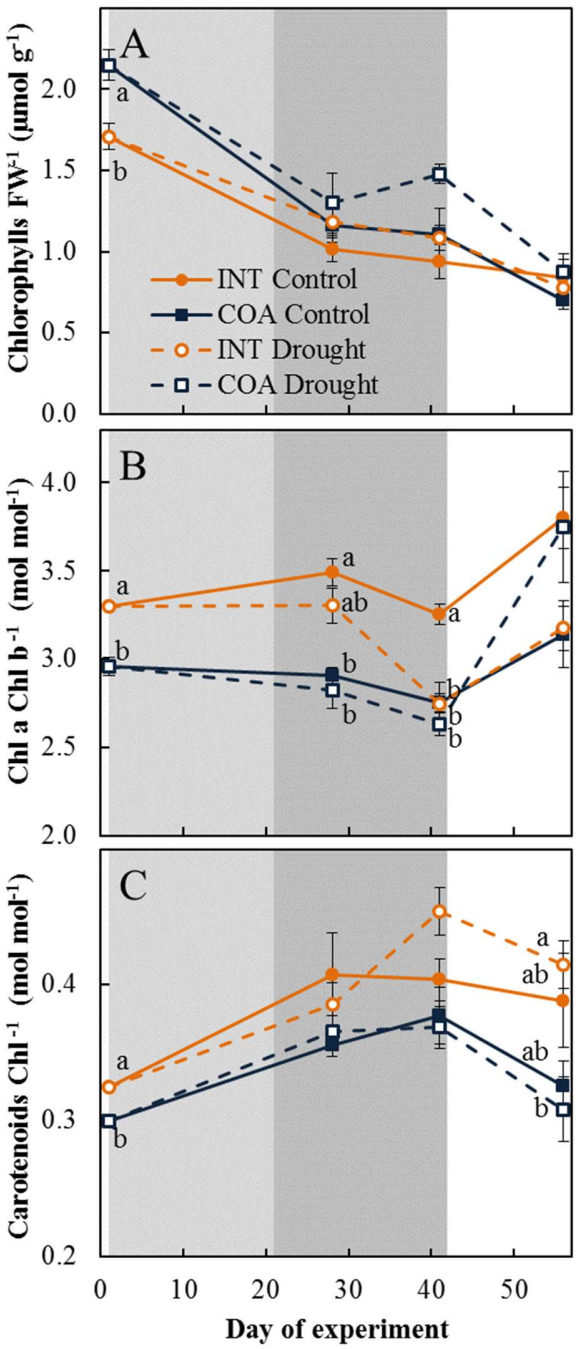

Fig. 3.5: Chlorophylls and carotenoids of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) Total chlorophylls per fresh weight (Chlorophylls FW^{-1}), B) chlorophyll a to chlorophyll b ratio (Chl a Chl b^{-1}) and C) carotenoids per total chlorophyll (Carotenoids Chl^{-1}). Data show mean of n = 5 samples (±SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

The pigments of the xanthophyll cycle in relation to total carotenoids showed an increase from 0.2 to 0.35 mol mol^{-1} Car in both provenances during the first month of the experiment (Fig. 3.6a). During the whole experiment, xanthophyll content of COA seedlings of the drought treatment was equal to control seedlings, with a decrease during the last two weeks of the experiment. In contrast, INT seedlings showed an increased xanthophyll content at the end of the drought treatment compared to control seedlings and had not recovered to control plant levels two weeks after rewatering. The deepoxidation state of the xanthophyll cycle (DEPS) also showed a general increase during the first month of the experiment (Fig. 3.6b). Both provenances showed a significant treatment effect, with increased DEPS at the end of the drought treatment, which was more pronounced for INT (Fig. 3.6b, Table 3.1).

β-carotene per carotenoids was relatively constant during the experiment, with a slight decrease by day 41 followed by an increase towards the end in both provenances (Fig. 3.6c). β-carotene was with 0.141 mol mol^{-1} Car in INT significantly higher compared to 0.12 mol mol^{-1} Car in COA, but did not vary between control and drought stressed seedlings (Table 3.1). Neoxanthin decreased in relation to total carotenoids over the course of the experiment, from initial values of 0.13 mol mol^{-1} Car to 0.05 mol mol^{-1} Car in control seedlings of both provenances (Fig. 3.6d). INT showed a slight, but significant higher neoxanthin content compared to COA, while no treatment effect was observed (Table 3.1). α-carotene decreased in all seedlings over the course of the experiment. α-carotene decreased from 0.10 to 0.03 mol mol^{-1} Car in COA and 0.13 to 0.01 mol mol^{-1} Car in INT, respectively (Fig. 3.6e, Table 3.1). Lutein per carotenoids increased in both provenances over the course of the experiment. COA had a significantly higher lutein content, which increased from 0.47 to 0.61 mol mol^{-1} Car, compared to INT, which had a lutein content increasing from 0.43 to 0.56 mol mol^{-1} Car (Fig. 3.6f). Lutein content was not affected by the drought treatment (Table 3.1).

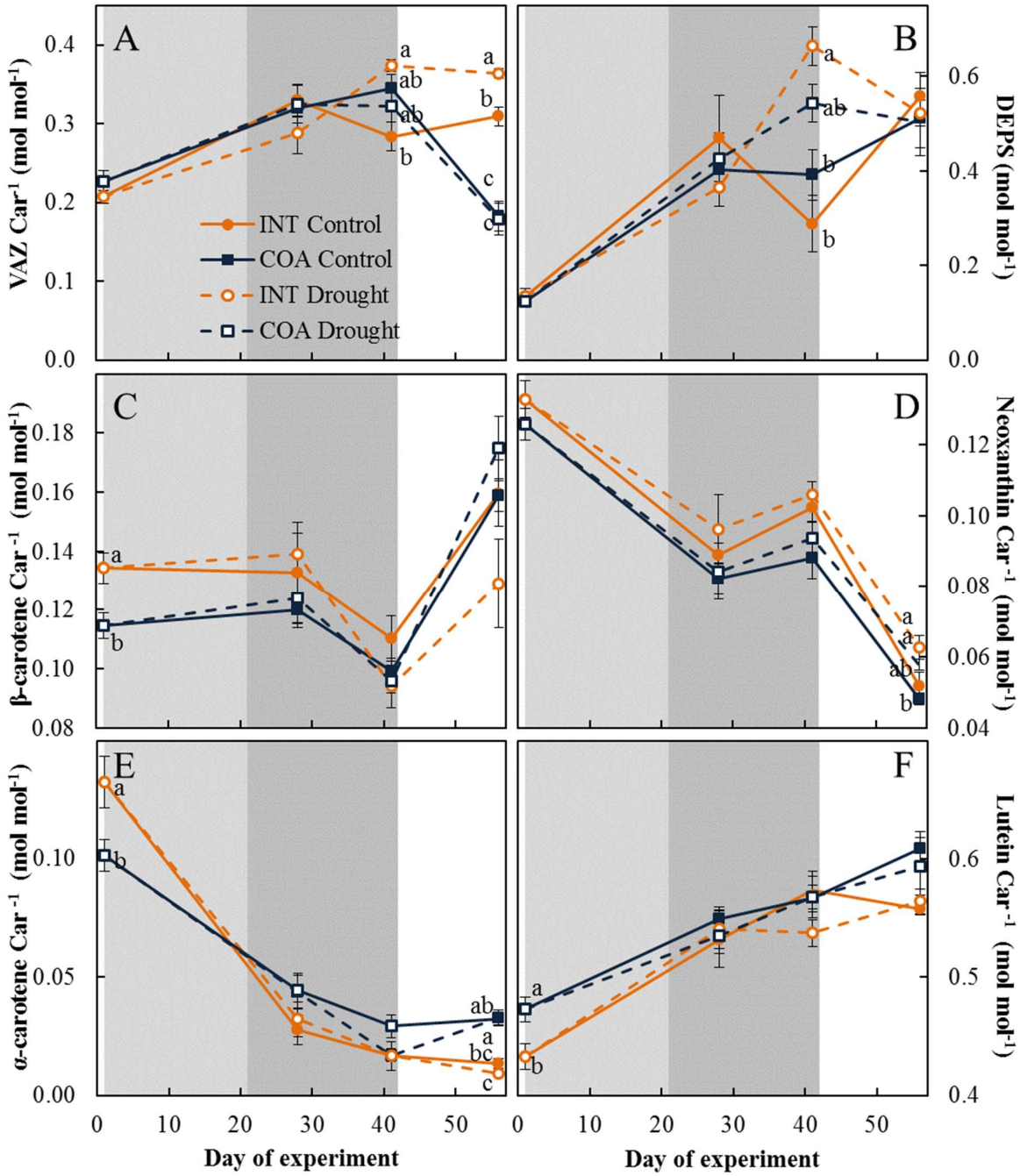

Fig. 3.6: Essential isoprenoids of interior (INT) and coastal (COA) Douglas-fir under control and drought conditions. A) Xanthophyll cycle pigments per total carotenoids (VAZ Chl^{-1}), B) de-epoxidation state of the xanthophyll cycle (DEPS, calculated as (0.5A+Z) (V+A+Z)$^{-1}$), C) β-carotene per total carotenoids, D) neoxanthin per total carotenoids, E) α-carotene per total carotenoids and F) lutein per total carotenoids. Data show mean of n = 5 samples (±SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.

3.4.4 Volatile isoprenoid pools and emissions

The monoterpene pool sizes in seedlings of both treatments remained stable over the course of the experiment, but revealed a significant *Provenance* effect (Fig. 3.7a, Table 3.1). INT pool sizes were 187 µmol g^{-1} FW and COA stored about 138 µmol g^{-1} FW monoterpenes. Sesquiterpene pool sizes of control seedlings of both provenances were relatively stable around 1.5 µmol g^{-1} over the course of the experiment (Fig. 3.7b). While INT seedlings of the drought treatment did not deviate from control seedlings, COA seedlings of the drought treatment showed increased sesquiterpene pool sizes with a maximum of 3.5 µmol g^{-1}, indicating a significant *Provenance* x *Treatment* interaction (Table 3.1). Emission levels of monoterpenes were quite stable and did not vary among treatments, but showed a high tree-by-tree variation. Monoterpene emissions of COA were significantly higher compared to INT (Fig. 3.7c; Table 3.1).

The composition of volatile isoprenoid pools and emission showed little variation between treatments but varied between provenances (Table 3.3). 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 3.3). Monoterpene emissions exhibited high variation between individual trees, but did not significantly differ between provenances.

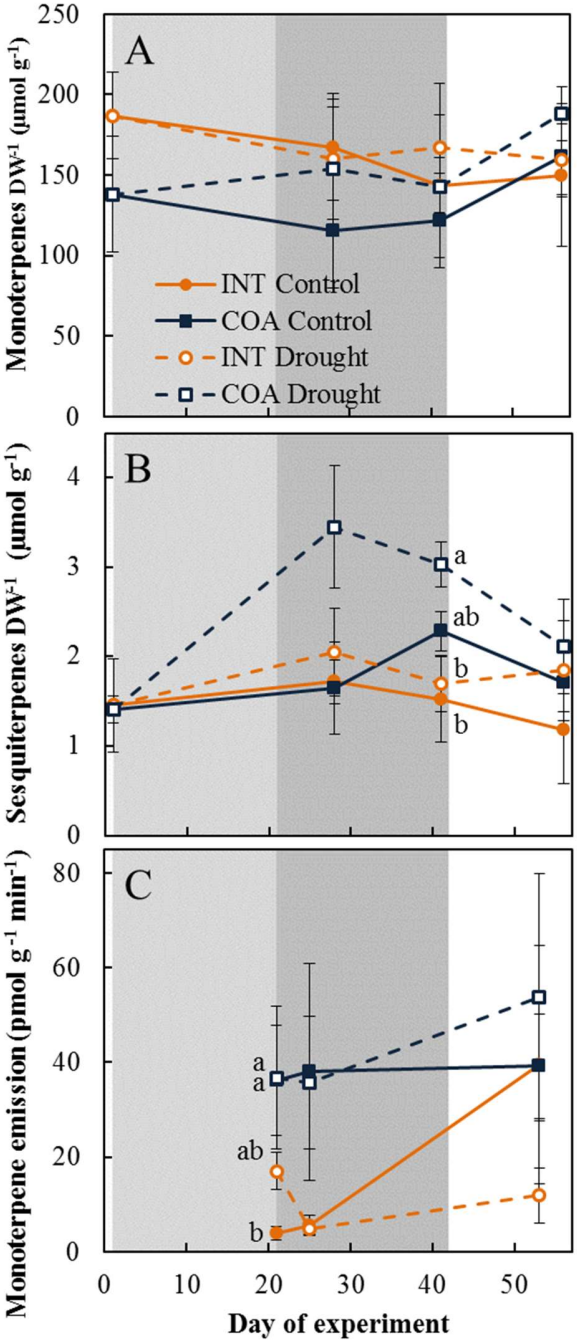

Fig. 3.7: Volatile isoprenoids of interior (INT) and coastal (COA) Douglas-fir seedlings under control and drought conditions. A) stored volatile monoterpenes, B) stored volatile sesquiterpenes and C) monoterpene emission. Data show mean of n = 5 samples (±SE). Significant differences ($p < 0.05$ using Kruskal-Wallis-rank-sum-test) are indicated by different letters. Light grey background indicates period of reduced watering, dark grey background indicates period of withheld watering, followed by rewatering.