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1 **Stem and crown growth of Japanese larch and its hybrid F₁ grown in two soils and**
2 **exposed to two free-air O₃ regimes**

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11 **ABSTRACT**

12 Ozone (O₃) pollution and soil infertility may negatively affect boreal forests across the
13 Northern Hemisphere. Impacts to economically and ecologically important larches (*Larix sp.*,
14 Pinaceae) are particularly concerning. Using a free air O₃ enrichment (FACE) system, we
15 investigated the effect of two-year elevated O₃ exposure ($\approx 66 \text{ nmol mol}^{-1}$) on Japanese larch
16 (*L. kaempferi*) and its hybrid larch F₁ (*L. gmelinii* var. *japonica* \times *L. kaempferi*) planted
17 directly into either fertile brown forest soil (BF) or BF mixed with infertile volcanic ash soil
18 (VA). Overall, photosynthetic pigmentation and the growth performance of the stem and
19 crown were reduced in both taxa exposed to elevated O₃. Furthermore, hybrid larch, in both
20 O₃ treatments, performed better than Japanese larch. This finding contradicts findings of prior
21 experiments with potential experimental artifacts of O₃ exposure facilities and root
22 restrictions. Elevated O₃ also disproportionately inhibited stem diameter growth and caused
23 an imbalance in chlorophylls *a/b* and chlorophylls/carotenoids ratios. Hybrid and Japanese

24 larches grown in BF and VA had a significantly lower drop of stem diameter over the run of
25 stem height (from base to top) when exposed to elevated O₃, compared to ambient O₃. This
26 finding indicates altered stem shape under elevated O₃. Among eleven response variables,
27 there were no significant interactions between O₃ treatment and taxa. There was also no
28 significant interaction of soil condition and taxa, suggesting that the two larches shared a
29 similar response to O₃ and soil type. Understanding the performance of hybrid larch in
30 relation to its parent species has ramifications for breeding success in an soil-degraded and
31 O₃-polluted environment.

32 **Keywords:** crown, ozone, O₃ biomonitoring, stem shape, abiotic stress, trioxygen

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50 **Conflict of Interest:** The authors declare that they have no conflict of interest.

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1. INTRODUCTION

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Tropospheric ozone (O₃) is a widespread air pollutant of increasing global significance as it is toxic to plants at species-specific thresholds (Cooley and Manning 1987; Chappelka and Samuelson 1998; Broberg et al. 2015). Ozone level was approximately 10 nmol mol⁻¹ during the pre-industrialization era, about 20 nmol mol⁻¹ up to 1930, and has since continued to increase exponentially in the Northern Hemisphere (Marenco et al. 1994). O₃ pollution is a particularly severe issue in East Asia (Yamaji et al. 2006; Feng et al. 2015; Kim et al. 2015; Koike et al. 2013) and the Mediterranean (Sicard et al. 2013; Kalabokas et al. 2013; Paoletti et al. 2014; Kopanakis et al. 2015). In these regions, sensitive cultivated and wild plant taxa are particularly vulnerable to the adverse effects of ambient O₃ phytotoxicity (Yamaguchi et al. 2011; Oksanen et al. 2013; Saitanis et al. 2015; Osborne et al. 2016).

Soil degradation is also having global ramifications for plant viability (Lal and Steward 1990; Chen et al. 2002; Schroeter et al. 2005; St.Clair and Lynch 2010). Importantly, phosphorus (P) scarcity threatens long term sustainability due to expected increases in demands and decreases in the stocks of phosphate rocks (Vance et al. 2003; Cordell et al. 2009; Van Vuuren et al. 2010; Cooper et al. 2011; Dawson and Hilton 2011). In the long term, soil fertility will be more dependent on qualitative properties of litter, as occurs in undisturbed forests where soils are poor in inorganic nutrients, and thus soil fertility depends on litter deposition (Cuevas and Medina 1986, 1988). However, elevated CO₂ and O₃ levels negatively affect litter quality, with likely implications to soil fertility (Loladze 2002; Deng et al. 2015; Agathokleous et al. 2016a; Shi et al. 2016). Although it is known that soil affects the responses of plants to other applied treatments (Körner 2011; Sigurdsson et al. 2013), the

84 interaction of O₃ with soil type is underexplored, with the majority of investigations
85 conducted using potted seedlings (Agathokleous et al. 2016).

86 Larches are deciduous trees within the genus *Larix* (Pinaceae) with high economic and
87 ecological value as they are primary components of Siberian and Canadian boreal forests,
88 and have a wide range across the Northern Hemisphere (Farjon 1990; Abaimov et al. 2000).

89 Larches hold an important role in Eurasia as maintainers of the CO₂ balance (Osawa et al.
90 2010). Japanese larch (*Larix kaempferi* (Lamb.) Carr.) is a light-demanding pioneer species.

91 As a major plantation species in Japan, significant efforts towards optimum breeding have
92 been conducted (Kurinobu 2005). Consequently, the hybrid larch F₁ was developed by

93 crossing Dahurian larch (*Larix gmelinii* var. *japonica* (Maxim. ex Regel) Pilg.) with Japanese
94 larch (Miyaki 1990). Properties of high growth, stem straightness and resistance to bark

95 gnawing by voles contribute to the viability of hybrid larch as a plantation taxon. In Hokkaido,
96 the northernmost island of Japan, 400 ha of hybrid plantations are established annually

97 (Kurinobu 2005; Kita et al. 2014).

98 However, breeding of hybrid larch has thus far occurred without taking into consideration
99 elevated O₃ levels. This hybrid has been found sensitive to elevated O₃ levels, experiencing

100 greater O₃-induced damage than its parental species (Ryu et al. 2009; Koike et al. 2012; Kam
101 et al. 2015; Wang et al. 2015). Additionally, under combined conditions of elevated O₃ and

102 CO₂ levels, the negative consequences of O₃ have been shown to dominate (Koike et al.
103 2012). These studies used: a) open-top chambers with plants directly planted in the ground

104 (Wang et al. 2015); b) open-top chambers with plants in pots (Koike et al. 2012); or c) free-

105 air O₃-concentration-enrichment (FACE) systems with plants grown in pots (Kam et al.
106 2015). The effect of O₃ on larch exposed to the natural environment (i.e. without chamber
107 effects), and without root limitation (i.e. planted directly in the ground) remains unknown.
108 Given the economic and ecological importance of larches in Eurasia, studies on the future
109 challenges of this hybrid larch are important to inform future afforestation practices.

110 The aims of this study were to investigate how two years of free-air O₃ enrichment influences
111 a) the growth and photosynthetic pigmentation of Japanese larch in comparison to its hybrid
112 when grown pot-free, and b) how O₃ impacts differ between two types of soil; fertile brown
113 forest soil (Dystric Cambisols) (BF) and BF mixed with infertile, immature volcanic ash plus
114 pumice soil (Vitric Andosols) (VA). We further aimed to quantify the magnitude of
115 difference among experimental groups to provide practical insights for these ecologically and
116 economically important taxa. We hypothesized that in these fast-growing taxa, O₃ effects on
117 growth would differ from previous findings derived from OTC experiments or pot-grown
118 plants in FACE systems. This prediction is based on the assumption that epigeous growth
119 depends on hypogeous growth and root expansion was restricted in potted experiments.
120 Based on previous findings (Pretzsch et al. 2010; Carriero et al. 2015), we hypothesized that
121 stem diameter growth would be reduced or inhibited by elevated O₃ and that this would occur
122 primarily in the lower third of the stem. Altered stem shape caused by uneven stem growth
123 is a critical issue as this pathological condition may reduce plant survival under windy
124 conditions (Pretzsch et al. 2010; Carriero et al. 2015). Plants grown in infertile soils often
125 allocate resources to root symbionts at the expense of photosynthetic products to overcome
126 nutrient deficiencies (Hermans et al. 2006; Smith and Read 2008). We therefore hypothesized

127 that larch responses to O₃ would be affected by soil fertility through shifts in photosynthesis
128 and nutritional demands.

129 **2. MATERIALS & METHODS**

130 **2.1. Experimental area and meteorological conditions**

131 The experiment was conducted in the FACE system of Sapporo Experimental Forest of
132 Hokkaido University, located at Sapporo, Japan (43°04' N, 141°20' E, 15 m a.s.l.), in the
133 years 2014-2015. The snow-free period lasted from early May to mid-November.
134 Temperature, wind speed, relative humidity, sunshine and precipitation were continuously
135 monitored by the Japan Meteorological Agency (2016) in Sapporo (WMO, ID: 47412,
136 43°03.6'N 141°19.7'E) (Table 1). The photosynthetic photon flux density (PPFD) was
137 recorded by a HOBO Pendant data logger (UA-002-64, Onset Computer, Co., MA, USA)
138 located in the center of each experimental plot at a height of two meters. During the period
139 June-October (2014 and 2015), an observation was recorded every 5 min. For both years, the
140 PPFD in the experimental plots exceeded 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (*i.e.* light compensation point of
141 photosynthesis, Koike (1988)) from 07:00 to 17:00. The daily 10-hr mean (07:00-17:00) for
142 the experimental plots (n=6) was $362.9 \pm 14.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ in 2014 and $354.3 \pm 25.8 \mu\text{mol m}^{-2}$
143 s^{-1} in 2015).

144 **2.2. Experimental Design**

145 The O₃ treatments were ambient O₃ (AOZ) and elevated O₃ (EOZ), with three plot replicates
146 for each treatment randomly scattered throughout the experimental forest. Each plot
147 (diameter=6.5 m) contained three soil sub-plots from which BF and VA sub-plots were used

148 in the present study. Each sub-plot covered an area of $\approx 42\%$ of the total plot area. For both
149 BF and VA sub-plots, 30 cm of soil was removed and placed back. BF is the native soil in
150 this experimental forest. To create VA, volcanic ash soil from the Tomakomai Experimental
151 Forest of Hokkaido University, Tomakomai, Japan, was mixed with the existing BF.
152 Volcanic ash soil, which is poor in P and N content and has high porosity, is widely
153 distributed throughout Hokkaido island, Japan. The pH of soil sub-plots was 5.69 ± 0.11 for
154 BF and 5.09 ± 0.09 for VA. These soils have been widely used in research (Koike et al. 2015)
155 and are considered free from toxic components. Details of chemical and physical properties
156 of BF and VA can be found in Eguchi et al. (2008).

157 Two-year old seedlings of Japanese and hybrid larches were directly planted into the soils on
158 May 15th, 2014. These seedlings were cultivated by the Forestry Research Institute of
159 Hokkaido Research Organization in Bibai city, ≈ 60 km from Sapporo. Agrochemicals or
160 fertilizers were not used during the experiment.

161 The FACE system was calibrated to maintain EOZ approximately twice the ambient O_3
162 concentrations, with a maximum of 80 nmol mol^{-1} . Ozone generated from pure oxygen was
163 diluted with ambient air in a pressurized tank and released into the rings by 2.5-m teflon tubes
164 suspended vertically 2.5 m above ground, and two horizontal Teflon tubes fixed around the
165 plots elevated to 0.5 and 1.5 m. An SM70 Fixed Ozone Monitor (Aeroqual Ltd., Auckland,
166 NZ) operated at the center of each EOZ plot, and a Hioki LR5042-20 (HIOKI E.E.
167 Corporation, Nagano, JP) recorded O_3 data in two-minute intervals. Plants were exposed to
168 EOZ from August 15th to October 26th in 2014 and from April 24th to October 26th in 2015,

169 during daylight hours with a PPFD higher than $70 \mu\text{mol m}^{-2} \text{s}^{-1}$ (07:00-17:00). The mean 10-
170 hr O_3 levels for EOZ plots were $60.1 \text{ nmol mol}^{-1}$ in 2014 and $72.1 \text{ nmol mol}^{-1}$ in 2015, which
171 are translated to accumulated exposure over a threshold of 40 nmol mol^{-1} (AOT40) values of
172 8.4 and $49.1 \mu\text{mol mol}^{-1} \text{ h}$ in 2014 and 2015. Although a critical AOT40 level of $8\text{-}15 \mu\text{mol}$
173 $\text{mol}^{-1} \text{ h}$ has been proposed for sensitive forest trees in Japan (Kohno et al. 2005), AOT40
174 values higher than $30 \mu\text{mol mol}^{-1} \text{ h}$ occur in Japan (Hoshika et al. 2012). Background O_3
175 concentrations, for the same hours of the corresponding months, were 20.4 and 33.8 nmol
176 mol^{-1} in 2014 and 2015, respectively, as continuously monitored by an O_3 monitor (Model
177 202, 2B Technologies, Boulder CO, USA).

178 **2.3. Data collection**

179 **2.3.1. Pre-treatment assessment**

180 At the time of planting, initial measurements of plant height (cm) and stem basal diameter
181 (mm) of the lignified stem at the base were taken from each plant. Plant height means were
182 $16.68 (\pm 0.34 \text{ se})$ and $14.57 (\pm 0.32 \text{ se}) \text{ cm}$ for hybrid larch and Japanese larch, respectively.
183 Basal diameter means were $2.26 (\pm 0.06 \text{ se})$, $2.16 (\pm 0.06 \text{ se}) \text{ mm}$ for hybrid larch and
184 Japanese larch, respectively.

185 **2.3.2. Post-treatment assessment**

186 Late in the second growing season (2015), O_3 visible foliar injury was assessed on a
187 continuous scale of 0-100 % for the foliage of each seedling. Each seedling was observed
188 from above and four cardinal directions and the total foliar injury was estimated as percentage
189 of injured needles per plant. Observations were done for both AOZ and EOZ plants.

190 Needles of similar maturity, randomized for O₃ symptom intensity, were destructively
191 sampled from each plant to assess chlorophyll-related characteristics as potential indicators
192 of physiological activity (Gottardini et al. 2014). Collected needles were stored in a deep
193 freezer until pigments analysis. For each plant extract, needles were immersed in dimethyl
194 sulfoxide (DMSO) solvent and incubated at 65 °C for 4 h under dark conditions, until
195 shadowed thalli became visible. After incubation, each extract was covered with aluminum
196 foil until its measurement. For the extracts, disposable 4.5 mL tubes were used (Nunc
197 CryoTube Vials, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).
198 Measurements were taken at 415, 435, 470, 648, 665 nm (GeneSpec III; Hitachi Genetic
199 Systems; MiraiBio, Alameda, CA). The ratio 435/415 (OD₄₃₅/OD₄₁₅) was calculated as a
200 potential index of phaeophytinization, which shows the relative proportion of *Chl_a* and
201 phaeopigment *a* (Ronen and Galun 1984). The total carotenoids content (*TCar*) was
202 calculated according to Lichtenthaler (1987). As we performed the extraction with dimethyl
203 sulfoxide as opposed to acetone, *TCar* should be interpreted for relative differences among
204 experimental groups rather than on absolute values which may slightly differ. The
205 chlorophyll *a* content (*Chl_a*), chlorophyll *b* content (*Chl_b*) and total chlorophyll content
206 (*TChl*) were estimated according to Barnes et al. (1992). The fractions *Chl_a*/*Chl_b* and
207 *TChl*/*TCar* were also calculated.

208 Growth measurements (cm) were taken at the end of the active growth period in the second
209 growing season (2015). Crown thickness (from the point to which the first lateral shoot is
210 attached on the stem to the highest point of the crown), and mean crown spread (distance
211 between the two farthest points of the crown, as observed from above) were measured

212 alongside height, using measuring tape. The middle of the stem was marked following plant
213 height measurements. Diameter of the lignified stem was measured at the base, middle and
214 top using a digital a caliber, by taking the mean of two crosswise measurements at each stem
215 level. Stem volume was also calculated for each plant as:

$$216 \quad Volume = \pi \times \frac{\left(\frac{diameter}{2}\right)^2}{10} \times height \text{ cm}^3$$

217 where diameter is the mean of two diameter measurements at the base of the stem and π is
218 set at 3.14.

219 **Statistical Analysis**

220 The level of significance for all statistical analyses was set at $\alpha=0.05$. The data of pre-
221 treatment plant height and diameter and post-treatment *Chl_a*, *Chl_b*, *TChl*, *Chl_a/Chl_b*, *TCar*
222 *OD₄₃₅/OD₄₁₅*, *TChl/TCar*, plant height, crown thickness, mean crown spread and stem
223 volume were transformed using the maximum likelihood approach of Box-Cox
224 transformation to approximate the Gaussian fit (Box and Cox 1964). General Linear Models
225 (GLMs) were conducted on transformed data of each pigment-related response variable with
226 Ozone, Soil and Taxa as fixed factors and randomized by plot (Kuehl 1999). Covariate-
227 adjusted GLMs were performed on transformed data for growth-related response variables
228 (Senturk and Muller 2009). The baseline data of plant height were used as a covariate for
229 post-treatment plant height, crown thickness, mean crown spread and stem volume, whereas
230 the baseline data of basal diameter were used as a covariate for post-treatment mean crown
231 spread, stem volume and diameters of stem. For testing diameter across the stem levels (low,
232 middle, upper), baseline-basal-diameter-adjusted GLM was applied using stem level as the

233 within effects dependent variable. Type 6 Sums of Squares, which uses the σ -restricted
234 coding of effects in order to provide unique effect estimates even for lower-order effects
235 (straightforward computing method), was employed. Post hoc Tukey's HSD test was
236 performed when required.

237 For the variables that GLMs revealed Ozone as a significant factor, simple linear regression
238 analysis was conducted between each pigment-related variable (predictor) and each growth-
239 related variable (criterion). The gain in plant height and stem basal diameter was calculated
240 by subtracting the pre-treatment score from the post-treatment score. Values of r within the
241 arbitrary segments [0.00, 0.10), [0.10, 0.30), [0.30-0.50), [0.50-0.70), [0.70-0.90) and 0.90+,
242 indicate trivial, low, moderate, large, very large and nearly perfect correlation magnitude,
243 respectively (Hopkins 2000).

244 Visible injury data were subjected to arcsine transformation before performing a Generalized
245 Linear Mixed Model (GLMM) with identity link function; random and main, two-way and
246 three-way fixed factors specified as in GLMs (Bolker et al. 2008). The variance component
247 was selected for random effect covariance type. An *a priori* decision was made to exclude
248 the two-way effects from the full factorial terms (full: Ozone + Soil + Taxa + Ozone \times Soil
249 + Ozone \times Taxa + Soil \times Taxa + Ozone \times Soil \times Taxa + (Plot)), despite their statistical
250 significance, and include only effects with biological importance. Plot was not significant
251 (Estimate = 17.8, se = 18.3, Z = 0.975, p=0.330, 95% CI [2.4, 133.0]) and excluded to
252 simplify the model; the corrected Akaike Information Criterion (AIC_c) was smaller without
253 Plot. The final model was Ozone + Soil + Taxa + Ozone \times Soil \times Taxa.

254 The unbiased Cohen's δ was estimated based on balanced real replicates (Hedges and Olkin
255 1985) so as to quantify the magnitude of difference when the predictor was statistically
256 significant. For covariate-adjusted response variables, δ was estimated by subtracting the
257 baseline effect from the post-treatment effect (Agathokleous et al. 2016c). Estimations of δ
258 were calculated according to Agathokleous et al. (2016b, 2016c). Values within the arbitrary
259 segments [0.00, 0.50), [0.50, 1.50), [1.50-3.00) and 3.00+, indicate neutral, small, moderate
260 and large magnitude of difference, respectively (Agathokleous et al. 2016b, modified from
261 Cohen 1988). Absolute δ values in the interval [0.50-1.50] indicate educational significance
262 while δ values >1.50 indicate practical significance (Agathokleous et al. 2016b, modified
263 from Wolf 1986).

264 Furthermore, δ was converted to overlapping coefficient (OVL), a potential measure of
265 bioequivalence under the name proportion of similar response (Reiser and Faraggi 1999),
266 using the formula

$$267 \quad OVL = 2\Phi(-|\delta|/2)$$

268 where, Φ is the cumulative distribution function of the standard normal distribution and δ the
269 population δ . This coefficient represents the common area under two probability density
270 curves and indicates the agreement between two distributions (Reiser and Faraggi 1999).

271 Log-transformed height of the plant at which stem diameter was measured was used to plot
272 height against stem diameter (n=3 for each taxon in each soil, O₃ treatment and stem level);
273 the equations are shown in Fig 3. The data of each Ozone group were pooled from taxa and
274 soils, for each stem level, and the slopes of AOZ and EOZ were tested ($\alpha=0.05$) using

275 comparison of regression lines slopes from two independent samples based on pooled
276 variance (Howell 2010).

277 For data presentation purposes, the untransformed values are presented. Data processing and
278 statistical analyses were conducted using MS EXCEL 2010 (Microsoft ©), PASW Statistics
279 18 (formerly SPSS Statistics, IBM ©) and STATISTICA v.10 (StatSoft Inc. ©) software.

280

3. RESULTS

281 3.1. Visible injury

282 Larch needles were discoloured in EOZ but not AOZ treatments. Discolouration was
283 observed as yellowish flecks initiating from the top and progressing towards the base of the
284 needles. The average values of needle areas affected by O₃ visible injury per plant (%) in
285 EOZ plants were 93 (± 3), 83 (± 7), 73 (± 3) and 84 (±5) for hybrid larch×BF, hybrid
286 larch×VA, Japanese larch×BF and Japanese larch×VA, respectively. The value of the
287 corrected AIC_c was 118.548. In the corrected model ($F=81.5$, $P<0.001$), only the predictor
288 Ozone had a significant ($F=564.2$, $P<0.001$) fixed effect. Soil ($F=0.02$, $P=0.895$), Taxa
289 ($F=1.5$, $P=0.239$), and Ozone×Soi×Taxa ($F=1.3$, $P=0.318$) were not significant. The
290 coefficient of AOZ was -67.42 ± 5.68 se, Wald t statistic (t) = -11.87 , $P<0.001$.

291 3.2. Pigments and plant size

292 Ozone was not a significant factor for OD₄₃₅/OD₄₁₅ (Table 2, Fig 1). However, EOZ had a
293 small negative effect on Chl_a/Chl_b ($\delta=-1.20$, OVL=0.550) and a moderate negative effect on
294 plant height ($\delta=-1.80$, OVL=0.362), crown thickness ($\delta=-2.26$, OVL=0.259), mean crown
295 spread ($\delta=-2.28$, OVL=0.255) and stem volume ($\delta=-2.89$, OVL=0.148) (Fig 1-2).

296 Furthermore, EOZ had a large negative effect on Chl_a ($\delta=-6.58$, OVL=0.001), Chl_b ($\delta=-5.86$,
297 OVL=0.002), $TChl$ ($\delta=-6.54$, OVL=0.001), $TCar$ ($\delta=-3.49$, OVL=0.081) and $TChl/TCar$
298 ($\delta=-3.46$, OVL=0.084). For Chl_a , Chl_b , $TChl$, $TCar$ and $TChl/TCar$, 10 % (maximum) of the
299 groups (AOZ and EOZ) overlap and there is a >99 % chance that a random observation from
300 EOZ would reveal a lower score than a random observation from AOZ (area under the
301 receiver operating characteristics (AUC); Ruscio and Mullen 2012).

302 The effect of soil type was significant only for Chl_a/Chl_b (Table 2, Fig 1) to which VA caused
303 a small positive effect ($\delta=0.83$, OVL=0.678).

304 Taxa differed in all the variables except the three pigmentation ratios (Chl_a/Chl_b ,
305 OD_{435}/OD_{415} , $TChl/TCar$), with hybrid larch having superior performance to Japanese larch
306 in all the measured characteristics (Table 2, Fig 1-2). In AOZ, hybrid larch and Japanese
307 larch had a difference of small magnitude in $TCar$ ($\delta=1.19$, OVL=0.550). A moderate
308 difference between species existed for Chl_b ($\delta=1.48$, OVL=0.458), mean crown spread
309 ($\delta=2.15$, OVL=0.283), Chl_a ($\delta=1.55$, OVL=0.438) and $TChl$ ($\delta=1.58$, OVL=0.431). Similarly,
310 in AOZ, they had a difference of large magnitude in plant height ($\delta=3.53$, OVL=0.078),
311 crown thickness ($\delta=3.70$, OVL=0.064) and stem volume ($\delta=4.08$, OVL=0.041). In EOZ,
312 hybrid larch and Japanese larch had a difference of small magnitude in Chl_b ($\delta=1.13$,
313 OVL=0.573) and $TChl$ ($\delta=1.43$, OVL=0.474) and a difference of moderate magnitude in all
314 the other characteristics including plant height ($\delta=1.72$, OVL=0.391), crown thickness
315 ($\delta=2.05$, OVL=0.304), mean crown spread ($\delta=1.61$, OVL=0.420), stem volume ($\delta=1.67$,
316 OVL=0.404), Chl_a ($\delta=1.64$, OVL=0.412) and $TCar$ ($\delta=1.66$, OVL=0.406).

317 Ozone×Soil was significant only for Chl_b and Chl_a/Chl_b , whereas only for Chl_a/Chl_b there
318 was a difference of interest in multiple comparisons. A moderately higher Chl_a/Chl_b was
319 observed in EOZ×VA compared with EOZ×BF ($\delta=-1.65$, OVL=0.410).

320 The interactions Soil×Taxa and Ozone×Taxa were insignificant, for all the pigment and plant
321 size-related variables.

322 Ozone×Soil×Taxa was significant only for stem volume. Hybrid larch had a smaller stem
323 volume in EOZ×BF compared with AOZ×BF ($\delta=-7.53$, OVL<0.001) and in EOZ×VA
324 compared with AOZ×VA ($\delta=-6.80$, OVL=0.001) with large differences in magnitude.
325 Similar large differences were observed for Japanese larch, which had a smaller stem volume
326 in EOZ×BF compared with AOZ×BF ($\delta=-3.03$, OVL=0.130) and in EOZ×VA compared
327 with AOZ×VA ($\delta=-6.83$, OVL=0.001). Finally, hybrid larch had a greater stem volume than
328 Japanese larch in AOZ×BF ($\delta=5.45$, OVL=0.006), AOZ×VA ($\delta=3.04$, OVL=0.129) and
329 EOZ×VA ($\delta=2.41$, OVL=0.229), of large, large and moderate magnitude, respectively.
330 Hybrid larch stem volume was 29% larger in EOZ×BF, however high RSD (35.5% and
331 28.4%) masked the difference.

332 GLM analysis of diameters across the stem (Fig 3) revealed Ozone ($F=102.13$, $P<0.001$),
333 Taxa ($F=5.30$, $P<0.05$) and Stem level ($F=9.02$, $P<0.001$) but not Soil ($F=0.01$, $P=0.924$) as
334 significant predictors. Stem diameter was higher in AOZ than in EOZ and higher in hybrid
335 larch compared with Japanese larch. Stem diameter was also higher at the lower part
336 compared with the middle part of the stem, and at the middle part compared with the upper
337 part of the stem. From second order interaction, only Ozone×Stem level ($F=46.38$, $P<0.001$)

338 and Taxa×Stem level ($F=9.70$, $P<0.001$) were significant. Stem diameter was decreased in
339 EOZ treatments at each level. Only the lower part of the stem was different between taxa,
340 where Japanese larch had lower stem diameter than hybrid larch. Ozone×Soil ($F=1.84$,
341 $P=0.195$), Ozone×Taxa ($F=0.75$, $P=0.401$), Soil×Taxa ($F=0.27$, $P=0.611$), Soil×Stem level
342 ($F=0.10$, $P=0.905$) were insignificant. There were no significant effects for third and fourth
343 order interactions: Ozone×Soil×Taxa ($F=2.33$, $P=0.148$), Ozone×Soil×Stem level ($F=2.52$,
344 $P=0.098$), Ozone×Taxa×Stem level ($F=1.72$, $P=0.196$), Soil×Taxa×Stem level ($F=0.48$,
345 $P=0.621$) and Ozone×Soil×Taxa×Stem level ($F=1.43$, $P=0.256$). The slope between the
346 height of the stem, at which diameter was measured, and the stem diameter was lower in
347 EOZ compared to AOZ ($T=-3.61$, $P<0.001$).

348 The simple linear regressions (Fig 4-5) showed that all the examined pigment-related
349 variables can effectively predict scores on all the growth-related variables ($P < 0.05$, adjusted
350 $R^2 < R^2$). Chl_a/Chl_b had a moderate correlation with all the growth-related variables except
351 stem basal diameter with which it had a large correlation, and $TChl/TCar$ had a large
352 correlation with plant height and mean crown spread (Fig 5). All the other correlations of
353 $TChl/TCar$ and the other pigment-related variables with growth-related variables were very
354 large or near-perfect.

355 4. DISCUSSION

356 The O₃-induced visual injury was high in both soil types, and of similar magnitude for both
357 larch taxa. This is consistent with the lower Chl_a , Chl_b , $TChl$, $TCar$ and $TChl/TCar$ of both
358 taxa in EOZ than in AOZ which was of large magnitude. Importantly, despite the large

359 differences in chlorophyll contents, the difference of hybrid larch and Japanese larch in terms
360 of Chl_a/Chl_b between EOZ and AOZ was small, with 55% of the groups overlapping and an
361 80% chance that a haphazard observation from EOZ will reveal a lower score than a
362 haphazard observation from AOZ. Chlorophyll production indicates nutritional,
363 photosynthetic and productive efficiency of plants and relates to a unique plant defensive
364 system against insect herbivory through the catalysis of chlorophyll by chlorophyll
365 chlorophyllidohydrolase to produce chlorophyllide and phytol (Curran et al. 1990; Hu et al.
366 2015). Therefore, chlorophyll molecules are of utmost importance for plant health. Loss of
367 chlorophyll may relate to chlorophyll oxidation or altered regulation of pigment biosynthesis
368 (Caregnato et al. 2013), where the synthesis of new chlorophyll *a* is inhibited or the synthesis
369 of chlorophyll *b* is increased as compared with uninjured needles (Knudson et al. 1977). The
370 present Chl_a/Chl_b findings are consistent with higher sensitivity of chlorophyll *a* than
371 chlorophyll *b* to O_3 in C3 plants reported in previous studies (Knudson et al. 1977; Saitanis
372 et al. 2001; Neufeld et al. 2006; Feng et al. 2008). However, they are inconsistent with other
373 studies where a higher sensitivity of chlorophyll *b* than chlorophyll *a* to O_3 was found in C3
374 plants in the framework of a rearrangement of the pigments ratio as an adaptive defense
375 mechanism within the chloroplasts to protect PSII reaction centers from photoinhibition
376 (Fernandez et al. 1993; Döring et al. 2013; Pellegrini 2014). The structure of a chlorophyll
377 molecule is characterized by a porphyrin head and a phytol tail with O_3 -sensitive double
378 bonds, however, while chlorophyll *a* has a $-CH_3$ group, chlorophyll *b* has an $-CHO$ group at
379 carbon atom 3 of pyrrole ring II. Despite their similar structure, chlorophylls *a* and *b* have
380 different locations, light absorption spectra and tasks within the photosystems, and these

381 dissimilarities may define their difference in sensitivity. The present and previous findings
382 (Manninen et al. 1999; Saitanis et al. 2001; Feng et al. 2008; Agathokleous et al. 2016c; Gao
383 et al. 2016) indicate that Chl_a/Chl_b is a more efficient indicator of O₃ stress when O₃ injury
384 is high than when O₃ injury is low, since a reduction of Chl_a associates with an increase in
385 foliar injury (Knudson et al. 1977; Neufeld et al. 2006; Caregnato et al. 2013; Gottardini et
386 al. 2014). Contrastingly, O₃ sensitivity of $TChl/TCar$ was 1.9 times higher (δ) than that of
387 Chl_a/Chl_b . Such observation is consistent with higher sensitivity of $TChl$ than $TCar$ found in
388 C3 plants (Agathokleous et al. 2014, 2016c; Pellegrini 2014). Alterations in $TChl/TCar$
389 indicate malfunctions in plant physiological and phenological status caused by stress-induced
390 photooxidation through breakdown of chlorophylls and a need for photoprotection (Peñuelas
391 et al. 1995; Pellegrini 2014). In addition to the higher sensitivity of $TChl/TCar$ compared
392 with Chl_a/Chl_b , the correlations of $TChl/TCar$ with the growth-related variables were large
393 or very large whereas those of Chl_a/Chl_b were moderate in four out of five cases. We thus
394 postulate that $TChl/TCar$ may be a more sensitive O₃ biomarker than Chl_a/Chl_b and suggest
395 further investigation of this index for O₃ pollution assessment studies of deciduous coniferous
396 species. It is also worthwhile to test whether Chl_a/Chl_b increases under low stress in the
397 framework of biological plasticity with the central player of Chl_b , and decreases under high
398 oxidation damage in Chl_a (Calabrese and Blain 2009; Pellegrini 2014).

399 Physiological malfunctions indicated by pigmentation assessment reflected in plant growth
400 through strong intercorrelations. Similarly to pigment-related traits, EOZ moderately
401 suppressed plant height, crown thickness, mean crown spread and stem volume. However,
402 heterosis was observed in the hybrid larch. Such a heterosis was previously observed in stem

403 and crown of the hybrid larch *Larix decidua* (Mill.) x *L. leptolepis* (Sieb and Zucc.) Gord.
404 (Matyssek and Schulze 1987). Hybrid larch heterosis, observed in this study, was maintained
405 in EOZ despite EOZ negatively affected hybrid growth too. Although vertical growth has
406 been extensively studied, horizontal growth is poorly understood in open-grown tree species
407 (Pretzsch et al. 2015). Tree crown size is an important index both for forest and urban trees
408 showing the epigeous space a tree occupies, which relates to the total leaf area and biomass
409 (Binkley et al., 2013; Forrester 2014; Pretzsch et al. 2015). An EOZ-induced decrease in
410 crown spread and thickness shows smaller crown size along with smaller epigeous space
411 occupied by a tree. Such a decrease may further show decreased light use efficiency, as the
412 latter may decrease with decreasing epigeous tree size (Gspaltl et al. 2013). Cachectic crown
413 size in forest trees may have long term implications for forest ecosystems. Changes in
414 epigeous competition between species of different O₃ sensitivity, undergrowth light intensity,
415 crown faunal composition and carbon sequestration may be affected. In urban environments,
416 suppressed crown size has implications of decreased particulate pollution absorption,
417 reduced shading and a greater risk of branches breaking in high winds (Pretzsch et al. 2015).
418 With EOZ, stem diameter was reduced at each stem level across taxa and soils. However,
419 plants in EOZ had a significantly lower drop of stem diameter over the run of stem height
420 (from base to top) compared to plants in AOZ. This drop indicates a disproportional loss of
421 stem diameter from the base to the top of the stem and may be attributed to a shorter exposure
422 of the upper stem to EOZ than the middle and base. Disproportional inhibition of stem
423 diameter growth across different heights indicates more warped growth and a possible
424 susceptibility to winds. An O₃-induced reduction in stem diameter at the lower third of the

425 stem was reported in a plantation of the O₃-sensitive Oxford clone of poplar (*Populus*
426 *maximoviczii* Henry x *berolinensis* Dippel) in a 6-year open-field study (Carriero et al. 2015).
427 A lower increment or failure to increase in stem diameter also occurred mostly at the lower
428 third of the stem of mature European beech trees (*Fagus sylvatica* L.) after a 7-year treatment
429 with elevated O₃ levels (see Pretzsch et al. 2010 for discussion and references). The findings
430 of this study support conclusions by Pretzsch et al. (2010) that a decline in diameter
431 increment at the lower third of the stem should not be interpreted as an overall growth decline
432 due to overestimations.

433 The present findings of an EOZ-induced diameter suppression of both hybrid and Japanese
434 larches contradict studies with potential experimental artifacts of O₃ facilities and root
435 restrictions. For example, in a study using a FACE system with 60 nmol mol⁻¹ daytime
436 exposure over a growing season, no significant effects of O₃ were found on growth of the
437 stem basal diameter and stem volume of potted hybrid and Japanese larch seedlings (Kam et
438 al., 2015). Contrastingly, in an OTC study where potted seedlings were exposed to 60 nmol
439 mol⁻¹, 7 h per day over a growing season, there was a significant O₃-induced reduction of
440 diameter growth increment and height of potted seedlings of hybrid larch but not its parental
441 species (Koike et al. 2012). However, the whole plant dry mass and the shoot/root ratio of all
442 taxa were unaffected (Koike et al. 2012). Another OTC study on hybrid larch seedlings
443 directly planted in the soil (i.e. no root limitations) with the same O₃ treatment as in Koike et
444 al. (2012), found an O₃-induced reduction in final stem dry mass after two growing seasons
445 of exposure to O₃ (Wang et al. 2015). However, O₃-induced reductions in plant height and
446 stem diameter in the first growing season did not translate to early or late in the second (Wang

447 et al. 2015). Thus, under experimental conditions with OTCs or potted plants in FACE
448 systems, O₃ appears to influence plant growth differently with altered conditions. The present
449 contradictory results in a system without containment artifacts and root restrictions reveal the
450 importance of free air studies in natural soils to understand how co-occurring environmental
451 changes will impact forest tree species.

452 Soil, independently from Taxa and Ozone, was a significant factor only for *Chl_a/Chl_b*. In
453 contrast to the EOZ-induced decrease in *Chl_a/Chl_b*, VA caused an increase in *Chl_a/Chl_b*,
454 showing higher *Chl_a* than *Chl_b*. This increase, was upon prevention of the EOZ-induced
455 imbalance in *Chl_a/Chl_b* by VA, which was the only difference of interest in the Soil×Ozone
456 interaction. *Chl_a/Chl_b* is an efficient indicator of the light environment of plants (Dale and
457 Causton 1992; Lei et al. 1996), and its increase associates with acclimation to high light
458 conditions and low N availability (Kitajima and Hogan 2003). Since the light environment
459 was similar for both soils in this experiment, the increase in *Chl_a/Chl_b* in VA may be
460 attributed to the lower N content in VA than in BF (Eguchi et al. 2008). It is also possible
461 that Mg content differed between soil types. As Mg is a central atom in chlorophyll porphyrin
462 ring, such a potential difference might have affected chlorophyll biosynthesis.

463 The complexity of using *Chl_a/Chl_b* as a measure of the O₃ impact on plants in *in vitro* studies
464 is demonstrated. Attention should be paid to the numerous factors influencing *Chl_a/Chl_b*, and
465 particularly site-specific soil, which may lead to erroneous conclusions. OD₄₃₅/OD₄₁₅ is an
466 ineffective biomarker of O₃ injury in these taxa. *TChl/TCar*, may be more accurate in

467 assessing O₃ effects on the studied taxa as it is more sensitive to EOZ than *Chl_a/Chl_b* and
468 consistently related to O₃ alone.

469 Diameter across stem height could be utilized as a low-cost assessment tool of O₃ stress in
470 large trees especially in remote areas where access to equipment and facilities is prohibitive.
471 Still, diameter across stem height and crown size should be taken into account by urban plant
472 pathologists and local authorities in risk analysis (Lorenzini and Nali 2014). It is also
473 recommended to avoid interpretation of O₃ impact on overall growth based on a decline in
474 diameter increment at the lower third of the stem.

475 **5. CONCLUSIONS**

476 The present findings provide evidence for sustained heterosis in hybrid larch under elevated
477 O₃ levels. It is concluded that hybrid larch is no more susceptible to elevated O₃ levels than
478 Japanese larch for the growth and health traits tested. These results differ from previous
479 studies conducted with the same taxa and at the same experimental area but with potential
480 experimental artifacts.

481 In contrast to the tested hypothesis of a soil-dependent response of larch to O₃, there was no
482 relevant difference except in *Chl_a/Chl_b*. It is recommended including tests with more infertile
483 soil in future studies.

484 Long-term studies and breeding programmes which account for improvement to O₃-tolerance
485 capacity are required. It is suggested to consider stem shape as well as vertical and horizontal
486 growth when making comparative viability assessments of rehabilitation and plantation

487 species. Superior growth, stem form and branching traits in changing environment should be
488 an additional goal in practice due to the ecological and economic importance of these traits.

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735 **Captions**

736 **Table 1** Means \pm s.e. of the main meteorological conditions at Sapporo, Japan, for the months
737 June-October, of the years 2014-2015.

738 **Table 2** Results of the GLM statistical analysis. All the pigment-related data subjected to
739 general linear models randomized by plot. Data obtained from Japanese larch and a hybrid
740 larch F₁ seedlings grown in brown forest soil (BF) or BF mixed with volcanic ash soil (VA)
741 and exposed to ambient air (AOZ) or O₃-enriched air (EOZ) (O₃≈66 nmol mol⁻¹) for two
742 growing seasons. Each treatment condition is consisted of three replicates.

743 **Fig. 1** Means (\pm se) of chlorophyll *a* content (*Chl_a*), chlorophyll *b* content (*Chl_b*), total
744 chlorophyll content (*TChl*), *Chl_a/Chl_b* ratio, ratio of optical densities 435 to 415
745 (OD₄₃₅/OD₄₁₅), total carotenoid content (*TCar*) and *TChl/TCar* ratio. Data obtained from
746 Japanese larch (light shading) and a hybrid larch F₁ (dark shading) seedlings grown in brown
747 forest soil (BF) or BF mixed with volcanic ash soil (VA) and exposed to ambient air (AOZ)
748 or O₃-enriched air (EOZ) (O₃≈66 nmol mol⁻¹) for two growing seasons. Different uppercase
749 letters show statistically significant differences within O₃×Soil interaction (Taxa pooled),
750 according to Tukey's HSD posthoc test. The significance of the main factors and O₃×Soil
751 interaction is indicated by ns (non-significant, $P>0.05$), * ($P<0.05$) ** ($P<0.01$) or ***

752 ($P < 0.001$). The interactions $O_3 \times \text{Taxa}$, $\text{Soil} \times \text{Taxa}$ and $O_3 \times \text{Soil} \times \text{Taxa}$ were insignificant for
753 all the response variables. Each value of the eight means resulted from three replicates.

754 **Fig. 2** Means (\pm se) of plant height, crown thickness, mean crown spread and stem volume.
755 Data obtained from Japanese larch (light shading) and a hybrid larch F_1 (dark shading)
756 seedlings grown in brown forest soil (BF) or BF mixed with volcanic ash soil (VA) and
757 exposed to ambient air (AOZ) or O_3 -enriched air (EOZ) ($O_3 \approx 66 \text{ nmol mol}^{-1}$) for two growing
758 seasons. Different lowercase letters indicate statistically significant differences among the
759 means, when $O_3 \times \text{Soil} \times \text{Taxa}$ was significant, and different uppercase letters show
760 statistically significant differences within $O_3 \times \text{Soil}$ (Taxa pooled), according to Tukey's HSD
761 posthoc test. The significance of the main factors is indicated by ns (non-significant, $P > 0.05$),
762 * ($P < 0.05$) ** ($P < 0.01$) or *** ($P < 0.001$). The interactions $O_3 \times \text{Soil}$, $O_3 \times \text{Taxa}$ and $\text{Soil} \times \text{Taxa}$
763 were insignificant for all the response variables. Each value of the eight means resulted from
764 three replicates.

765 **Fig. 3** Means (\pm se) of diameter across stem as measured at the base (Lower), middle (Middle)
766 and top (Upper) of the stem. Data obtained from Japanese larch (JL, dashed lines) and a
767 hybrid larch F_1 (HL, solid lines) seedlings grown in brown forest soil (BF, lower figure) or
768 BF mixed with volcanic ash soil (VA, upper figure) and exposed to ambient air (AOZ) or
769 O_3 -enriched air (EOZ) ($O_3 \approx 66 \text{ nmol mol}^{-1}$) for two growing seasons. Different uppercase
770 letters A, B and C show statistically significant differences among stem levels (other
771 treatments pooled). Different lowercase letters between means of taxa in each stem level
772 show statistically significant differences within the interaction $O_3 \times \text{Stem level}$ (Soil and Taxa

773 pooled). Different uppercase letters L, M, N and O above means of taxa in each stem level
774 show statistically significant differences within the interaction Taxa×Stem level (O3 and Soil
775 pooled). Differences are marked according to Tukey's HSD posthoc test. The significance of
776 the main factors and the interactions O3×Stem level and Taxa×Stem level is indicated by ns
777 (non-significant, $P>0.05$), * ($P<0.05$) ** ($P<0.01$) or *** ($P<0.001$). The remaining second
778 order interactions and the third and fourth order interactions were insignificant. Each mean
779 is the product of three replicates.

780 **Fig. 4** Regression analysis of chlorophyll *a* content (Chl_a), chlorophyll *b* content (Chl_b) and
781 total chlorophyll content ($TChl$) with each growth-related variable. Each data point is the
782 average of the plants in each research condition (N=24). The regressions were tested at an α
783 level of 0.05.

784 **Fig. 5** Regression analysis of total carotenoid content ($TCar$), Chl_a/Chl_b ratio and $TChl/TCar$
785 ratio with each growth-related variable. Each data point is the average of the plants in each
786 research condition (N=24). The regressions were tested at an α level of 0.05.

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794 **Table 1**

	2014	2015
Daily average air temperature (°C)	18.60 ±2.04	17.92 ±2.05
Daily maximum air temperature (°C)	23.08 ±2.03	22.22 ±2.03
Daily minimum air temperature (°C)	14.96 ±2.21	14.50 ±2.22
Daily wind speed (m s⁻¹)	3.40 ±0.16	3.32 ±0.23
Daily relative humidity (%)	69.6 ±1.69	70.2 ±2.33
Total sunshine duration (h)	181.96 ±11.04	157.54 ±4.59
Total precipitation (mm)	132.6 ±24.23	111.6 ±24.85

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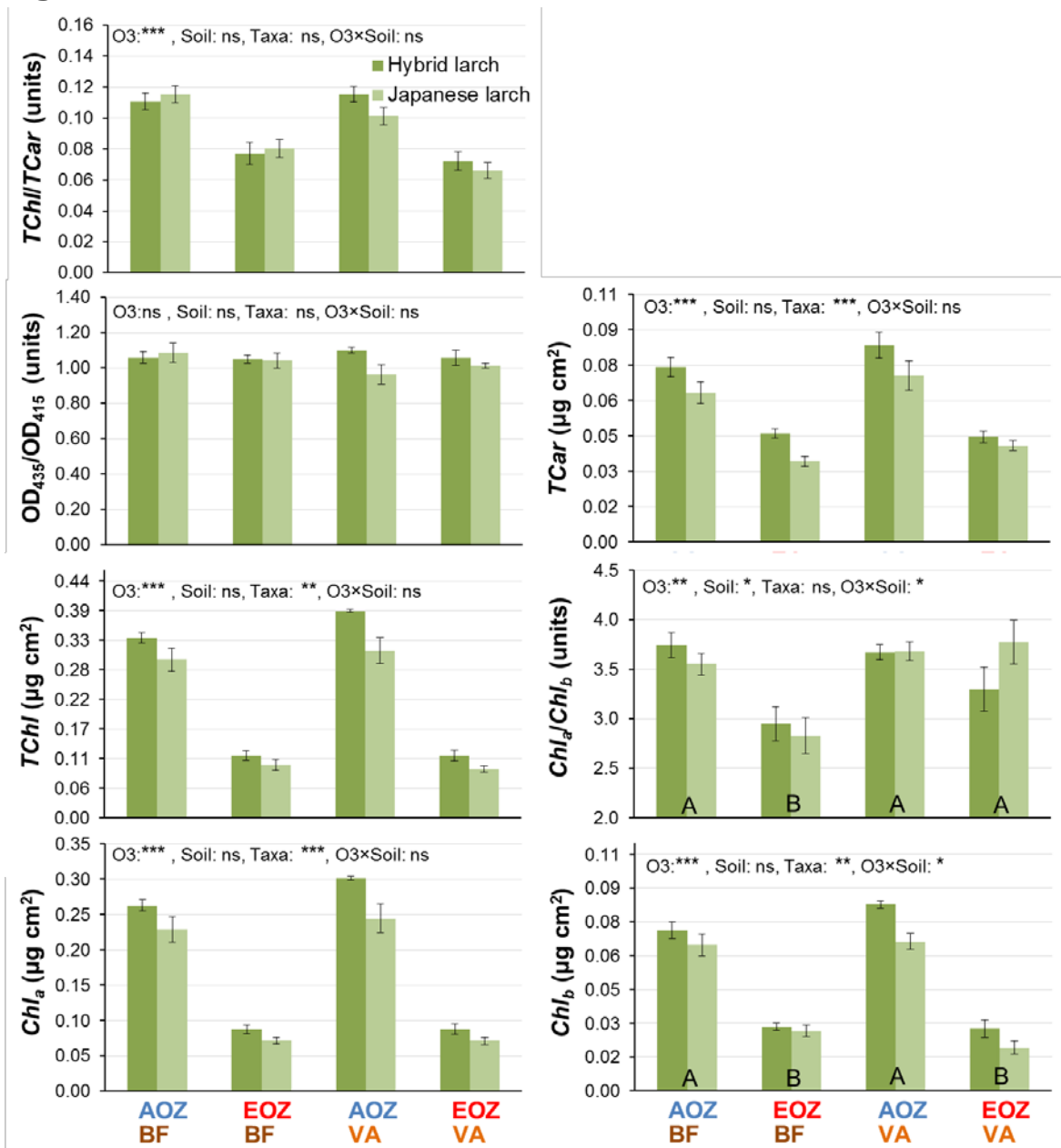
808 **Table 2**

	Ozone	Soil	Taxa	OzonexSoil	OzonexTaxa	SoilxTaxa	OzonexSoilxTaxa
Plant height	<i>F</i> =67, <i>P</i><0.001	<i>F</i> =0.06, <i>P</i> =0.807	<i>F</i> =32, <i>P</i><0.001	<i>F</i> =0.38, <i>P</i> =0.547	<i>F</i> =0.15, <i>P</i> =0.703	<i>F</i> =0.38, <i>P</i> =0.546	<i>F</i> =0.04, <i>P</i> =0.844
Crown thickness	<i>F</i> =89, <i>P</i><0.001	<i>F</i> =0.30, <i>P</i> =0.591	<i>F</i> =42, <i>P</i><0.001	<i>F</i> =0.62, <i>P</i> =0.443	<i>F</i> =0.02, <i>P</i> =0.894	<i>F</i> =0.40, <i>P</i> =0.536	<i>F</i> =0.30, <i>P</i> =0.592
Mean crown spread	<i>F</i> =54, <i>P</i><0.001	<i>F</i> =0.01, <i>P</i> =0.907	<i>F</i> =19, <i>P</i><0.001	<i>F</i> =0.14, <i>P</i> =0.711	<i>F</i> =0.01, <i>P</i> =0.938	<i>F</i> =0.19, <i>P</i> =0.669	<i>F</i> =0.01, <i>P</i> =0.938
Stem volume	<i>F</i> =221, <i>P</i><0.001	<i>F</i> <0.01, <i>P</i> =0.975	<i>F</i> =42, <i>P</i><0.001	<i>F</i> =1.8, <i>P</i> =0.200	<i>F</i> =0.53, <i>P</i> =0.477	<i>F</i> <0.00, <i>P</i> =0.958	<i>F</i>=4.79, <i>P</i><0.050
Chl_a	<i>F</i> =633, <i>P</i><0.001	<i>F</i> =1.13, <i>P</i> =0.304	<i>F</i> =17, <i>P</i><0.001	<i>F</i> =1.33, <i>P</i> =0.266	<i>F</i> =0.04, <i>P</i> =0.855	<i>F</i> =0.22, <i>P</i> =0.649	<i>F</i> =0.11, <i>P</i> =0.750
Chl_b	<i>F</i> =333, <i>P</i><0.001	<i>F</i> =0.21, <i>P</i> =0.655	<i>F</i> =11, <i>P</i><0.010	<i>F</i> =5.2, <i>P</i><0.050	<i>F</i> <0.01, <i>P</i> =0.957	<i>F</i> =2.94, <i>P</i> =0.106	<i>F</i> =0.153, <i>P</i> =0.701
TChl	<i>F</i> =557, <i>P</i><0.001	<i>F</i> =0.38, <i>P</i> =0.544	<i>F</i> =15, <i>P</i><0.010	<i>F</i> =2.2, <i>P</i> =0.156	<i>F</i> =0.03, <i>P</i> =0.867	<i>F</i> =0.7, <i>P</i> =0.422	<i>F</i> =0.01, <i>P</i> =0.940
Chl_a/Chl_b	<i>F</i> =13, <i>P</i><0.010	<i>F</i> =7.8, <i>P</i><0.050	<i>F</i> =0.23, <i>P</i> =0.636	<i>F</i> =6.6, <i>P</i><0.050	<i>F</i> =1.18, <i>P</i> =0.182	<i>F</i> =3.3, <i>P</i> =0.087	<i>F</i> =0.66, <i>P</i> =0.430
TCar	<i>F</i> =161, <i>P</i><0.001	<i>F</i> =4.3, <i>P</i> =0.055	<i>F</i> =16, <i>P</i><0.001	<i>F</i> =0.19, <i>P</i> =0.669	<i>F</i> =0.07, <i>P</i> =0.794	<i>F</i> =1.2, <i>P</i> =0.282	<i>F</i> =1.55, <i>P</i> =0.230
OD₄₃₅	<i>F</i> =0.32, <i>P</i> =0.582	<i>F</i> =0.85, <i>P</i> =0.370	<i>F</i> =1.91, <i>P</i> =0.186	<i>F</i> =0.24, <i>P</i> =0.631	<i>F</i> =0.15, <i>P</i> =0.700	<i>F</i> =3.39, <i>P</i> =0.084	<i>F</i> =1.23, <i>P</i> =0.285
TChl/TCar	<i>F</i> =82, <i>P</i><0.001	<i>F</i> =3.25, <i>P</i> =0.090	<i>F</i> =0.56, <i>P</i> =0.464	<i>F</i> =0.47, <i>P</i> =0.503	<i>F</i> =0.17, <i>P</i> =0.690	<i>F</i> =2.95, <i>P</i> =0.106	<i>F</i> =0.23, <i>P</i> =0.635

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811 **Fig 1**

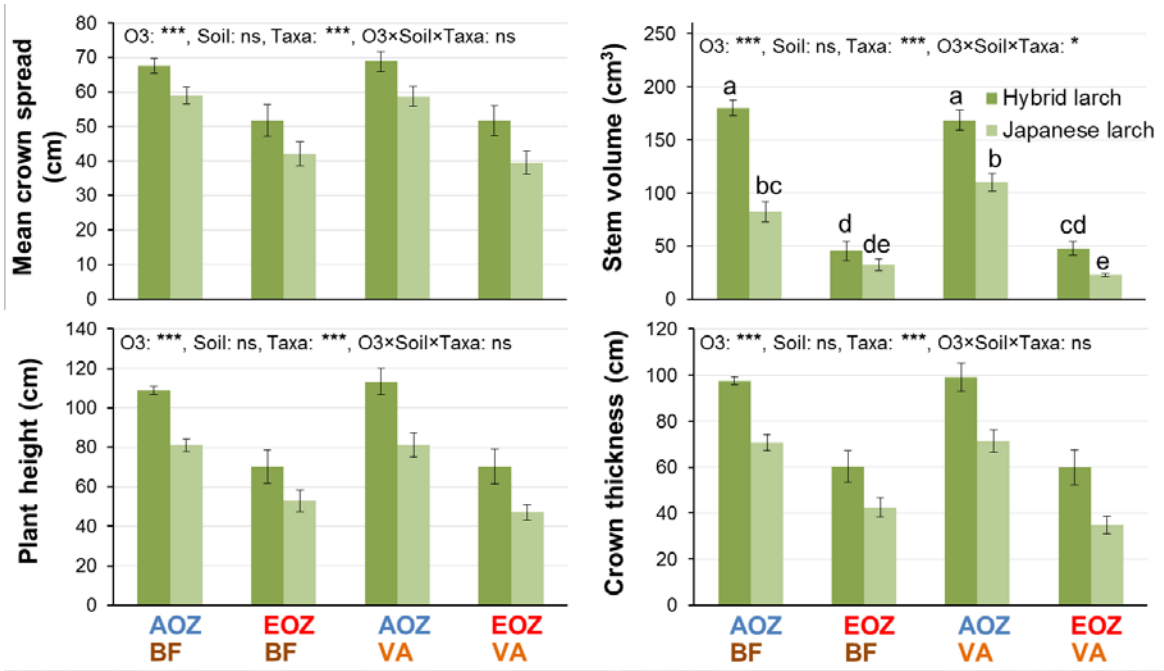


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815 **Fig 2**



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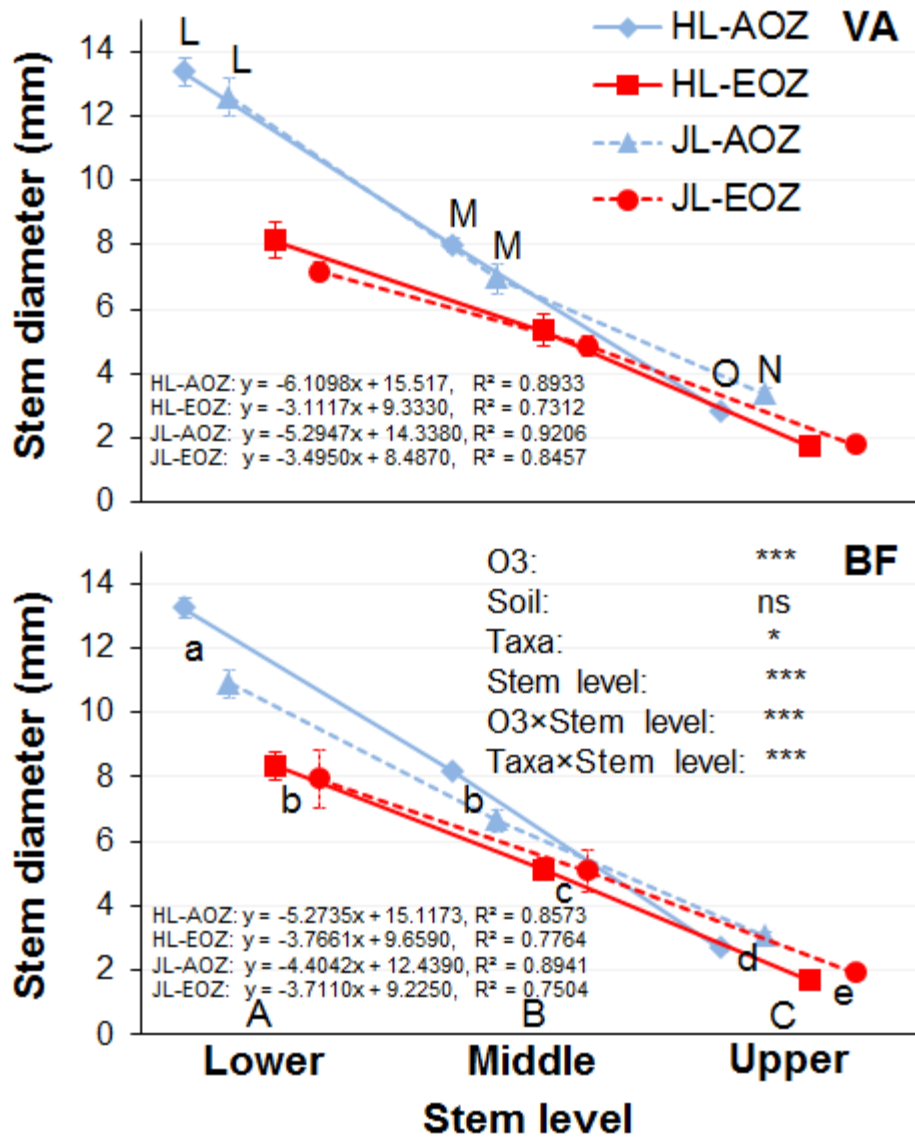
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824 **Fig 3**



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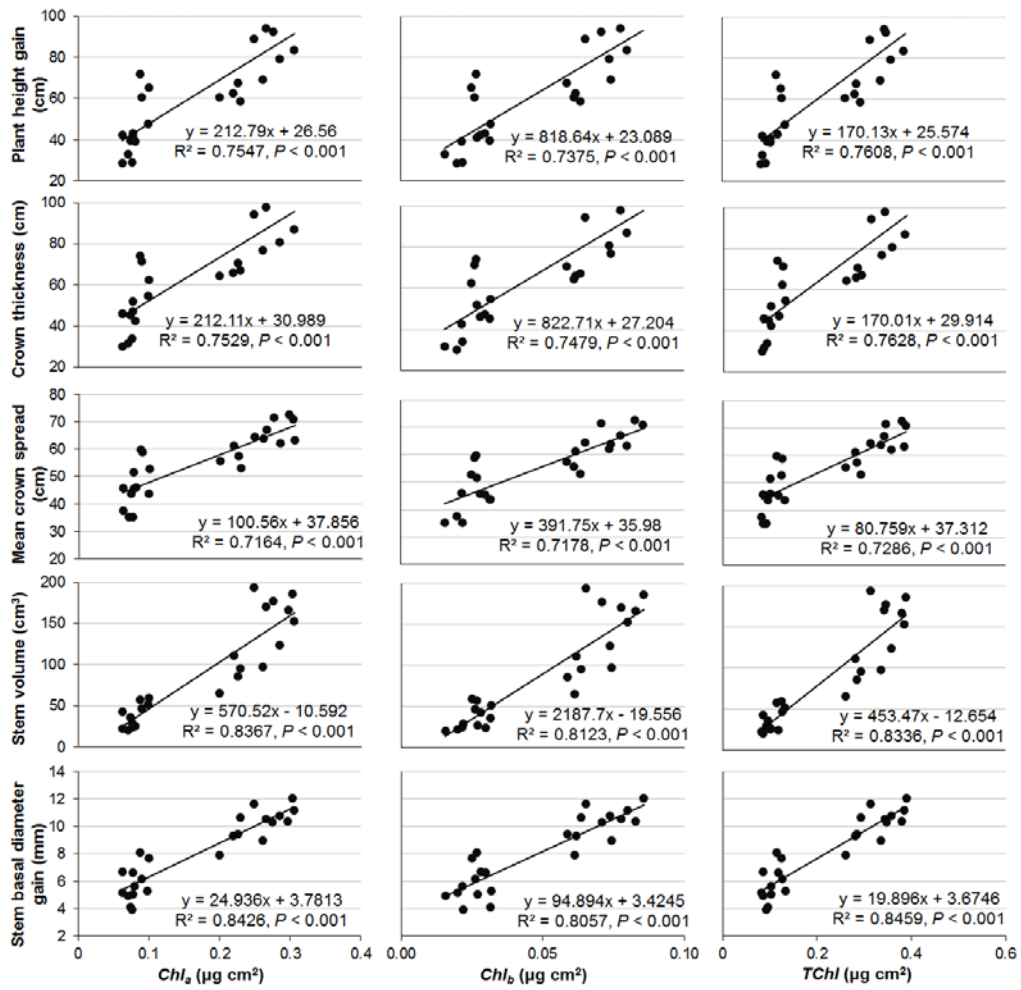
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830 **Fig 4**



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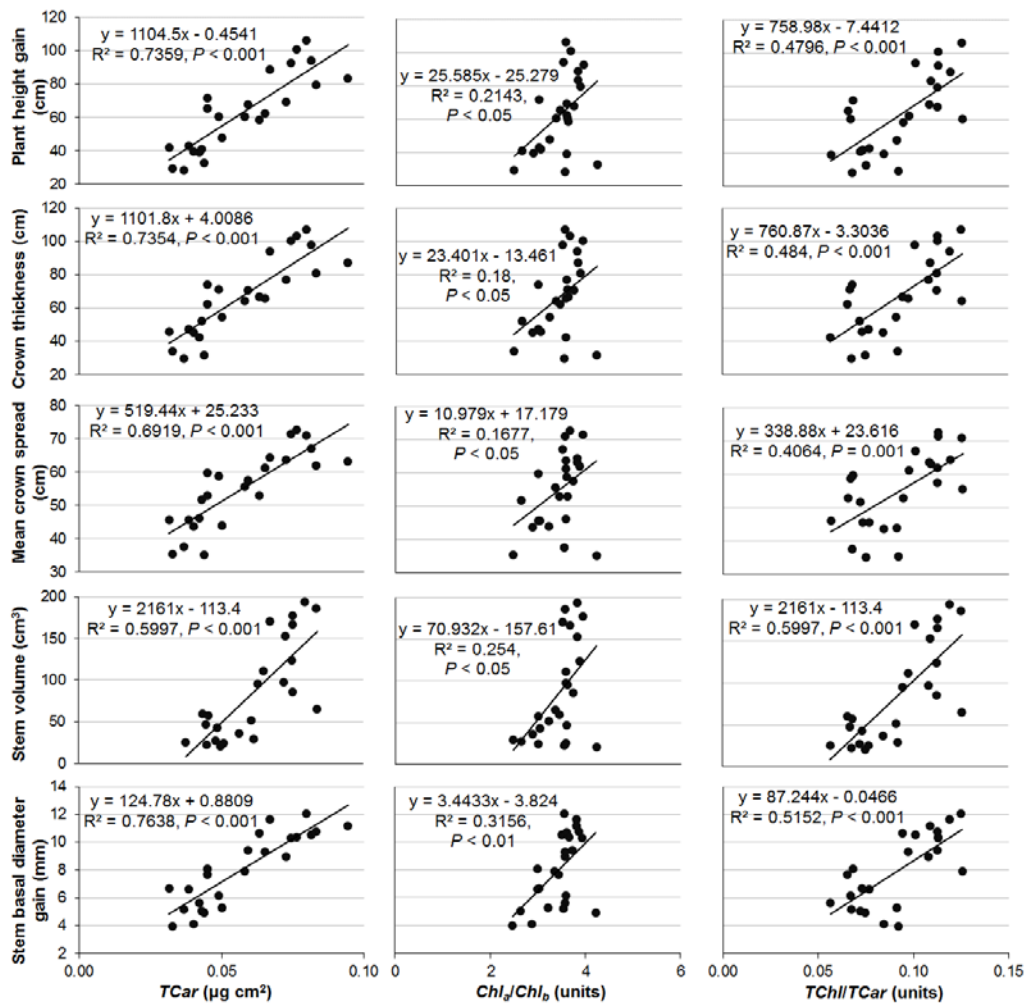
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Fig 5



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