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8 **Differential root responses in two cultivars of winter wheat (*Triticum aestivum* L.) to**
9 **elevated ozone concentration under fully open-air field conditions**

10

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15

16 **Abstract** The effect of elevated tropospheric ozone concentration [O₃] on root processes in
17 wheat systems of different O₃-sensitivity is not well understood. Two wheat cultivars (cv. Y15
18 and YN19) with contrasting O₃-tolerance were grown in a fully open-air O₃ enrichment
19 platform for one season. We found that elevated O₃ (EO₃) (50% above the ambient O₃)
20 significantly decreased the total biomass at all key growth stages and the yield of the
21 O₃-sensitive cultivar YN19 but did not affect those of the O₃-tolerant cultivar Y15. EO₃
22 significantly decreased the root biomass of two wheat cultivars at the jointing and grain filling
23 stages. EO₃ significantly decreased the root length, length density, surface area and volume of
24 the two cultivars at the jointing stage but increased those of YN19 at the grain filling stage.
25 EO₃ significantly increased the root activities (specific root respiration rates) of Y15 and YN19
26 at the jointing, heading and grain filling stages. EO₃ significantly decreased the contribution of

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27 fresh root respiration to soil respiration (CRS) of YN19 at the jointing stage but increased it at
28 the heading stage; however, it did not change the CRS of Y15 at any growth stages. The
29 present study indicates that the effects of EO₃ on root morphology and activity varied among
30 wheat cultivars, and suggest that we can breed O₃-tolerant cultivars to maintain crop yield
31 under higher [O₃] scenarios.

32
33 **Keywords** ozone pollution; root morphology; root activity; wheat; climate change

34 **Abbreviation**

35 O₃: ozone

36 EO₃: elevated O₃

37 AO₃: ambient O₃

38 O₃FACE: fully open-air O₃ enrichment

39 RPC: root-split growth and respiration partitioning chamber

40 RRS: ratios of root to shoot

41 R_{root}: root respiration rates

42 SR_{root}: specific root respiration rates

43 CRS: contribution of root respiration to soil respiration

44 **Introduction**

45 The tropospheric [O₃] has been increasing rapidly in many Asian countries in recent years
46 (Fowler et al. 2008, IPCC 2013). It is predicated that in less than 40 years, [O₃] in East and
47 South Asia will be 25 nL L⁻¹ higher than the current level of 30 – 50 nL L⁻¹ (Fowler et al. 2008).
48 Negative effects of EO₃ on the growth and production of main crops (e.g. wheat, rice, etc)
49 have been noted since the 1990s, which may get worse in the future (Feng and Kobayashi
50 2009, Avnery et al. 2011, Clausen et al. 2011, Burkart et al. 2013).

51 Previous studies mainly concentrated on the effects of O₃-stress on plant photosynthesis,
52 growth and production (Andersen 2003, Feng et al. 2003, Ainsworth and McGrath 2010),
53 highlighting that the EO₃ effects varied with O₃ level, plant species (Michael et al. 2012, Sarkar
54 and Agrawal 2012) and cultivars (Kou et al. 2012). For example, Kou et al. (2012) observed
55 that the effects of EO₃ on aboveground biomasses and yields differed among five wheat
56 cultivars. On the other hand, EO₃ decreased (Andersen 2003) or did not affect (Edwards 1991,

57 Coleman et al. 1996) the root biomass of trees. EO₃ alters the physiological processes of
58 plants (Nie et al. 1993, Soja and Soja 1995, Feng et al. 2008, Kou et al. 2012), thereby
59 affecting their root morphology and functions. Roots control plant growth through absorbing
60 soil moisture and nutrients (Casper and Jackson 1997), and influence nutrient (including
61 carbon) cycling in plant-soil systems (Schimel 1995, Lambers et al. 1996, Andersen 2003, Kou
62 et al. 2015). Root autotrophic respiration accounts for 12-50% of soil carbon emission
63 (Andrews et al. 1999, Jia et al. 2006). Cooley and Manning (1987) reported that EO₃ had larger
64 influence on roots than shoot.

65 Limited studies on EO₃ effects on crop roots indicated that EO₃ decreased soybean root
66 length (Wang et al. 2004) and volume (Zhao et al. 2012) but increased rice root activities (Kou
67 et al. 2013). However, the effects of EO₃ on wheat root morphology (root surface, root diameter,
68 root volume, or root length density) or activity (respiration) have rarely been studied, let alone
69 the potentially differential responses between O₃-tolerant and O₃-sensitive cultivars (Biswas et
70 al. 2008, Kou et al. 2012). This information is critical for the understanding of the influences of
71 increasing [O₃] on the response mechanisms of roots and soil C cycling in wheat systems.
72 Two local major wheat cultivars (cv. Yangmai-15 (Y15) and Yangnong-19 (YN19)) in
73 Southeast China were examined in this study as O₃-tolerant and -sensitive cultivar,
74 respectively. The former cultivar showed less decrease in yield in response to EO₃ than the
75 latter according to our previous research (Kou et al. 2012). The objectives of this study were to
76 investigate the effects of open-air O₃ enrichment on (i) the yield and dry matter belowground
77 partitioning, (ii) the root morphology (root length, surface area, average diameter, volumes and
78 length density), and (iii) the root activity (root respiration) and the CRSs of two wheat cultivars
79 with contrasting O₃ tolerance.

80

81 **Materials and methods**

82

83 Experimental site and ozone fumigation

84 The field trial was conducted at Jiangdu, Jiangsu Province, China (N 32°35', E 119°42'), in
85 which summer rice-winter wheat rotation has long been practiced. The site was located at the
86 subtropical marine climatic zone with a mean annual rainfall of 980 mm and a mean annual

87 temperature of 14.9 °C. The average 7 h mean AO₃ from March to May was 45 nL L⁻¹ during
88 the wheat growing season and varied little in recent years (Tang et al. 2011). The soil in the
89 region is a sandy-loam according to the US classification (Soil Survey Staff 2003). The topsoil
90 (0–15 cm) properties are as follows: clay (<0.002 mm) of 13.6%, silt (0.002–0.02 mm) of
91 28.5%, bulk density of 1.16 g cm⁻³, pH of 7.2, organic carbon of 18.4 g kg⁻¹, total N of 1.45 g
92 kg⁻¹, available N of 106 mg kg⁻¹, and Olsen P of 33.8 mg kg⁻¹.

93 An O₃FACE system was used in this study. This system includes three rings under ambient
94 O₃ concentration (hereinafter referred to as AO₃) and three rings under enriched O₃
95 concentration (hereinafter referred to as EO₃). Each ring was separated from the adjacent
96 rings by > 70 m to avoid O₃ cross contamination. The O₃ exposure (from 9:00 hours to sunset
97 every day) was continuous from March 1 to May 28 (i.e. the regreening stage to harvest)
98 during the wheat growth season in 2012. To estimate the effects of higher O₃ concentration
99 projected for 2050, the target O₃ concentration for EO₃ treatment was 50% higher than AO₃.
100 The O₃ release was halted on rainy day to limit acute damage. The 24-h average O₃
101 concentrations from initial fumigation to harvest were 27.5 and 40.3 nL L⁻¹ at AO₃ and EO₃,
102 respectively. The details of the experiment design and the performance are described in Tang
103 et al. (2011) and Kou et al. (2014).

104

105 Wheat cultivation and root-split growth and respiration partitioning chamber

106 Two major local wheat cultivars (cv. Y15 and YN19) were grown in each ring. To ensure the
107 measurements of morphology and physiological function of roots of field-growth wheat, the
108 RPC was used in the study. The RPC could provide favorable soil conditions for the growth of
109 wheat and maintain normal wheat growth without root growth restriction as reported in Kou et
110 al. (2007). The design of RPC and the effectiveness evaluation of respiration (root and soil
111 respiration) partitioning are described in Kou et al. (2007).

112 After the harvest of the rice plants (previous crop), the topsoil in the ambient plot was
113 collected, separated from visible roots and residues, and then mixed through a 2-cm sieve.
114 The bottom of each RPC was wrapped with a nylon cloth (aperture 0.1 mm) to keep the roots
115 within the chamber. Eight RPCs were buried into the field at 15 cm depth in each subplot of the
116 three EO₃ and AO₃ rings. Soil was packed in the RPC to a bulk density of approximately 1.16

117 g cm⁻³ and watered to 60% of soil water-filled pore space (WFPS). Eight wheat seeds for each
118 cultivar (Y15 and YN19) were sown in each seed chamber of RPC on November 15, 2011,
119 with each cultivar sown to four RPCs in each subplot. Six wheat seedlings were maintained in
120 each chamber to mimic the sowing density of the local practice. Urea nitrogen (148.1 mg N
121 kg⁻¹ air-dried soil), phosphorus (44.4 mg P₂O₅ kg⁻¹ air-dried soil) and potassium (44.4 mg K₂O
122 kg⁻¹ air-dried soil) fertilizers were applied to each treatment, equivalent to 225 kg N ha⁻¹, 75 kg
123 ha⁻¹ P₂O₅ and 75 kg ha⁻¹ K₂O, respectively. Fertilizer application and management were the
124 same as the standard cultivation practices in the region. Wheat plants were harvested on June
125 5, 2012. Grain yield of each cultivar was calculated based on the sample from the rest PRC
126 each subplot at ripening stage.

127

128 Respiration and root morphology and biomass measurement

129 Soil and root respiration rates, specific root respiration rate, and CRS were measured at the
130 jointing (March 25), heading (April 27), and grain filling stages (May 21). During the
131 measurement of fresh root respiration, the RPC was dug out (three for each cultivar each
132 treatment) and immediately transferred to an irrigation ditch (30 m from the treatment). The
133 roots for measuring root respiration in RPC were extracted by washing soil off with water.
134 Three gas samples for CO₂ analysis were collected using the static opaque chamber
135 technique at 0, 20, and 40 min. after chamber closure (Wang and Wang 2003). Before the
136 RPC was dug out, soil respiration was measured in situ as described above. Gas samples of
137 root and soil respiration were analyzed using a gas chromatograph (Shimadzu, GC-14B)
138 equipped with a thermal conduction detector (TCD). After the measurement of root respiration,
139 the roots in RPC were extracted to measure root morphology with a root scanner (EPSON
140 Perfection V700 Photo). The above- and belowground biomass of wheat grown in the RPC
141 was oven-dried at 80°C for 72 h and weighed. Whole plants were divided into root, straw and
142 grain.

143

144 Statistical analysis

145 All data were subjected to ANOVA using the SPSS 11.5 (Windows version 11.5; SPSS, Inc.,
146 Chicago, IL). Statistically significant differences were determined using analysis of variance

147 (ANOVA) and least significant difference (LSD) calculations at $p = 0.05$.

148

149 **Results**

150

151 Yield and biomass partitioning

152 EO_3 did not affect the grain yield of Y15 but significantly decreased that of YN19 by 14.4% (Fig.
153 1). Similar trend was observed for the total biomass of each cultivar at the three key stages
154 (Table 1). EO_3 significantly increased the shoot biomass of Y15 at the jointing stage but
155 significantly decreased that of YN19 at all the three growth stages. EO_3 significantly
156 decreased the root biomass of Y15 and YN19 at the jointing and grain filling stages. EO_3
157 significantly decreased the RRS of Y15 and YN19 by 43.1% and 26.6% at the jointing stage,
158 but significantly increased that of YN19 by 9.1% and 5.9% at the heading and grain filling
159 stages, respectively. Significant interaction between O_3 and cultivar was noted only at the
160 jointing stage (Table 1).

161

162 Root morphology

163 From the jointing stage to the heading stage, root morphology index (lengths, length densities,
164 surface areas and volumes) of two cultivars at EO_3 and AO_3 increased rapidly (Table 2). From
165 the heading stage to the grain filling stage, the root length and root length density of Y15 were
166 increased at both EO_3 and AO_3 , but those of YN19 showed opposite responses. EO_3
167 significantly decreased root lengths, root length densities, root surface areas and root volumes
168 of Y15 and YN19 by 44.7-45.8% and 34.6-37.8%, respectively, at the jointing stage, but
169 significantly increased those of YN19 by 71.8-91.4% at the grain filling stage (Table 2). EO_3
170 significantly increased the average root diameter of Y15 by 7.8% at the grain filling stage and
171 that of YN19 by 17.8% at the jointing stage, but the interactive effects of O_3 and cultivar on
172 root diameter were not significant at any growth stage (Table 2).

173

174 Root activity and respiration

175 The R_{root} s of two cultivars at EO_3 and AO_3 increased from the jointing stage to the heading
176 stage and then decreased after the heading stage (Fig. 2). EO_3 significantly increased the

177 R_{root} of Y15 by 12.3% at the grain filling stage but not at the jointing or heading stage. EO_3
178 significantly decreased the RR of YN19 by 38.4% at the jointing stage but increased the R_{root} s
179 by 55.2% and 35.9% at the heading and grain filling stages, respectively. The SR_{root} s of two
180 cultivars at EO_3 and AO_3 declined from the jointing stage to the grain filling stage. EO_3
181 significantly increased the SR_{root} s of Y15 and YN19 at all growth stages, by 9.8-69.5% and
182 8.2-62.2%, respectively.

183 Contribution of root respiration to soil respiration

184 The CRSs of wheat cultivar Y15 and YN19 were 19.4-41.6% and 15.3-45.8%, respectively,
185 across the three key growth stages (Fig. 3). The CRSs of two cultivars increased from the
186 jointing stage, peaked at the heading stage, followed by a sharp decline, regardless of O_3
187 concentrations. EO_3 had no significant effect on the CRS of Y15 at any growth stages (Fig. 3).
188 EO_3 significantly decreased the CRS of YN19 by 15.7% at the jointing stage but increased it
189 by 13.3% at the heading stage.

190

191 Discussion

192

193 Dry matter accumulation and partitioning

194 The present study suggests that there exists an intraspecific variation in the grain yield and
195 biomass responses to EO_3 (Table 1). In terms of grain yield and biomass production, wheat
196 cultivar Y15 is more tolerant to O_3 pollution than YN19. The decrease in total and shoot
197 biomass of wheat cultivar YN19 in response to EO_3 was in line with previous findings (Feng et
198 al. 2003, 2008, Emberson et al. 2009). This reduction in grain yield and biomass has been
199 attributed to impaired photosynthesis (Nie et al., 1993) and C assimilation under EO_3 (Cooley
200 and Manning 1987, Fiscus et al. 2005). Grain yield is dependent on root activity/ nutrient
201 uptake and the allocation of nutrients among shoot and grain (Lambers et al., 1996, Zheng et
202 al., 2013, Kou et al., 2017). Our study supports the proposition that EO_3 had larger influence
203 on root than shoot (Cooley and Manning 1987). This occurred at the early and late wheat
204 growth stages (Table 1), which indicates that the two wheat cultivars reduced the allocation of
205 assimilated C to the roots at these stages. This was probably because the plant increases the
206 allocation of assimilated C to the aboveground parts to satisfy the respiratory demands for the

207 maintenance and repair of damaged leaf tissues under EO₃ (Andersen 2003). The decrease in
208 root biomass at the late growth stage could also be due to the senescence of wheat grown
209 under EO₃ (Soja and Soja 1995). Unlike the O₃-tolerant cultivar Y15, YN19 significantly
210 increased the RRS at the middle and late growth stages. This further highlights that the two
211 cultivars may have different thresholds to O₃-resistance (Ewert and Pleijel 1999), and that
212 more assimilated C may be allocated to belowground parts in O₃-sensitive cultivar than
213 O₃-tolerant cultivar at the middle and late growth stages. Other studies have also reported that
214 crops grown under O₃ would alter C assimilation and partitioning (Fiscus et al. 2005, Kou et al.
215 2015).

216

217 Root morphology

218 The decreases in root lengths, root length densities, root surface areas and root volumes of
219 the two cultivars at the jointing stage further demonstrated the inhibitory effect of EO₃ on root
220 growth at the early growing stage. This explains the reduction in the root biomass and RRS of
221 the two wheat cultivars in response to EO₃ at the jointing stage. A reduction in root volume
222 (Zhao et al. 2012) and root length (Wang et al. 2004) was also observed for soybean under
223 EO₃. The O₃-induced stress on root morphology impaired the ability of roots to absorb water
224 and nutrients in soil. Our previous studies reported that EO₃ decreased the K concentrations
225 of the two wheat cultivars (Kou et al. 2014). The negative effects of EO₃ on root morphology
226 were weakened at the later growth stage, evidenced by the increase in root average diameter
227 of Y15 ($p < 0.05$) and YN19 ($p = 0.085$) at the grain filling stage (Table 2). This would improve the
228 ability of root to water and nutrient uptake and delay the senescence of root.

229

230 Root respiration and its contribution to soil respiration

231 Root respiration is a physiological process and is affected mainly by plant development. Our
232 results suggest that cultivar and growth stage are important factors for the physiological
233 response of wheat to EO₃. The differential responses of root respiration to EO₃ between
234 wheat cultivars and growth stages were in contrast to the pervious findings which
235 demonstrated either increase (Edwards 1991, Nouch et al. 1991, Scagel and Andersen 1997)
236 or decrease (Hofstra et al. 1981, Nobel and Palta 1989) in root respiration in other plants under

237 EO_3 . The neutral (Y15) or negative (YN19) effects of EO_3 on root respiration at the jointing
238 stage was due to reduced biomass and carbohydrate allocation to belowground (Table 1). At
239 the heading stage, EO_3 had no effects on root respiration of Y15 but increased that of YN19.
240 The former might be explained by the neutral effects of EO_3 on root biomass and RRS of Y15,
241 whereas the latter could be attributed to increased RRS with unaffected root biomass of YN19
242 in response to O_3 (Fig. 2). At the grain filling stage, the increase in root respiration of two
243 cultivars under EO_3 was associated with enhanced root morphology, which facilitated greater
244 moisture and nutrient uptake to maintain normal reproductive growth. These results
245 highlighted that the influence of EO_3 on plant physiology varied with cultivars and growth
246 stages, and that these factors should be considered when studying the effect of EO_3 on root
247 system including root respiration, root morphology and other belowground processes.

248 Root activity can be represented by specific root respiration (SR_{root}) (Kou et al. 2007). The
249 positive effects of EO_3 on the SR_{root} s of two wheat cultivars (Fig. 2) were also observed for rice
250 plants (Kou et al. 2013). These results highlight that the C assimilation and allocation to the
251 belowground parts satisfied the increased respiratory demands of root tissues under EO_3 , and
252 that EO_3 enhanced root activity and increased more water and nutrient uptake to maintain
253 plant growth.

254 The effect of EO_3 on CRS was either negative (jointing stage) or positive (heading stage)
255 for YN19 but not significant for Y15 at any growth stages, which further suggests that Y15 is an
256 O_3 -tolerant cultivar, compared with YN19.

257

258 **Conclusions**

259

260 The effects of EO_3 on wheat root morphology and activity, and CRS varied with growth stages
261 and cultivars. EO_3 decreased biomass partitioning to belowground but enhanced root activity
262 at three growth stages. Differential responses to EO_3 were observed between O_3 -sensitive
263 and tolerant wheat cultivars. EO_3 decreased the CRS of O_3 -sensitive cultivar YN19 at the
264 early stage but adversely affected it at the middle growth stage. However, the CRS of
265 O_3 -tolerant cultivar Y15 was unaffected by EO_3 at any growth stages. Understanding these
266 differential responses to EO_3 in a longer term at various temperature and rainfall conditions is

267 critical for evaluating the belowground processes and C cycling in cropping systems in a high
268 O₃ world.

269

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275

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1 Table 1-2:

2 **Table 1** Biomass and ratio of root to shoot of two wheat cultivars as affected by elevated O₃ at

3 different growth stages

Stage	Item	Y15		YN19		ozone		cultivar		ozone* cultivar	
		AO ₃	EO ₃	AO ₃	EO ₃	F value	p value	F value	p value	F value	p value
Jointing stage	Shoot biomass (g m ⁻²)	169.0±2.9	181.3±3.9	162.9±7.4	145.1±2.3	0.359	0.566	21.185	0.002	10.834	0.011
	Root biomass (g m ⁻²)	19.5±0.7	11.9±1.8	12.9±1.1	8.4±0.5	27.865	0.001	19.174	0.002	1.855	0.210
	Total biomass (g m ⁻²)	188.5±2.6	193.2±4.0	175.8±8.4	153.5±1.8	3.228	0.110	28.353	0.001	7.586	0.025
	Root/ shoot ratio	0.116±0.006	0.066±0.01	0.079±0.004	0.058±0.004	30.129	0.001	11.463	0.010	5.695	0.045
Heading stage	Shoot biomass (g m ⁻²)	732.7±32.4	744.8±51.4	868.1±43.2	793.7±7.5	0.692	0.430	6.045	0.039	1.329	0.282
	Root biomass (g m ⁻²)	93.1±1.5	90.7±2.4	95.3±2.5	95.0±3.5	0.119	0.739	0.702	0.426	0.072	0.795
	Total biomass (g m ⁻²)	825.8±33.8	835.4±57.1	963.4±45.4	888.7±5.3	0.651	0.443	5.609	0.045	1.094	0.326
	Root/ shoot ratio	0.127±0.004	0.122±0.004	0.110±0.003	0.120±0.005	0.167	0.694	4.167	0.076	1.500	0.256
Grain filling stage	Shoot biomass (g m ⁻²)	1410.1±98.7	1460.9±69.2	1711.9±24.1	1549.7±18.2	0.804	0.396	9.864	0.014	2.934	0.125
	Root biomass (g m ⁻²)	84.3±1.7	78.4±2.0	88.1±2.7	84.4±0.3	6.258	0.037	6.697	0.032	0.330	0.581
	Total biomass (g m ⁻²)	1494.4±98.4	1539.3±70.5	1799.9±25.3	1634.1±18.5	0.935	0.362	10.244	0.013	2.840	0.130
	Root/ shoot ratio	0.060±0.004	0.054±0.002	0.051±0.001	0.054±0.001	0.200	0.667	1.800	0.217	1.810	0.212

4 Data are expressed as means ± 1 SE (n=3). AO₃ refers to ambient O₃ concentration. EO₃

5 refers elevated O₃ concentration with 150% daily average AO₃.

6 **Table 2** Root morphology index of two wheat cultivars as affected by elevated O₃ at different

7 growth stages

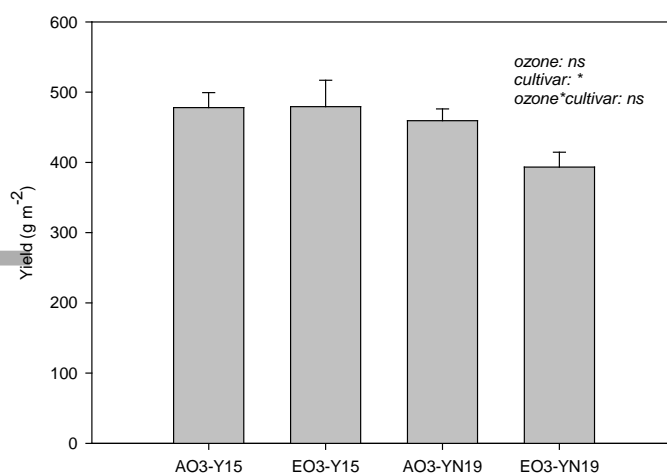
Stage	Item	Y15		YN19		ozone		Cultivar		ozone* Cultivar	
		AO ₃	EO ₃	AO ₃	EO ₃	F value	p value	F value	p value	F value	p value
Jointing stage	Length (m m ⁻²)	4695.2±156.0	2569.2±472.6	3117.3±276.4	1939.3±57.0	33.345	<0.001	14.889	0.005	2.745	0.136
	Surface area (dm ² m ⁻²)	511.8±23.7	281.1±48.6	329.8±29.8	210.6±6.7	31.734	<0.001	16.517	0.004	3.224	0.11
	Diameter (cm)	0.350±0.006	0.354±0.001	0.342±0.008	0.403±0.051	1.602	0.241	0.620	0.454	1.233	0.299
	Length density (m m ⁻³)	2086.8±69.3	1141.9±210.1	1385.5±122.9	861.9±25.3	33.345	<0.001	14.889	0.005	2.745	0.136
	Volume (cm ³ m ⁻²)	445.1±27.8	246.2±40.3	278.9±26.4	182.5±7.1	27.699	0.001	16.782	0.003	3.342	0.105
Heading	Length (m m ⁻²)	15856.9±1370.3	16272.7±2286.7	19169.3±1359.6	19599.3±880.6	0.074	0.793	5.43	0.046	0.001	0.996

stage	Surface area (dm ² m ⁻²)	1603.6±151.4	1690.5±237.8	2020.2±88.4	2099.8±74.7	0.299	0.600	7.345	0.027	0.001	0.981
	Diameter (cm)	0.328±0.005	0.339±0.005	0.335±0.012	0.346±0.004	0.731	0.418	2.588	0.146	0.540	0.483
	Length density (m m ⁻³)	7047.5±609.0	7232.3±1016.3	8519.7±604.3	8710.8±391.4	0.074	0.793	5.43	0.046	0.001	0.996
	Volume (cm ³ m ⁻²)	1302.6±136.7	1411.4±204.0	1708.1±25.9	1802.5±55.3	0.645	0.445	9.916	0.014	0.003	0.956
	Length (m m ⁻²)	18682.8±1264.3	17738.4±607.6	11659.9±815.3	20034.0±516.4	19.041	0.002	7.709	0.024	29.953	0.001
Grain	Surface area (dm ² m ⁻²)	1708.2±92.7	1666.7±63.6	1010.5±80.5	1833.7±49.8	28.269	0.001	13.035	0.007	34.6	0.001
filling	Diameter (cm)	0.293±0.004	0.316±0.007	0.281±0.005	0.294±0.008	1.873	0.208	1.767	0.22	0.887	0.374
stage	Length density (m m ⁻³)	8303.5±561.9	7883.7±270.0	5182.2±362.3	8904.0±229.5	19.041	0.002	7.709	0.024	29.953	0.001
	Volume (cm ³ m ⁻²)	1252.0±52.0	1252.6±52.0	701.4±63.0	1342.5±42.7	36.772	<0.001	18.962	0.002	36.644	<0.001

8 Data are expressed as means ± 1 SE (n=3). AO₃ refers to ambient O₃ concentration. EO₃

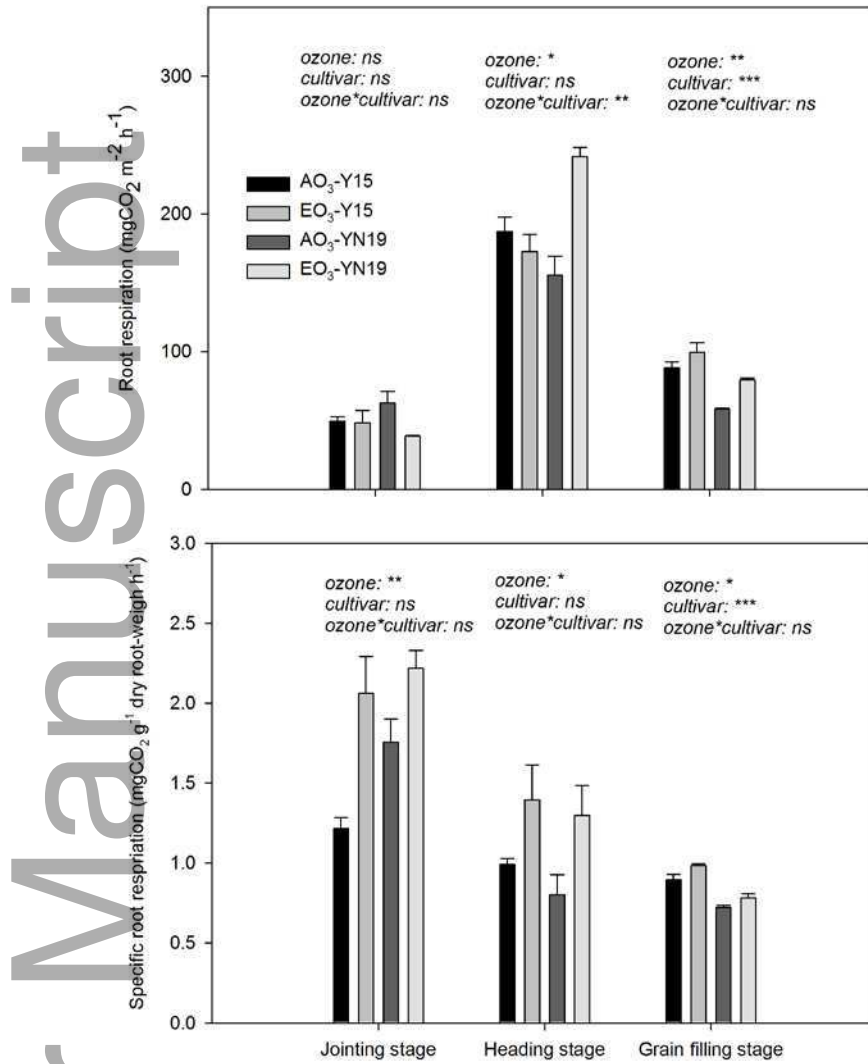
9 refers elevated O₃ concentration with 150% daily average AO₃.

1 Fig. 1-3:



2

3 **Fig. 1** Grain yields of two wheat cultivars (Y15 and YN19) under AO₃ (ambient O₃)
4 concentration) and EO₃ (elevated O₃ concentration with 150% daily average AO₃) during the
5 jointing stage to harvest of wheat in 2012. For ANOVA results, * and *ns* are significant at $p <$
6 0.05 and not significant, respectively. Values are means \pm 1SE (n=3)



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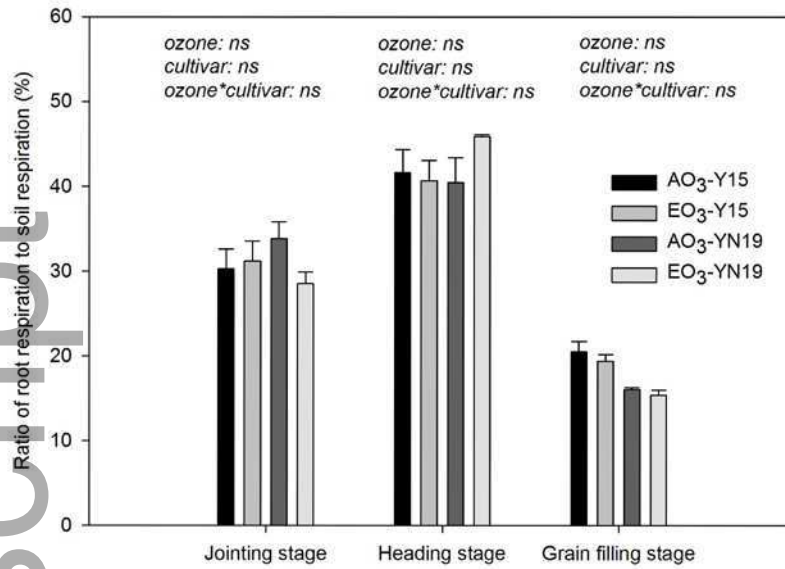
8 **Fig. 2** Root respiration rate and specific root respiration rate (root respiration rate per unit root

9 biomass) of two wheat cultivars at different growth stages. AO₃ refers to ambient O₃

10 concentration. EO₃ refers elevated O₃ concentration with 150% daily average AO₃. For

11 ANOVA results, *, **, and *** significant differences are at $p < 0.05$, $p < 0.01$ and $p < 0.001$,

12 respectively. ns, not significant. Values are means \pm 1SE (n=3)



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Fig. 3 Contribution of root respiration to soil respiration of two wheat cultivars at different growth stages. AO₃ refers to ambient O₃ concentration. EO₃ refers elevated O₃ concentration with 150% daily average AO₃. For ANOVA results, *ns* is not significant. Values are means ± 1SE (n=3)