

1 **Thirsty tree roots exude more carbon**

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10 **Abstract**

11 Root exudation is an important input of carbon into soils and affects plant and soil  
12 communities, but little is known about the effect of climatic factors such as drought on  
13 exudation and its ability to recover. We studied the impact of increasing drought on root  
14 exudation and its subsequent recovery in the Mediterranean tree species *Quercus ilex* in a  
15 greenhouse study by measuring the amount of total organic carbon in exudates. The amount  
16 of exudation per unit root area increased with drought duration and was 21% higher under  
17 the most extreme drought scenario compared with the non-droughted control. The amount of  
18 root exudation did not differ between the treatments following six weeks of re-watering,  
19 indicating a strong capacity for recovery in this species. We concluded that drought could  
20 affect the amount of root exudation which could in turn have a large impact on microbial  
21 activity in the rhizosphere, and alter these microbial communities, at least in the short term.  
22 This tree species may be able to return to normal levels of root exudation after a drought  
23 event, but long-term exudate-mediated impacts on Mediterranean forest soils may be an  
24 unforeseen effect of drought.

25

26 **Key words:** Carbon; Drought; Mediterranean; *Quercus ilex*; Root Exudates;  
27 Rhizodeposition.

## 28 **Introduction**

29 Drought intensity is increasing in many parts of the globe, through more frequent and longer  
30 periods of water stress (Dai 2011; Field et al. 2014; Touma et al. 2015), and this is likely to  
31 have negative impacts on plants and soils in both natural and agricultural habitats. Much  
32 remains to be understood about the impacts of water stress on plant-soil interactions and  
33 belowground processes. Rhizodeposition is the release of a wide range of compounds from  
34 roots into the soil (Bais et al. 2006), and the characterisation of rhizodeposits of tree species  
35 has become an important research focus (Prescott and Grayston 2013). Compounds released  
36 from roots as rhizodeposits can be divided into water-soluble exudates (e.g. sugars, amino  
37 acids, organic acids, and enzymes) and water-insoluble materials (e.g. mucilage, sloughed  
38 cells, and dying roots) (Merbach et al. 1999; Wichern et al. 2008).

39 Rhizodeposition can represent an important loss of carbon (C) from plants, estimated  
40 at 2-11% of the C fixed during photosynthesis (Jones et al. 2004; Jones et al. 2009; Pinton et  
41 al. 2007). However, the significance of rhizodeposition is not only the amount of C that is  
42 released, but the further impacts it has on microbial activity in the rhizosphere (Bais et al.  
43 2006; Finzi et al. 2015; Huang et al. 2014). The majority of this C efflux is likely passively  
44 diffused, but roots may also increase C efflux under certain conditions, such as in response to  
45 toxic compounds (Badri and Vivanco 2009; Morel et al. 1986) or to increase nutrient  
46 mobilisation from the soil (Farrar et al. 2003). Root exudation is an important C source for  
47 soil microorganisms, and changes in the amount and composition of root exudates can have  
48 further effects on the characteristics of soil communities (Dennis et al. 2010; Haichar et al.  
49 2008; Paterson et al. 2007), making it a key linkage between plants and soils. Overall, root  
50 exudation and other rhizosphere processes are increasingly being seen as important drivers of  
51 terrestrial C and nutrient cycling on an ecosystem scale (Bardgett et al. 2014; Finzi et al.  
52 2015).

53           The effect of drought on root exudation is not well understood, with varied responses  
54 reported amongst studies, so effects may be difficult to predict (Preece and Peñuelas 2016).  
55 For example, C inputs (per individual and per gram of plant biomass) from rhizodeposits  
56 increased for *Lolium perenne*, *Festuca arundinacea*, and *Medicago sativa* in a 40-day  
57 drought pot experiment (Sanaullah et al. 2012) and for a mixture of perennial grasses and  
58 herbs water stressed for 56 days in a field experiment in a mountain meadow (Fuchslueger et  
59 al. 2014). In contrast, rhizodeposition decreased for *Calluna vulgaris* during drought in a 56-  
60 day field study with sites in the UK and Denmark (Gorissen et al. 2004). A recent review,  
61 summarising drought studies that measured root exudation, suggested that low-moderate  
62 drought increases exudation, but this effect is more variable under extreme water stress  
63 (Preece and Peñuelas 2016).

64           Periods of drought are predicted to increase in the Mediterranean region in the next  
65 decades (Field et al. 2014), but the effects of drought on root exudation in Mediterranean  
66 species have not been investigated. The holm oak, *Quercus ilex* L., is a key tree species  
67 throughout the Mediterranean Basin and has been well-studied, so it is a good candidate for  
68 investigating the impacts of drought on plant-soil interactions throughout this region. Both  
69 experimental and natural droughts have had negative impacts on survival and growth of this  
70 species, for example, a five-year experimental drought decreased stem diameter and  
71 increased stem mortality (Ogaya and Peñuelas 2007). Recurrent natural droughts have had a  
72 negative impact on survival and regeneration of the species, leading to changes in the forest  
73 canopy and loss of resilience (Lloret et al. 2004; Peñuelas et al. 2001), although there is  
74 evidence that a dampening of the drought effects occurs over longer periods of more than a  
75 decade (Barbeta et al. 2013).

76           We thus determined the effect of increasing drought intensities on *in situ* root  
77 exudation in a greenhouse experiment with three-year-old *Q. ilex* saplings. We hypothesised

78 that: (1) low-intensity (short duration) drought would increase exudation, whereas high-  
79 intensity (long-duration) drought might decrease it, based upon patterns found in the existing  
80 literature (Preece and Peñuelas 2016); (2) root exudation would be able to recover when the  
81 drought conditions ended, as this species is adapted to the drought-prone Mediterranean  
82 climate, but perhaps not following high intensity drought, which could lead to irreversible  
83 damage of the roots.

84

## 85 **Materials and methods**

### 86 *Plant and soil material*

87 A greenhouse experiment was established at the Autonomous University of Barcelona  
88 (Spain) in May 2015. The experiment comprised 180 three-year-old *Quercus ilex* L. (holm  
89 oak) saplings (provided by Forestal Catalana, Barcelona, Spain). Plants were re-potted in 3.5  
90 l pots, with a substrate consisting of 45% autoclaved peat, 45% sand, and 10% natural soil  
91 inoculum. The soil was collected from a natural holm oak forest on a south-facing slope (25%  
92 slope) in the Prades Mountains in north-eastern Spain (41°13'N, 0°55'E; 930 m a.s.l.). The  
93 experiment was designed to include three soil types of control, droughted, and sterilised, with  
94 60 plants in each soil. Therefore, the natural soil inoculum varied amongst the treatments:  
95 topsoil was collected from the control and drought plots of the long-term drought experiment  
96 for inoculating the corresponding control and drought soil treatments, respectively, in the  
97 current experiment. The sterilised soil treatment received autoclaved soil from the control  
98 plots. However, there was no difference in exudation between soil types, (data with points  
99 separated by soil is shown in Supplementary Figure S1) so data was pooled into one group  
100 for all remaining analyses. The roots of the *Q. ilex* saplings were carefully washed in water  
101 prior to replanting to remove all soil from the previous potting mix, so that the soil  
102 communities were representative of the new soil treatments. All plants were then given

103 adequate water, to maintain soil moisture between 20-25%, for six weeks (until the end of  
104 June 2015) to allow them to adjust to the greenhouse environment.

105

### 106 *Experimental design*

107 The drought treatment was applied by stopping water addition. Ten levels of drought were  
108 applied by varying the length of time without water - 0, 2, 4, 7, 9, 11, 14, 16, 18 and 21 days.  
109 Each drought level therefore had 18 pots, divided into six blocks. Samples of root exudates  
110 were collected (described below) from half of the pots at the end of each drought period, and  
111 the plants were harvested to obtain plant biomass and root:shoot ratio. The remaining pots  
112 were re-watered at optimal amounts (in order to achieve soil moisture of 20-25%) for six  
113 weeks, after each respective drought period, to represent a recovery phase, after which  
114 exudate and biomass measurements were collected (details given below). This amount of  
115 time for recovery was chosen as it should have been sufficient to allow the soil moisture  
116 return to normal and for roots to recover function, but still within the time-frame of the same  
117 summer period, allowing us to determine if recovery could happen in the same growing  
118 period.

119 Mean air temperature during the experiment (monitored using EL-USB-2 data logger,  
120 Lascar Electronics, Wiltshire, UK) was 26.7 °C. Soil temperature was monitored at a fine  
121 scale in five pots, across the different soil types (using a Decagon Em50 data logger with  
122 5TM soil probes, Decagon Devices, Pullman, USA), and averaged 27.0 °C throughout the  
123 experiment (see Supplementary Figure S2). Soil moisture in each pot was measured at the  
124 start of the experiment and at the end of its drought period, and recovery period if relevant  
125 (using ML3 Theta Probe connected to a HH2 Moisture Meter from Delta-T Devices,  
126 Cambridge, UK). Mean soil moisture was 22.6% at the start of the experiment and decreased  
127 exponentially throughout the 21-day drought period to 0.3% at the end of the drought

128 treatment (see Supplementary Figure S3a). Soil moisture recovered quickly to about 20%  
129 within one week of re-watering and was successfully maintained at non-drought levels at a  
130 mean of 24.7% (see Supplementary Figure S3b).

131

### 132 *Plant measurements*

133 Root exudates were measured at the end of each drought period and again at the end of each  
134 six-week recovery period using an *in situ* measuring technique developed from that of  
135 Phillips *et al.* (2008). Briefly, a root was carefully excavated from the soil, cleaned, to  
136 remove any attached soil that could have affected the later measurement of carbon, placed in  
137 moist sand, and then wrapped in aluminium foil. This step allows the root to acclimate to  
138 being moved, and keeps it protected from physical damage and desiccation. The root was  
139 cleaned again after one day of acclimation and placed in a cuvette containing small glass  
140 beads (to apply physical pressure to the root to simulate soil) and a C-free nutrient solution  
141 (0.5 mM NH<sub>4</sub>NO<sub>3</sub>, 0.1 mM KH<sub>2</sub>PO<sub>4</sub>, 0.2 mM K<sub>2</sub>SO<sub>4</sub>, 0.4 mM CaCl<sub>2</sub>, 0.15 mM MgSO<sub>4</sub>) to  
142 prevent desiccation. The nutrient solution was replaced after two days with fresh solution (0.2  
143 mM K<sub>2</sub>SO<sub>4</sub>, 0.4 mM CaCl<sub>2</sub>, 0.15 mM MgSO<sub>4</sub>), and the new solution was collected  
144 approximately 24 h later for the analysis of total organic C (non-purgeable organic C, using  
145 the Analytik Jena-Analyzer multi N/C 3100, Analytik Jena, Jena Germany). Three control  
146 cuvettes (without roots) were used for each drought level, and the mean C contents per hour  
147 of collection were subtracted from the C contents of the cuvettes containing roots. A few  
148 cuvettes with roots reported less C than the control cuvettes, implying that they were either  
149 subject to methodological problems or that there may have been re-uptake of the exuded C,  
150 and they were removed from further analysis.

151 The roots from the cuvettes were taken for measuring surface area (on fresh roots)  
152 using Image J software (Schneider *et al.*, 2012) and dry root biomass. The amount of C

153 released from the root was thus calculated per root area, as  $\mu\text{g C cm}^{-2} \text{ h}^{-1}$ , and per root mass,  
154 as  $\mu\text{g C g}^{-1} \text{ dry mass h}^{-1}$ . Total plant biomass and the root:shoot ratio did not differ by the  
155 length of the drought treatment or between drought and recovery. Mean plant biomass was  
156 50.1 g ( $\pm 1.95$ ) and the mean root:shoot ratio was 1.2 ( $\pm 0.03$ ).

157

### 158 *Statistical analyses*

159 The data for the root exudates were log-transformed to correct for positive skewness and to  
160 achieve normality of the residuals. The data were then analysed with linear mixed-effects  
161 models, to assess the effect of the duration of drought on the amount of C released. The  
162 difference between exudation during the drought and recovery treatments was also tested, as  
163 well as the interaction with drought duration. Block was included as a random factor, and  
164 analyses were performed using the nlme package in R (R Core Team, 2016).

165

## 166 **Results**

### 167 *Root exudation under drought*

168 Mean root exudation per unit root area under control conditions (no drought) was  $0.80 \mu\text{g C}$   
169  $\text{cm}^{-2} \text{ h}^{-1}$  (SE = 0.33), and the median value was  $0.52 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ . The raw data for the  
170 drought treatment varied greatly, with the first quartiles of  $0.23 \mu\text{g C cm}^{-2} \text{ h}^{-1}$  and third  
171 quartile of  $1.02 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ . Exudation was positively linearly correlated with drought  
172 duration ( $P < 0.01$ ) (Fig. 1). Mean exudation was 21% higher for the highest drought  
173 intensity ( $0.97 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ ) compared with the control, and median exudation was 38%  
174 higher for the highest drought level ( $0.72 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ ) compared with the control.

175 Exudation per unit of root biomass was not correlated with drought duration (see  
176 Supplementary Figure S4), and mean exudation for all drought levels was  $56.7 \mu\text{g C g}^{-1} \text{ h}^{-1}$   
177 (SE = 8.2) and the median value was  $31.0 \mu\text{g C g}^{-1} \text{ h}^{-1}$ .



178

### 179 *Recovery from drought*

180 Root exudation following a six-week period of recovery was lower during the recovery than  
181 the drought per unit root area ( $P < 0.0001$ ). Exudation was not correlated with drought  
182 duration, indicating that the plant roots were able to return to non-drought levels of exudation  
183 even after the most severe drought stress. Mean exuded C per unit root area (across all  
184 drought levels) was  $0.35 \mu\text{g C cm}^{-2} \text{ h}^{-1}$  (SE = 0.05) and median was  $0.26 \mu\text{g C cm}^{-2} \text{ h}^{-1}$  (see  
185 Supplementary Figure S4). There was an interaction between the duration of drought and  
186 type of experiment (drought versus recovery,  $P < 0.05$ ) due to the drought and recovery  
187 exudation having different slopes in relation to duration of drought.

188 Exudation per unit root mass was also lower under recovery conditions compared  
189 with drought ( $P < 0.0001$ ). It had a mean value of  $25.2 \mu\text{g C g}^{-1} \text{ h}^{-1}$  (SE = 4.2) and median of  
190  $15.2 \mu\text{g C g}^{-1} \text{ h}^{-1}$ . There was no interaction between the duration of drought and type of  
191 experiment (drought or recovery experiment).

192

## 193 **Discussion**

### 194 *Root exudation under drought*

195 This result generally corroborates the few previous studies that have also measured  
196 rhizodeposition or root exudation under drought (Preece and Peñuelas 2016), which have  
197 reported generally higher C release under water stress. The amounts of C exuded in our study  
198 were similar to those in previous studies using a variety of methods (e.g. Brzostek et al.,  
199 2013; Meier et al., 2013; Baptist et al., 2015; Tückmantel et al., 2017), but were lower than  
200 those in other studies (e.g. Yin et al. 2013; Zhang et al. 2016).

201 Higher root exudation under drought could be an adaptation that helps the survival of  
202 roots, by creating better conditions for growth and survival. For example, increasing the

203 release of mucilage lubricates the roots so that they can pass through the soil more easily  
204 (Ahmed et al. 2014; Czarnes et al. 2000; Huang 2000) and have a higher chance of reaching  
205 areas with more water. Increased production of organic acids or extracellular enzymes  
206 involved in the release of soil nutrients may provide a short-term burst of available nutrients  
207 for plant uptake (Dakora and Phillips 2002; Jones 1998; Paterson 2003), which could also  
208 enable plants to increase root length and be more likely to arrive to soil that is less water  
209 stressed.

210           Alternatively, the increase in the amount of C detected in the root exudates with  
211 drought stress may have been due to increased cell damage and leakage of cell contents. A  
212 previous drought study of rhizodeposition also offered this suggestion (Henry et al. 2007),  
213 because water stress can induce root desiccation, mortality, and leakage of organic solutes  
214 (Huang and Gao 2000). Our experimental design did not allow us to conclusively determine  
215 the cause of the changes in root exudation, but the cause may differ between mild and  
216 extreme droughts. For example, seeking a better environment may be most likely at lower  
217 levels of water stress, and root cell damage may be most likely in extreme water stress. Our  
218 data tentatively support these different causes; plants in drought of less than 16 days  
219 maintained green leaves, but in the most extreme drought intensities (16-21 days without  
220 water) leaves were badly damaged or lost, suggesting root damage. Additionally, changes in  
221 root morphology may partly drive the exudation response to drought, if roots are desiccated,  
222 and therefore lead to a relative increase in root exudation per root area.

223           A previous literature review of the impacts of drought on rhizodeposition found that C  
224 release decreased at high levels of water stress (Preece and Peñuelas 2016), but overall our  
225 data did not provide supporting evidence. Although exudation seemed to drop at day 14,  
226 mean C exudation was highest in the three most intense drought levels (soil moisture <5%).  
227 Our results for *Q. ilex* highlight the importance of performing experiments at varying drought

228 intensities, because the response, even within a species, can vary considerably with the level  
229 of water stress.

230

### 231 *Recovery from drought*

232 Exudation of C following a six-week period of recovery did not differ depending on the  
233 length of the drought treatment, indicating that the plant roots were able to return to non-  
234 drought levels of exudation even after the most severe drought stress. To the best of our  
235 knowledge, only one study about root exudation under drought has included a recovery or re-  
236 wetting treatment. In that study, plant-derived extractable organic C in the soil in an Austrian  
237 mountain meadow with mostly perennial grasses and herbs was higher in a drought treatment  
238 and decreased to control levels after re-wetting (Fuchslueger et al. 2014). This result is in  
239 accordance with our findings, but the plants in the study by Fuchslueger *et al.* (2014) were  
240 mowed immediately before the re-wetting, so completely separating the effects of these two  
241 treatments is impossible.

242

### 243 *The future of root exudate studies*

244 This study is the first to use a relatively new and simple technique to assess the  
245 changes in the amount of root exudation over the course of increasing drought and  
246 subsequent recovery. It is also the first to measure C exudation from *Q. ilex* and thus provides  
247 novel information about the efflux of C from an important Mediterranean tree species. The  
248 method used allowed us to measure exudation *in situ* on many plants and without expensive  
249 equipment, and similar protocols have been used previously to measure the effect of water  
250 stress on exudates (Canarini et al. 2016; Karst et al. 2017), and give results that agree with  
251 findings using different methods (e.g. Fuchslueger et al. 2014; Sanaullah et al. 2012). For  
252 further explanation of the different methods available for measuring root exudation see

253 Kuzyakov (2006) which gives a good overview of the fate of C within soils and appropriate  
254 methods to measure this, and Oburger and Schmidt (2016) which is a more recent review of  
255 techniques, including an emphasis on imaging methods. The quantity of C in the root  
256 exudates returned to non-drought levels when re-watering commenced, but we do not know if  
257 the composition of exudates varied under water stress, and if so, how long the differences  
258 persisted. Exudate composition is therefore a very important area for future studies.

259         The general conclusion of this study is that the efflux of C from *Q. ilex* by root  
260 exudation increased during increasing drought, and C release was >20% higher than normal  
261 at the most extreme intensities of drought stress (soil moisture <5%). This increase in  
262 exudation may help the species to survive extreme droughts and represents an important  
263 change in the rhizosphere of this species, which could have further impacts on the soil  
264 microbial community. Moreover, we demonstrated that plants can have a large capacity for  
265 the recovery of root exudation, and this ability may be part of the strategy of drought  
266 tolerance in this key Mediterranean tree species.

267

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276

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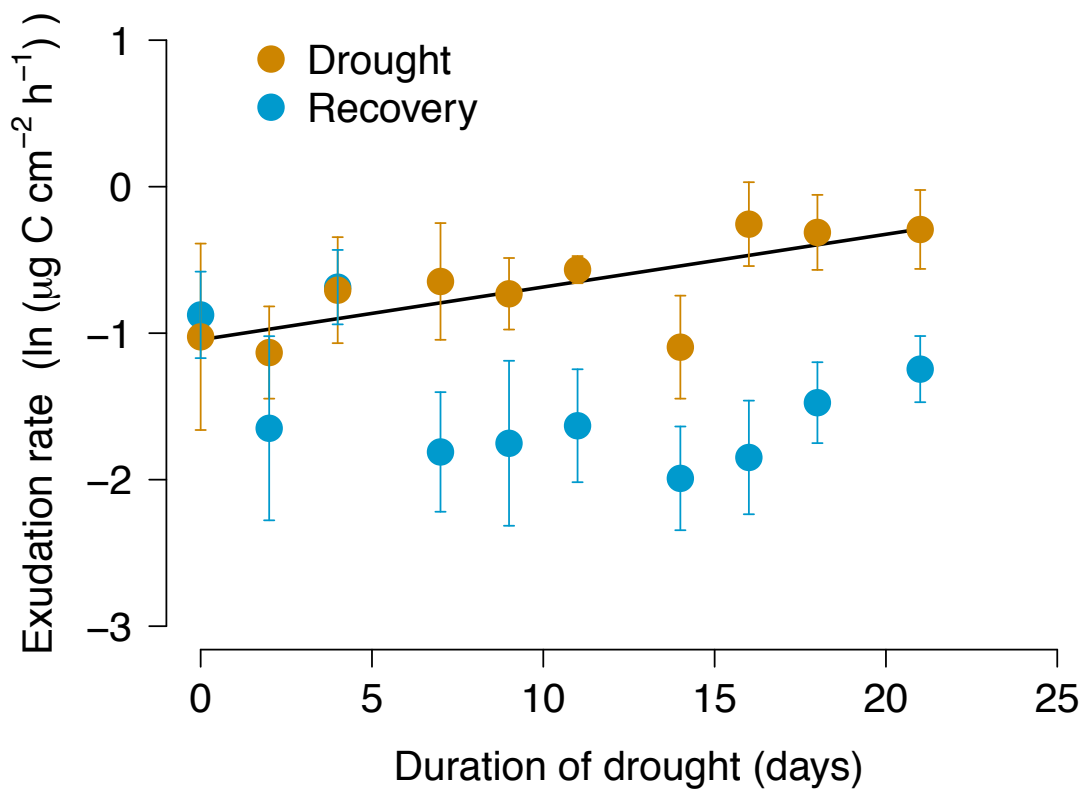
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399 **Figures**

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401 Fig. 1. Exudation of carbon (natural logged) from plant roots under increasing drought  
402 duration, shown as carbon per cm<sup>2</sup> root area. Orange points are mean exudations at the end of  
403 the drought periods and blue points are mean exudations after six weeks of re-watering  
404 (recovery). Error bars are one standard error. Carbon exudation after drought increased with  
405 the duration of drought ( $P < 0.05$ , slope = 0.04). Carbon exudation after recovery was not  
406 affected by the duration of the previous drought. Exudation was higher during the drought  
407 than the recovery period ( $P < 0.0001$ ), and there was a significant interaction between the  
408 duration of drought and whether plants were measured after drought or after recovery ( $P <$   
409 0.05).

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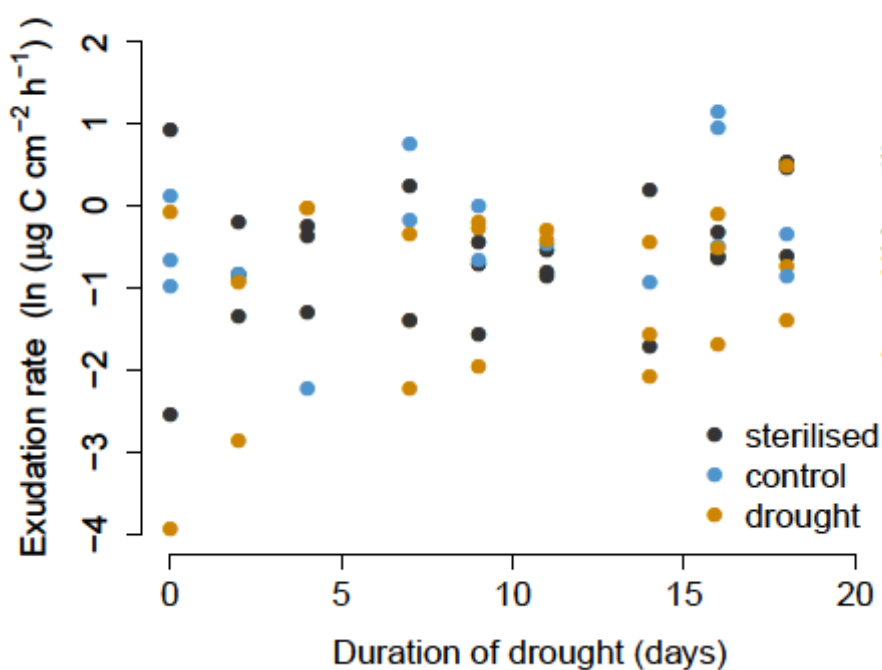
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412 **Electronic Supplementary Data**

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414 **Fig. S1.** Exudation of carbon (natural logged) from plant roots during the drought treatment,  
415 at different durations of drought, shown as carbon per cm<sup>2</sup> root area for individual plants.  
416 Data is colour-coded by soil type (sterilised, control or droughted) and there was no  
417 significant effect of soil type on exudation.



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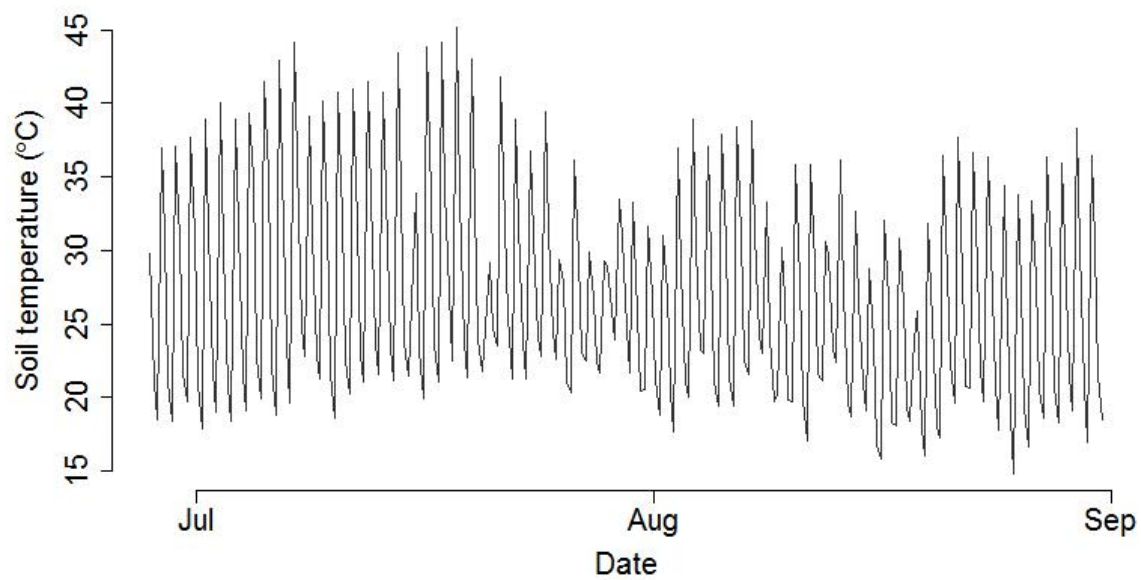
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425 **Fig. S2.** Mean soil temperature throughout the experiment measured in five pots (due to  
426 availability of soil moisture probes).

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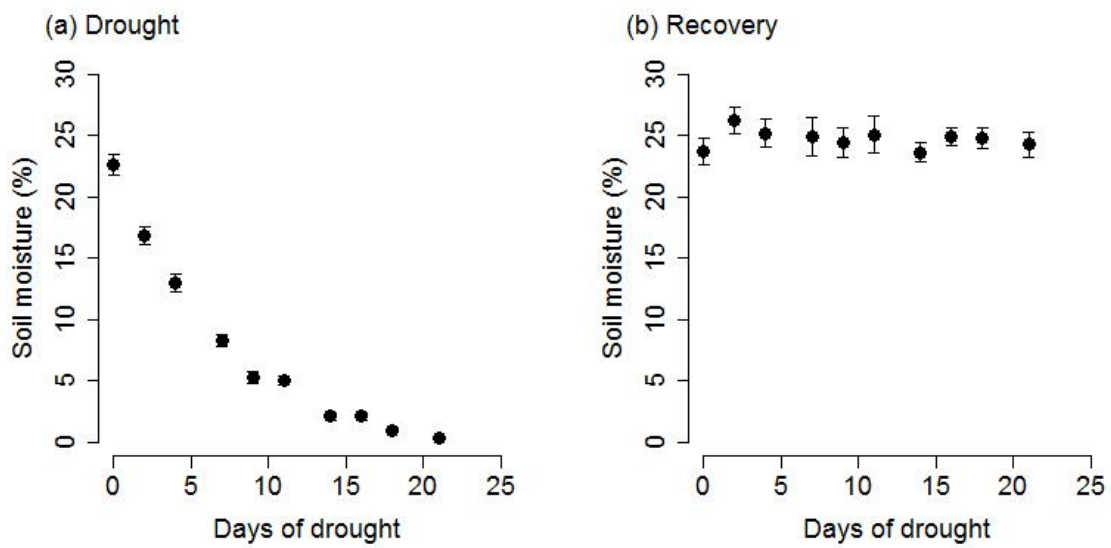
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431 **Fig. S3.** Percentage soil moisture for the drought levels (a) at the end of the drought treatment  
432 and (b) at the end of the recovery period. Points are means for each drought level and  
433 standard error bars are shown (n = 36 for drought measurements and n = 18 for recovery  
434 measurements).

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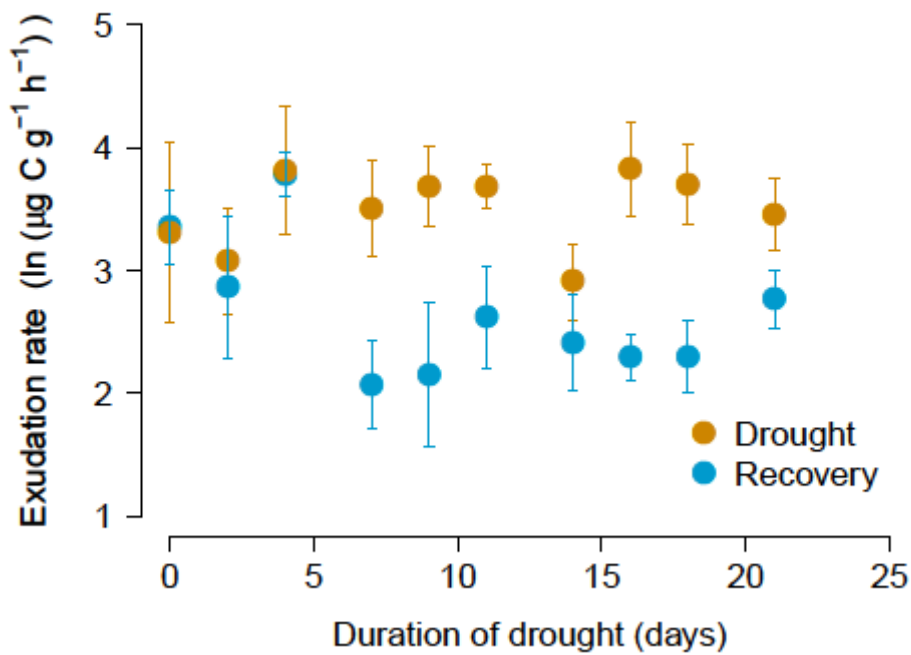
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447 **Fig. S4.** Exudation of carbon (natural logged) from roots under increasing drought duration,  
448 shown as carbon per gram of roots. Orange points are mean exudations at the end of the  
449 drought periods and blue points are mean exudations after six weeks of re-watering  
450 (recovery). Standard error bars are shown. There was no effect of drought duration on carbon  
451 exudation. Exudation was higher during the drought than the recovery period ( $P < 0.0001$ ),  
452 but there was no significant interaction between the duration of drought and whether plants  
453 were measured after drought or after recovery).



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