1   REGULAR ARTICLE

Chicory demonstrates substantial water uptake from below 2 m depth, but still did not escape topsoil drought

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Key-words

Cichorium intybus L.; Deep root growth; Deep water uptake; Drought resistance; Intercropping; hydrological tracer

Abstract

Aims Deep-rooted agricultural crops can potentially utilize deep water pools and thus reduce periods where growth is water limited. Chicory (*Cichorium intybus* L.) is known to be deep-rooted, but the contribution of deep roots to water uptake under well-watered and drought conditions by the deep root system has not been studied. The aim of this study was to investigate whether chicory could reach 3 m depth within a growing season and demonstrate significant water uptake from the deeper part of the root zone.

Methods We tested if chicory exposed to either topsoil drought or resource competition from the shallow-rooted species ryegrass (*Lolium perenne* L.) and black medic (*Medicago lupulina* L.) would increase deep water uptake in compensation for reduced topsoil water uptake. We grew chicory in 4 m deep soil filled rhizotrons and found that the roots reached 3 m depth within a growing season.

Results Water uptake from below 1.7 m depth in 2016 and 2.3 m depth in 2017 contributed significantly to chicory water use. However, neither drought nor intercropping increased the deep water uptake.

Conclusion Chicory benefits from being deep-rooted during drought events, yet deep water uptake cannot compensate for the reduced topsoil water uptake during drought.

Introduction

Minimizing water limitation during growth of agricultural crops is crucial to unlocking full yield potential. Crop yield losses vary according to the timing and severity of water limitations, but even short-term drought can be a major cause of yield losses (Zipper et al. 2016). Deep-rooted crops can potentially utilize otherwise inaccessible deep-water pools and thus reduce periods where crop production is water limited. In areas where precipitation is sufficient to rewet the soil profile during a wet season, shallow-rooted crops might still experience water limitation during the growing season, as they do not have access to the water stored deeper in the profile.

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42   The potential influence of deep roots on water uptake has been highlighted numerous times

43   (e.g. Canadell et al. 1996; Lynch and Wojciechowski 2015), still, information about the actual

44   contribution of deep roots to water uptake remains scarce. Maeght et al. (2013) suggest that this

45   is related to the absence of tools to measure deep root activity with sufficient throughput and

46   standardization at affordable costs, and to the widespread assumption that as deep roots only

47   represent a small fraction of the overall root system their contribution to root system function is

48   marginal. It has also been questioned whether deep root growth under field conditions is too

49   restricted by high soil strength, and unfavourable conditions such as e.g. hypoxia, acidity, and low

50   nutrient availability, to substantially benefit the crop (Lynch and Wojciechowski 2015; Gao et al.

51   2016).

52   While some soils definitely restrict deep root growth, others have shown to allow roots to

53   grow in the deeper soil layers (Sponchiado et al. 1989; Thorup-Kristensen and Rasmussen 2015). In

54   addition, even though the majority of the root biomass is found in the topsoil, deep roots can

55   contribute significantly to water supply in crops, as there is little connection between root biomass

56   and root activity (Mazzacavallo and Kulmatiski 2015). Gregory et al. (1978) found that in the field,

57   winter wheat had less than 5 % of its root biomass below 1 m depth, and as long as the water

58   supply was sufficient in the upper meter, the biomass reflected the water uptake well. However,

59   when the topsoil dried, the roots between 1 and 2 m depth supplied the plants with up to 20 % of

60   the total water use. In a study conducted in an Amazonian tropical forest, Nepstad et al. (1994)

61   found that they would have underestimated the evapotranspiration by 60 % in a dry year, had

62   they not considered roots below 2 m depth.

63   Indirectly deeper root growth in crops has also been associated with deep-water uptake, as

64   rooting depth has been shown to correlate positively with yield under drought in the field in e.g.

65   wheat (Lopes and Reynolds 2010), bean (Sponchiado et al. 1989; Ho et al. 2005), rice (Uga et al.

66   2013) and maize (Zhu et al. 2010). In addition, modeling studies indicate that selection for deeper

67   roots in grain crops could significantly improve deep-water acquisition and thereby yield in water

68   deficit seasons (Manschadi A et al. 2006; Lilley and Kirkegaard 2011). Common to most of these

69   studies is that deep root growth is considered to be in the range of 0.5 to 1.5 m depth. But several

70   agricultural crops have the capability to grow roots below 2 m depth or even deeper within a

71   season (Canadell et al. 1996; Ward et al. 2003; Thorup-Kristensen 2006; Rasmussen et al. 2015),

72   and thereby get access to an extra pool of water originating from wet season surplus precipitation

73   stored in the soil. For example, lucerne has shown to decrease the soil water content at 5 m depth

74   (Fillery and Poulter 2006).

75   Hydrological isotope tracer techniques have over the last two decades become an

76   increasingly popular tool to acquire information on temporal and spatial water use patterns in

77   plants (e.g. Bishop and Dambrine 1995; Peñuelas and Filella 2003; Beyer et al. 2016). Injection of

78   tracer into specific soil depths has proven to be a precise method to detect the relative water

79   uptake from the chosen depth (Kulmatiski et al. 2010; Kulmatiski and Beard 2013; Bachmann et al.

80   2015; Beyer et al. 2016). The hydrological tracer techniques utilize the fact that no isotopic

81   fractionation against isotope forms of hydrogen or oxygen occurs during soil water uptake by

82   roots (Wershaw et al. 1966; Dawson and Ehleringer 1991; Bariac et al. 1994; Mensforth and

83   Walker 1996).

84   The anthropocentric discussion of the importance of deep root growth in crop production is

85   put in perspective by the fact that some plant species have evolved the potential to grow deep

86   roots. Under what circumstances is that strategy beneficial? In this study, we hypothesize that

87   deep root growth can help plants escape topsoil drought. More specifically, we aimed at testing

88   the following hypotheses, using chicory (*Cichorium intybus L.*) as an example plant: 1) Chicory can

89   grow roots below 3 m depth within a growing season. 2) Chicory has a significant water uptake

90   from the deeper part of the root zone despite low root intensity. 3) When chicory is exposed to

91   either topsoil drought or resource competition from shallow-rooted species, deep water uptake

92   increases in compensation for the decreased topsoil water uptake.

93   Chicory is commonly grown in pasture mixtures for animal fodder or as a cash crop to

94   produce inulin (Meijer et al. 1993). It is known to be able to reach at least 2.5 m depth (Thorup-

95   Kristensen and Rasmussen 2015) and to be drought resistant (Monti et al. 2005; Skinner 2008;

96   Vandoorne et al. 2012a). To test the hypotheses we grew chicory as a sole crop and in an

97   intercropping with the two shallow-rooted species ryegrass (*Lolium perenne L.*) and black medic

98   (*Medicago lupulina L.*) in 4 m deep rhizotrons. We allowed extensive root development before

99   imposing a drought, as our focus was on the potential of deep roots to acquire water and not on

100   deep root growth during drought.

101

102 **Materials and Methods**

103 *Experimental facility*

104 We conducted the experiment in a semi-field facility at University of Copenhagen, Taastrup

105 (55°40'08.5"N 12°18'19.4"E), Denmark and repeated it for the two consecutive seasons, 2016 and

106 2017. We grew the crops in 4 m deep rhizotrons placed outside on a concrete foundation. The

107 rhizotrons where 1.2 x 0.6 m rectangular columns constructed of steel frames. A waterproof

108 plywood plate divided the rhizotrons lengthwise into an east- and a west-facing chamber with a

109 surface area of 1.2 x 0.3 m. The rhizotrons stood on a north-south axis, narrow side facing towards

110 one another (Fig. 1). On the east- and the west facing fronts of the rhizotrons, 20 transparent

111 acrylic glass panels allowed inspection of root growth at the soil-rhizotron interface on the entire

112 surface. Each panel was 1.2 m wide and could be removed to allow direct access to the soil

113 column. Every third panel was 0.175 m tall, and the rest were 0.21 m tall. We used the narrow

114 panels for placement of equipment and soil sampling. The tall panels were used only for root

115 observations. To avoid disturbance of root growth, we never removed these panels during the

116 experiment. All sides of the rhizotrons where covered in white plates of foamed PVC of 10 mm

117 thickness to avoid light exposure of soil and roots. On the fronts, the foamed PVC plates were also

118 divided into 20 panels. These were fixed in metal rails, allowing them to be slid off whenever we

119 had to observe the roots. A wick in the bottom of the rhizotrons allowed water to drain out.

120   We used field soil as a growth medium. The bottom 3.75 m of the rhizotrons was filled with

121 subsoil taken from below the plough layer at Store Havelse, Denmark (Table 1). We filled the

122 upper 0.25 m with a topsoil mix of sandy loam and fine sandy soil, half of each, both from the

123 University's experimental farm in Taastrup, Denmark. To reach a soil bulk density comparable to

124 field conditions we filled the soil into the rhizotrons stepwise at portions of approximately 0.15 m

125 depth and used a steel piston to compact each portion by dropping it several times on the soil. We

126 filled the rhizotrons in August 2015 and did not replace the soil during the two years. At the time

127 of the experiment, average subsoil bulk density was 1.6 g m^{-3}, which is close to field conditions for

128 this soil type.

129   We constructed rainout shelters to control water supply in the drought stress treatment. In

130 2016, we covered the soil with a transparent tarpaulin that had a hole for each plant stem. The

131 tarpaulins were stretched out and fixed with a small inclination to let the water run off. It turned

132　　out that this design failed to keep out water during intense precipitation events, which happened

133　　twice during the season. Thus in 2017, we designed barrel roof rainout shelters instead, using the

134　　same clear tarpaulin and placed them on all rhizotrons. The rain-out shelters were open in the

135　　ends and on the sides to allow air circulation but were wider than the rhizotrons to minimize that

136　　water reached the chambers during precipitation under windy conditions.

137　　　　We installed a drip irrigation pipe (UniRam™ HCNL) with a separate tap in each chamber.

138　　The pipe supplied 5 l hour^{-1}, equivalent to 14 mm hour^{-1} according to the surface area of the

139　　growth chambers.

140

141　　*Experimental design*

142　　We had two treatments in 2016 and four in 2017. In both years we grew chicory (*C. intybus L.*,

143　　2016: cv Spadona from Hunsballe frø. 2017: cv Chicoree Zoom F1 from Kiepenkerl) in monoculture

144　　under well-watered (WW) and drought stress (DS) conditions. In 2017, we also grew chicory

145　　intercropped with either ryegrass (*L. perenne L.*) or black medic (*M. lupulina L.*), both in a WW

146　　treatment. For chicory, we chose to work with a hybrid vegetable type cultivar in the second year

147　　to reduce the variation among plants in size and development speed seen in the forage type used

148　　the first year. In 2016, we transplanted four chicory plants into each chamber. In 2017, we

149　　increased the number to six in order to reduce within-chamber variation. For the two

150　　intercropping treatments in 2017, we transplanted five plants of ryegrass or black medic in

151　　between the six chicory plants.

152　　　　For the 2016 season, chicory plants were sown in May 2015 in small pots in the greenhouse

153　　and were transplanted into the rhizotrons 30 September. Despite our attempt to compact the soil

154　　inside the rhizotron chamber, precipitation made the soil settle around 10 % during the first

155　　winter. Therefore, 29 February 2016, we carefully dug up the chicory plants, removed the topsoil,

156　　filled in subsoil before filling topsoil back in and replanting the chicory plants. A few chicory plants

157　　did not survive the replanting and in March we replaced them with spare plants sown at the same

158　　time as the original ones and grown in smaller pots next to the rhizotrons. In 2017, we sowed

159　　chicory in pots in the greenhouse 11 April and transplanted them to the rhizotron chambers 3 May

160　　(Table 2). Chicory is perennial, it produces a rosette of leaves the first year and the second year it

161　　grows stems and flowers.

162   We grew all treatments in three randomized replicates. The soil inside the six chambers not

163   used for the experiment in 2016 but included in 2017 had also sunken during the 2015/2016

164   winter and the same procedure was used to top up soil in these chambers before transplanting

165   the chicory plants.

166   In 2016, we fertilized all chambers with NPK 5-1-4 fertilizer equivalent to 100 kg N ha^{-1}, half 1

167   April and the other half 21 June. In 2017, we fertilized all chambers 3 May and 1 June following the

168   same procedure. Two chambers were accidentally over irrigated mid-June 2017 and we re-

169   fertilized them 16 June.

170   In 2016, we pruned the plants at 0.5 m height, several times between 24 May and 12 July to

171   postpone flowering and induce leaf and root growth.

172   We started drying out the DS treatments 26 June in 2016 and 13 July in 2017, where we

173   stopped irrigation and mounted the rainout shelters. In 2016, we kept irrigating the WW

174   treatments whenever precipitation was considered insufficient to meet plant needs. In 2017,

175   where the rainout shelters excluded precipitation in all chambers we kept irrigating all treatments

176   apart from the DS to ensure sufficient water supply. However, we chose to supply the same

177   amount of water in all the irrigated chambers, which led to different levels of soil water content.

178

179   *Biomass and ^{13}C*

180   We harvested aboveground biomass 28 July in 2016 and 11 September in 2017. We dried the

181   biomass at 80°C for 48 hours. The biomass was analysed for $^{13}C/^{12}C$ ratio on an elemental analyser

182   interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) at the University of

183   California Stable Isotope Facility (Davis, California, USA). Isotope values are expressed in delta

184   notation (δ) in per mill [‰] following the definition of Coplen (2011):

$$\delta = \frac{R_{sample}}{R_{standard}} - 1 \#(1)$$

185

186   where R_{sample} is the ratio of the less abundant to the more abundant isotope ($^{13}C/^{12}C$) in the

187   sample and $R_{standard}$ the ratio in a standard solution. For $\delta^{13}C$ the international standard Vienna

188   PeeDee Belemnite ($R_{standard} = 11180.2 \times 10^{-6}$) was used. Analytical precision (σ) was 0.2‰.

189   The $^{13}C/^{12}C$ ratio in plants is directly related to the average stomatal conductance during

190   growth, as discrimination between $^{12}CO_2$ and $^{13}CO_2$ during photosynthesis is greatest when

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191   stomatal conductance is high. When stomates are partially or completely closed, a greater part of

192   the CO_2 inside the leaf is absorbed resulting in less fractionation and thereby higher $\delta^{13}C$ values of

193   the plant tissue (Farquhar and Richards 1984; Farquhar et al. 1989).

194

195   *Root measurements*

196   We documented the development in root growth by taking photos of the soil-rhizotron interface

197   through the transparent acrylic glass panels. For this purpose, we designed a "photo box" that

198   could be slid on the metal rails in place of the foamed PVC panels, and thereby excluded the

199   sunlight from the photographed area. We placed a light source consisting of two bands of LED's

200   emitting light at 6000 K in the photo box. We used a compact camera (Olympus Tough TG 860).

201   For each 1.2 m wide panel we took four photos to cover the full width of the panel. We

202   photographed the roots 21 June and 18 July 2016 and 6 July, 16 August and 12 September 2017,

203   corresponding to the time of drought initiation in the DS treatment, ^2H tracer injection (see below)

204   and for 2017, harvest. In 2017, harvest was postponed until 20 days after the ^2H tracer-

205   experiment, due to other tests running in the facility.

206   We recorded the roots using the line intersects method (Newman 1966) modified to grid

207   lines (Marsh 1971; Tennant 1975) to calculate root intensity, which is the number of root

208   intersections m−1 grid line in each panel (Thorup-Kristensen 2001). To make the counting process

209   more effective we adjusted the grid size to the number of roots, i.e. we used coarser grids when

210   more roots were present and finer grids for images with only a few roots. This is possible because

211   root intensity is independent of the length of gridline. We used four grid sizes: 10, 20, 40 and 80

212   mm. To minimize the variance of sampled data we used grid sizes that resulted in at least 50

213   intersections per panel (Ytting 2015).

214

215   *Soil water content*

216   We installed time-domain reflectometry sensors (TDR-315/TDR-315L, Acclima Inc., Meridian,

217   Idaho) at two depths to measure volumetric water content (VWC) in the soil. In 2016, the sensors

218   were installed at 0.5 and 1.7 m depth. In 2017, the sensors were installed at 0.5 and 2.3 m depth.

219   Soil water content was recorded every 5 min in 2016 and every 10 min in 2017 on a datalogger

220   (CR6, Campbell Scientific Inc, Logan, Utah). Discrepancies in measured VWC among the sensors at

8

221 field capacity (FC) let us conclude that the sensors were precise but not particularly accurate,

222 meaning that the change over time in VWC was reliable but not the measured actual VWC. We

223 have therefore estimated a sensor reading for each sensor at FC and reported changes in VWC

224 from FC. We estimated FC as the mean VWC over a 48-hour interval. In 2017, the measurement

225 was made in the autumn after excess water from a heavy rainfall had drained away. In the

226 autumn, there is little evaporation and no plant transpiration to decrease VWC below FC, making

227 it an optimal time to estimate FC. We did not have data from autumn 2016, so instead, we

228 estimated FC in early spring.

229

230 *Water uptake*

231 We estimated water uptake from the VWC readings. We assume that water movement in the soil

232 is negligible when VWC is below FC. Hence, the decrease in VWC can be interpreted as plant water

233 uptake. Water uptake is therefore estimated as the mean decrease in VWC over a given time

234 interval. We attempted to use intervals corresponding to the time of the ^{2}H tracer studies. In

235 2016, the interval was a postponed a few days and in 2017, the time interval did not cover the first

236 two days of the tracer study.

237 For the period from onset of drought to harvest 2017, we tested whether the daily water

238 uptake at 2.3 m depth was affected by daily mean VWC at 0.5 m depth across all treatments. For

239 this period, the VWC at 2.3 m depth was close to FC in all treatments and therefore unlikely to

240 affect the water uptake. As transpiration demand is high at this time of the year and plants are

241 large, we assumed that topsoil water limitations would limit total water uptake unless it is

242 balanced by an increased water uptake lower in the profile. We excluded days in which the

243 chambers were irrigated and one day after irrigation events to exclude periods with large soil

244 water movement.

245

246 ^{2}*H tracer*

247 We used ^{2}H labeled water injected into 2.3 m depth to trace water uptake from this depth. We

248 mixed 90 % ^{2}H$_2$O tracer with tap water 1:1, to achieve an enrichment of δ 5,665,651 ‰ and

249 injected 100 ml per chamber. We removed one of the acrylic panels in each chamber temporarily

250 to allow tracer injection and distributed it over 100 injection points in the soil. The injections were

9

251   made at two horizontal rows of each 10 equally distributed holes 5 cm above and below 2.3 m

252   depth respectively. In each of these 20 holes, we injected 5 ml tracer distributed between five

253   points: 5, 10, 15, 20 and 25 cm from the horizontal soil surface. Tracer injection was made 19 July

254   2016 and 15 August 2017.

255   We captured the tracer signal by collecting transpiration water using plastic bags. For studies

256   using tracers, collecting transpiration water is considered valid, as the tracers increase the

257   enrichment level several orders of magnitudes, which make the fractionation negligible (Thorburn

258   and Mensforth 1993; Beyer et al. 2016). We sampled the transpiration water one day before

259   tracer injection as a control and one, two, three, four and six days after in 2016, and three and six

260   days after in 2017. We fixed a plastic bag over each plant with an elastic cord that minimized air

261   exchange with the surroundings. Transpiration water condensed on the inside of the plastic bag,

262   which was folded inwards under the elastic cord to create a gutter for the water drops. Plastic

263   bags were mounted on the plants two hours before noon and removed at noon.

264   We removed the plastic bags one by one, shook them to unite the drops, and transferred

265   each sample to a closed plastic beaker. Later we filtered the samples through filter paper to

266   remove soil and debris contamination and transferred the samples to glass vials.

267   We collected water from all plants and in most cases mixed the individual plant samples

268   before analysis, taking equal amounts of water from each sample. Day 2 in 2016 and day 6 in

269   2017, we analysed the samples from each plant separately to get data on within chamber

270   variation. For the control samples in 2017, we only collected water from two plants of each

271   species per chamber. Single plant sample sizes varied from almost nothing to up to around 60 ml

272   in 2016 and 30 ml in 2017. The amount did not only reflect differences in transpiration rate, as it

273   was impossible to avoid spill when removing the plastic bags, and therefore we choose to use

274   equal amounts of water from each plant. For the control samples where variation was small, this is

275   of minor importance. The relatively large sample sizes for most samples limited the concerns of

276   fractionation due to evaporation during filtering and sample transfer.

277   The samples were analysed for ^{2}H at Centre for Isotope Research, University of Groningen,

278   The Netherlands on a continuous flow isotope ratio mass spectrometer (IRMS, Isoprime 10)

279   combined with a chromium reduction system (Europa PyrOH, Gehre et al. 1996).Isotope values are

280   expressed in delta notation (δ) as given in equation 1. R_{sample} is the ^{2}H/^{1}H ratio in the sample and

281 $R_{standard}$ for δ^2H is Vienna standard mean ocean water ($R_{standard} \approx 1/6412$). Analytical

282 precision (σ) was 0.7 ‰.

283   In order to identify whether tracer was present in a sample, we adapted the criteria

284 proposed by Kulmatiski (2010). If a sample had a δ^2H-value at least two standard deviations

285 higher than the control samples, tracer was assumed to be present.

286

287 *Statistics*

288 Data analyses were conducted in R version 3.4.4 (R Core team 2018). The effect of treatment on

289 aboveground biomass of chicory, black medic and ryegrass was tested in a mixed effects one-way

290 ANOVA. Separate harvest of single plants allowed the inclusion of chamber as random effect to

291 account for the fact that the two intercropped species are not independent.

292   The effect of soil depth and treatment on root intensity was tested in a mixed effects two-

293 way ANOVA. We included chamber as random effect to account for the fact that the different

294 depths are not independent. To meet assumptions of normality, depths where at least one of the

295 treatments had no roots in any of the replicates, were excluded from the model. Separate

296 analyses were made for each date.

297   The effect of soil depth and treatment on water uptake during a given time interval was

298 tested in a mixed effect three-way ANCOVA with time as covariate. In 2016, we excluded the

299 sensors from one replicate of the DS treatment because water reached it during a cloudburst. In

300 2017, we excluded two of the sensors at 0.5 m depth from the analysis, one in a chicory and

301 ryegrass intercropping treatment and one in a chicory and black medic intercropping treatment.

302 The first due to noise in the readings and the second due to readings showing a pattern in VWC

303 that did not resemble the pattern of any of the other sensors.

304   The effect of treatment and time on ^{2}H concentration in transpiration water was tested in a

305 mixed effect two-way ANOVA. We log-transformed the response variable to meet the assumptions

306 of homoscedasticity.

307   The effect of treatment on δ^{13}C was tested in a one-way ANOVA. For 2017, the model is a

308 mixed effects model because samples for each plant were analysed separately.

309   In all cases, separate analyses were made for each year. All models used met the

310 assumptions of normality and homoscedasticity. Differences were considered significant at P

311 <0.05. Tukey test P-values for pairwise comparisons were adjusted for multiplicity, by single step

312 correction to control the family-wise error rate, using the multcomp package (Hothorn et al.

313 2008). For root intensity, we decided to control the family-wise error rate for each root depth. For

314 the ^{2}H concentration, we only made pairwise comparisons for the last date.

315

316 **Results**

317 Plants grew well both years, and as hypothesized, roots were observed below 3 m depth by the

318 end of the growing season. Both the uptake of ^{2}H tracer and sensor readings showed that chicory

319 acquired water from 2.3 m depth. However, our results do not suggest that compensation takes

320 place, i.e. deep water uptake was not increased to balance the decreased topsoil water uptake

321 during drought.

322

323 *Biomass*

324 Plant development differed between the two experimental years. In 2016, the chicory plants were

325 in their second growth year and went into the generative stage right from the start of the growing

326 season. They started flowering in late May. In 2017, the chicory plants were in their first year of

327 growth and stayed in the vegetative state. Aboveground biomass of chicory did not differ

328 significantly between the two treatments in 2016 and was 6.52 and 6.85 t ha^{-1} in the WW and DS

329 treatment, respectively. In 2017, chicory biomass was 4.65 and 3.64 ton ha^{-1} in the WW and DS

330 treatment respectively and 2.80 and 2.21 t ha^{-1} when intercropped with either black medic or

331 ryegrass. Biomass of black medic and ryegrass was 5.89 and 7.68 ton ha^{-1} respectively. Both

332 intercropping treatments significantly reduced chicory biomass compared to the WW treatment.

333 Ryegrass produced significantly more biomass than black medic (Fig. 2).

334

335 *Root growth*

336 Root growth showed a similar pattern across the four treatments; however intercropping

337 decreased total root intensity down to around 2 m depth (only significant in few depths), except

338 for 0.11 m depth, where the chicory and ryegrass intercropping treatment had a significantly

339 higher root intensity than the other treatments. Roots of intercropped species could not be

340 distinguished and the reported root intensities are thus the sum of two species in the

12

341 intercropping treatments. The month-long summer drought did not influence root intensity in any

342 depths.

343   In 2016, roots had reached 2 m depth at the time of drought initiation, which was 3.5

344 months after transplanting. A month later, at the time of tracer injection the rooting depth of

345 chicory had increased below 3 m depth (Fig. 3a and b). In 2017, roots were observed almost to the

346 bottom of the rhizotrons already at drought initiation, 2 months after transplanting. However,

347 only a few roots were present below 2 m depth. At the time of tracer injection, which was again

348 3.5 months after transplanting root intensity had started to increase down to 2.5 m depth, and at

349 harvest, 4.5 months after transplanting this was the case down to around 3 m depth (Fig. 3c, d,

350 and e).

351

352 *Soil moisture and water uptake*

353 During the drought, 135 and 97 mm of water were excluded from the DS treatment in 2016 and

354 2017, respectively compared to the other treatments. In 2016, the soil dried out gradually at both

355 0.5 and 1.7 m depth in the DS treatment and in the WW treatment between the precipitation and

356 irrigation events. As a result, the soil was drier in the DS than in the WW treatment at both depths

357 recorded at the time of the tracer- experiment (Fig. 4a and b).

358   Although chicory WW and the two intercropping treatments in 2017 received the same

359 amount of water, less water reached the sensors at 0.5 m in the chicory and black medic

360 intercropping than in the WW and the chicory and ryegrass intercropping. This indicates that the

361 soil above the sensors was drier and therefore could withhold more water compared to the two

362 other irrigated treatments. At the time of the tracer-experiment, soil water content under the

363 chicory and black medic intercropping was similar to the DS treatment, which was lower in

364 comparison to two other treatments (Fig. 4c and d).

365   During the tracer-experiment, chicory plants in the WW treatment acquired 3.7 and 2.3 mm

366 water m^{-1} soil column day^{-1} from 0.5 m in 2016 and 2017, respectively. The uptake from 0.5 m

367 depth was reduced by more than 50 % in the DS treatment compared to the WW treatment in

368 both years. In the WW treatment, chicory took up 1.9 mm water m^{-1} soil column day^{-1} from 1.7 m

369 depth in 2016, whereas the uptake was 0.44 mm water m^{-1} soil column day^{-1} from 2.3 m depth in

370 2017. In 2016, drought significantly reduced water uptake of chicory from 1.7 m depth, whereas

13

371 no effect of drought was observed at 2.3 m depth in 2017. Common for both years was that the

372 amount of water taken up from 0.5 m depth in the DS treatment was equal to the uptake from 1.7

373 and 2.3 m depth in 2016 and 2017 respectively. Both intercropping treatments significantly

374 reduced water uptake at 0.5 m depth compared to the WW treatment, but no effect was seen at

375 2.3 m depth (Fig. 5).

376 We did not find any effect of mean daily soil VWC at 0.5 m depth on water uptake at 2.3 m

377 depth, giving no indication of compensatory deep water uptake (Data not shown).

378

379 *^{2}H enrichment*

380 Chicory took up ^{2}H tracer from 2.3 m depth in both years (Fig. 6a). Two days after tracer

381 application in 2016, 21 out of 23 chicory plants demonstrated isotope ratios that were two

382 standard deviations or more above controls. Six days after tracer application in 2017, it was 30 out

383 of 64 chicory plants that showed the enrichment. No ryegrass or black medic plants indicated

384 tracer uptake (Fig. 6b).

385 In 2016, the ^{2}H concentration in chicory plants in the DS treatment tended to be higher

386 compared with the WW treatment, but the difference was not significant. In 2017, no differences

387 were seen in tracer concentration among chicory plants across the treatments. Black medic and

388 ryegrass plants revealed significantly lower ^{2}H enrichment in comparison to intercropped chicory.

389

390 δ^{13}C enrichment

391 In 2016, there was no effect of drought on the ^{13}C concentration of the chicory biomass (Fig. 7).

392 Similarly, there was neither an effect of drought nor intercropping with ryegrass in 2017. However,

393 intercropping with black medic increased the ^{13}C concentration in chicory indicating that chicory

394 was more drought stressed in this treatment than in any of the other treatments.

395

396 **Discussion**

397

398 *Deep root growth*

399 In accordance with our hypothesis, chicory demonstrated its capability to grow roots below 3 m

400 depth and did so within 4.5 months. However, root intensity decreased markedly below 2 m in

14

401   2016 and below 2.5 m depth in 2017. The root intensity below 2 m depth at drought initiation, 2.5

402   m depth at tracer injection and 3.5 m depth at harvest in 2017 was very low and could be a result

403   of roots from the 2016 crop still visible on the rhizotron surface. Studies covering a longer growing

404   season have found extensive root growth in chicory down to 2.5 m, where equipment limitations

405   prevented observations deeper down (Thorup-Kristensen 2006; Thorup-Kristensen and Rasmussen

406   2015). In the field, higher soil bulk density (Stirzaker et al. 1996; Gao et al. 2016) and other factors

407   might restrict deep root growth, which is less likely in our semi-field facility with repacked soil.

408   However, we did use field soil with a soil bulk density comparable to field soils.

409   Both intercropping treatments decreased total root intensity especially from 0.5 to 2 m

410   depth. This has to be seen in the light of a total aboveground biomass that was twice as high as in

411   the WW sole crop treatment. Observing that chicory biomass, on the other hand, was reduced to

412   almost half when intercropped, suggests that both black medic and ryegrass had much lower root

413   intensity below 0.3 m depth than sole cropped chicory and that the interspecific competition

414   reduced both above- and belowground growth of chicory. Black medic and ryegrass are both

415   shallow rooted and are unlikely to reach below 1 m depth (Kristensen and Thorup-Kristensen

416   2004; Thorup-Kristensen and Rasmussen 2015), thus the deep roots observed in the intercropping

417   treatments are assumed to be chicory roots.

418

419   *Deep water uptake*

420   The sensors documented water uptake in all treatments from 1.7 m depth in 2016 and 2.3 m

421   depth in 2017. In fact, the sensors showed that in 2016, chicory water uptake at 1.7 m depth was

422   c. 30 % of its water uptake at 0.5 m depth when well-watered. In 2017, chicory water uptake at 2.3

423   m depth was c. 10 % of its uptake at 0.5 m depth when well-watered. In absolute terms, water

424   uptake from 1.7 m depth in 2016 was in the range of 1.5 mm m^{-1} soil column day^{-1} and from 2.3 m

425   depth in 2017, it was 0.5 mm m^{-1} soil column day^{-1}. Due to the small-sized plot placed at a windy

426   position at 4 m height, evapotranspiration must have been substantially higher than the potential

427   evapotranspiration measured nearby of 3.3 and 2.1 mm day^{-1} for the same periods in 2016 and

428   2017 respectively. Even though we did not estimate the total evapotranspiration, it is clear that

429   the water uptake from the deeper part of the root zone substantially contributed to the total plant

430   water balance.

431   The ^{2}H tracer uptake by chicory from 2.3 m depth both years support the sensor-based

432   water uptake calculations. Furthermore, the tracer study confirmed that neither black medic nor

433   ryegrass had roots deep enough to acquire water from 2.3 m depth. This is a clear example of

434   resource complementarity in root competition in intercropping (Tilman et al. 2001; Postma and

435   Lynch 2012).

436

437   *Response to water stress and intercropping*

438   Water uptake from 0.5 m depth was significantly reduced in the DS treatment compared to the

439   WW treatment indicating that the soil water potential was low enough to limit plant water uptake

440   in the DS treatment. Contrary to our expectations, we did not find a higher water uptake neither

441   at 1.7 m depth in 2016 nor at 2.3 m depth in 2017 when plants were water limited in the topsoil.

442   As biomass was not significantly reduced, whereas water uptake was reduced by 59 and 74 %, the

443   reduction in water uptake cannot be explained by a reduced water need.

444   Although not significant, the ^{2}H tracer study indicated a higher ^{2}H concentration in the

445   transpiration water in the DS compared to the WW treatment in 2016. This suggests a higher

446   relative water uptake from 1.7 m depth. A higher relative uptake from a certain depth can logically

447   be explained by an increase in water uptake from the given depth, a decrease in water uptake

448   somewhere else in the soil profile or a combination of both. As the water uptake based on the

449   sensor calculations show a significantly lower water uptake from 0.5 m depth in the DS than in the

450   WW treatment in 2016, it is likely that what we observed was the effect of decreased uptake in

451   the topsoil.

452   We only observed a significant increase in ^{13}C concentration in chicory when intercropped

453   with black medic. Samples were taken from the total biomass, and not from plant parts developed

454   during the drought, which might explain why the treatment effects were only captured in the

455   chicory and black medic intercropping, where black medic appeared to have induced drought

456   stress in chicory even before the onset of the drought stress we induced.

457   Intercropping reduced total root intensity at 0.5 m depth by over 40 %. Still, water uptake

458   from this depth was only slightly decreased indicating that the lower root intensity did not restrict

459   water uptake in well-watered conditions. Root density in upper soil layers of well-established

460   crops does not correlate well with water uptake (Anblin and Tennant 1987; Katayama et al. 2000),

16

461   which can be explained by the high mobility of water in the soil, making a dense root system

462   superfluous. Following the logic behind Walter's two-layer hypothesis (Walter 1939, 1971; Walker

463   and Noy-Meir 1982), intercropping would lead to a vertical niche partitioning resulting in

464   increased water uptake by the deep-rooted chicory when intercropped with a shallow-rooted

465   species. However, we did not observe an increase in deep water uptake.

466

467   *Absence of a deep water compensation effect*

468   We suggest three possible explanations for why we did not observe the hypothesized increase in

469   deep water uptake during drought or intercropping.

470   *1) The hydraulic resistance is too high to increase deep water uptake.* Theoretically, the

471   ability of root systems to extract water from deep roots depends not only on root system depth

472   but also on root system hydraulics (Javaux et al. 2013). Root hydraulic conductivity limits the

473   potential water uptake, and differs among species, but also among different roots in a root system

474   (Ahmed et al. 2018; Meunier et al. 2018). The ability of a root system to compensate, i.e. extract

475   water where it is easily available, for instance from deeper soil depths, is, therefore, a function of

476   (1) the xylem conductance between the roots in the extraction zone and the root crown and (2)

477   the radial root conductance in the wet zone. Compensation has been observed in chicory below

478   0.6 m depth, but this was in a study allowing root growth down to only 1.5 m depth (Vandoorne et

479   al. 2012a). In our experiment, the xylem conductance might simply have been too low in the

480   deeper part of the root zone to allow compensation, possibly because the deep soil

481   measurements were made in a zone with a low density of young roots (McCully 1995; Meunier et

482   al. 2018). However, chicory had 31 % fewer roots in the chicory and black medic intercropping

483   than in the WW treatment at 2.3 m, with no reduction in water uptake, not supporting such a

484   relationship between root density and water uptake.

485   *2) Insufficient water supply in the topsoil induces root-to-shoot signalling causing stomatal*

486   *closure, despite sufficient water supply in deeper soil layers.* Signals by phytohormones like Abscisic

487   acid (ABA), produced when parts of the root system are under low water potential, might reduce

488   plant transpiration and consequently root water uptake also from deeper depths by triggering

489   stomatal closure (Zhang and Davies 1990a, b; Tardieu et al. 1992; Dodd et al. 2008). Split-root

490   experiments, where one side of the root system is under low water potential, have found reduced

17

491   stomata conductance, despite sufficient water supply (Blackman and Davies 1985; Zhang and

492   Davies 1990b). However, experiments with vertical heterogeneity in soil water content yield

493   ambiguous results (Puértolas et al. 2015; Saradadevi et al. 2016). The hormonal signalling during

494   topsoil drying has not been tested for chicory. But chicory does show an isohydric behaviour,

495   decreasing stomatal conductance and maintaining leaf water potential during moderate drought

496   stress (Vandoorne et al. 2012b).

497   *3) Deep water uptake compensation might have occurred, but was not captured in this*

498   *experimental set-up.* Water uptake compensation could have happened between or below the

499   depths covered by the sensors. In 2016, VWC was not only lower at 0.5 m depth in the DS

500   treatment compared to the WW treatment but also in 1.7 m depth, which could have impaired the

501   water uptake from this depth, too. Water uptake could also have been confounded with water

502   redistribution in the soil column, leading to an underestimation of water uptake in depths where

503   water is moving to, and an overestimation in depths where water is moving from.

504   In summary, chicory can grow roots down to 3 m depth within 4.5 months and benefit from

505   a significant water uptake from below 2 m depth both during well-watered and drought

506   conditions. Our study highlights the benefit of deep root growth for crop water uptake, but

507   questions whether further compensation in deep water uptake takes place when water is limited

508   in the topsoil. A compensation might however, be pronounced for other crop species or for crops

509   which have had more time to establish a deep root system.

510

511   **Acknowledgments**

518

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689

690   Table 1: Main characteristics of the soil used in the rhizotrons

Depth (m)	Organic matter[1] (%)	Clay (%) <0.002 mm	Silt (%) 0.002-0.02 mm	Fine sand (%) 0.02-0.2 mm	Coarse sand (%) 0.2-2.0 mm	pH[2]
0.00-0.25	2.0	8.7	8.6	46.0	35.0	6.8
0.25-4.00	0.2	10.3	9.0	47.7	33.0	7.5

691   [1] Assuming that organic matter contains 58.7 % carbon.

692   [2] pH = Reaction Number (Rt) – 0.5. Measured in a 0.01 M $CaCL_2$ suspension, soil:suspension ratio 1:2.5.

693   Table 2: Timeline of the experiments in 2016 and 2017

	2016	2017
Sowing	May 2015	11 April
Transplanting	29 February	3 May
Onset of drying out	26 June	13 July
H^2 tracer study	19-25 July	15-21 August
Water uptake calculations	24-27 July	17-21 August
Harvest	28 July	11 September

694

695   **Fig. 1** The rhizotron facility, consisting of 12 columns of 4 m height each divided into an east- and a

696   west-facing chamber. See text for a detailed description

697

698   **Fig. 2** Biomass harvested 28 July 2016 and 11 September 2017 in the well-watered (WW) and

699   drought stressed (DS) chicory sole crop treatments, and the chicory intercropping treatments with

700   ryegrass and black medic respectively. Error bars denote standard errors, and letters indicate

701   significant differences among treatments for each year. Part of the data has also been published in

702   (Rasmussen et al. 2018).

703

704   **Fig. 3** Root intensity in the well-watered (WW) and drought stressed (DS) chicory sole crop

705   treatments and in the chicory intercropping treatment with ryegrass and black medic respectively

706   in (a) 21 June 2016, (a) 18 July 2016, (c) 6 July 2017, (d) 16 August 2017 and (e) 12 September

707   2017, corresponding to the time of drought initiation in the DS treatment, ^{2}H tracer injection and

708   for 2017, harvest. Letters indicate significant differences among treatments in the given depth.

709   Arrows indicate the depth of TDR sensors and ^{2}H tracer injection.

710

711   **Fig. 4** Difference in soil volumetric water content from field capacity at 0.5 and 1.7 m depth in

712   2016 and 0.5 and 2.3 m depth in 2017 in the well-watered (WW) and drought stressed (DS) chicory

713   sole crop treatments and in the chicory intercropping treatment with ryegrass and black medic

714   respectively. Line segments represent the outcome of a three-way ANCOVA on the time interval

715   from 24 to 27 July in 2016 and 17 to 21 August in 2017. The slope of the segments gives the daily

716   decrease in volumetric water content and is interpreted as daily plant water uptake. See also Fig. 5

717

718   **Fig. 5** Mean daily decrease in soil volumetric water content at 0.5 and 1.7 m depth 24 to 28 July

719   2016 and 0.5 and 2.3 m depth 17 to 21 August 2017 in the well-watered (WW) and drought

720   stressed (DS) chicory sole crop treatments and in the chicory intercropping treatment with

721   ryegrass and black medic respectively. All days included. The daily decrease in volumetric water

722   content is interpreted as daily plant water uptake. Error bars denote standard errors, and letters

723   indicate significant differences among treatments in a three-way ANCOVA, with depth and

724   treatment as factors and time as covariate. Separate analyses were made for each year

725

726 **Fig. 6** ^{2}H concentration in transpiration water before and after application of tracer at 2.3 m depth

727 in (A) 2016 and (B) 2017 in the well-watered (WW) and drought stressed (DS) chicory sole crop

728 treatments and in the chicory intercropping treatment with ryegrass and black medic respectively.

729 We tested significant differences in a mixed effects two-way ANOVA. To meet the assumptions of

730 homoscedasticity data were log-transformed. Separate analyses were made for each year and

731 pairwise comparisons were only made for the last date. There was no effect of treatment in 2016.

732 In 2017, the ^{2}H concentration in chicory and in black medic in the intercropping treatment

733 differed. Likewise in the chicory and ryegrass intercropping. Differing treatments are marked with

734 identical symbols

735

736 **Fig. 7** ^{13}C concentration in chicory harvested 28 July 2016 and 11 September 2017 in the well-

737 watered (WW) and drought stressed (DS) chicory sole crop treatments and in the chicory

738 intercropping treatment with ryegrass and black medic respectively. Error bars denote standard

739 errors, and letters indicate significant differences among treatments in a one-way ANOVA.

740 Separate analyses were made for each year. For 2017, the model is a mixed effects model because

741 samples for each plant in a chamber were analysed separately

742   Figure 1
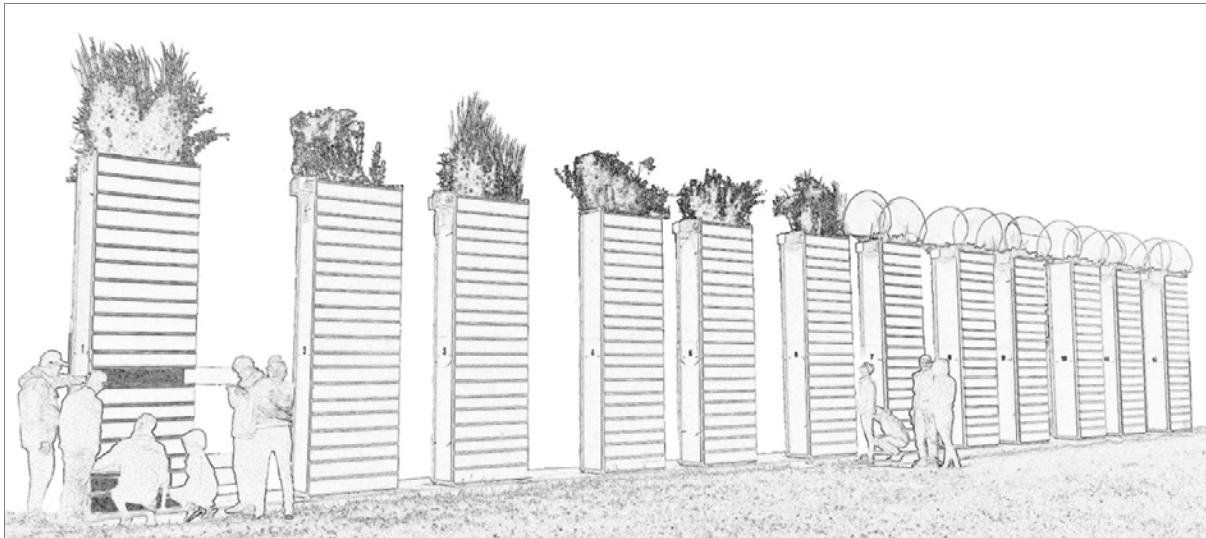

743
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745   Figure 2
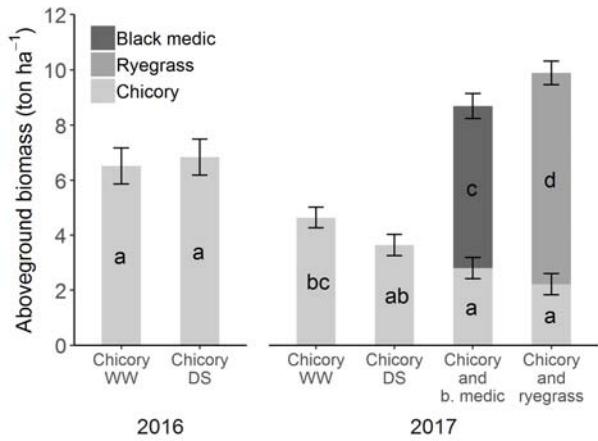
746

747   Figure 3
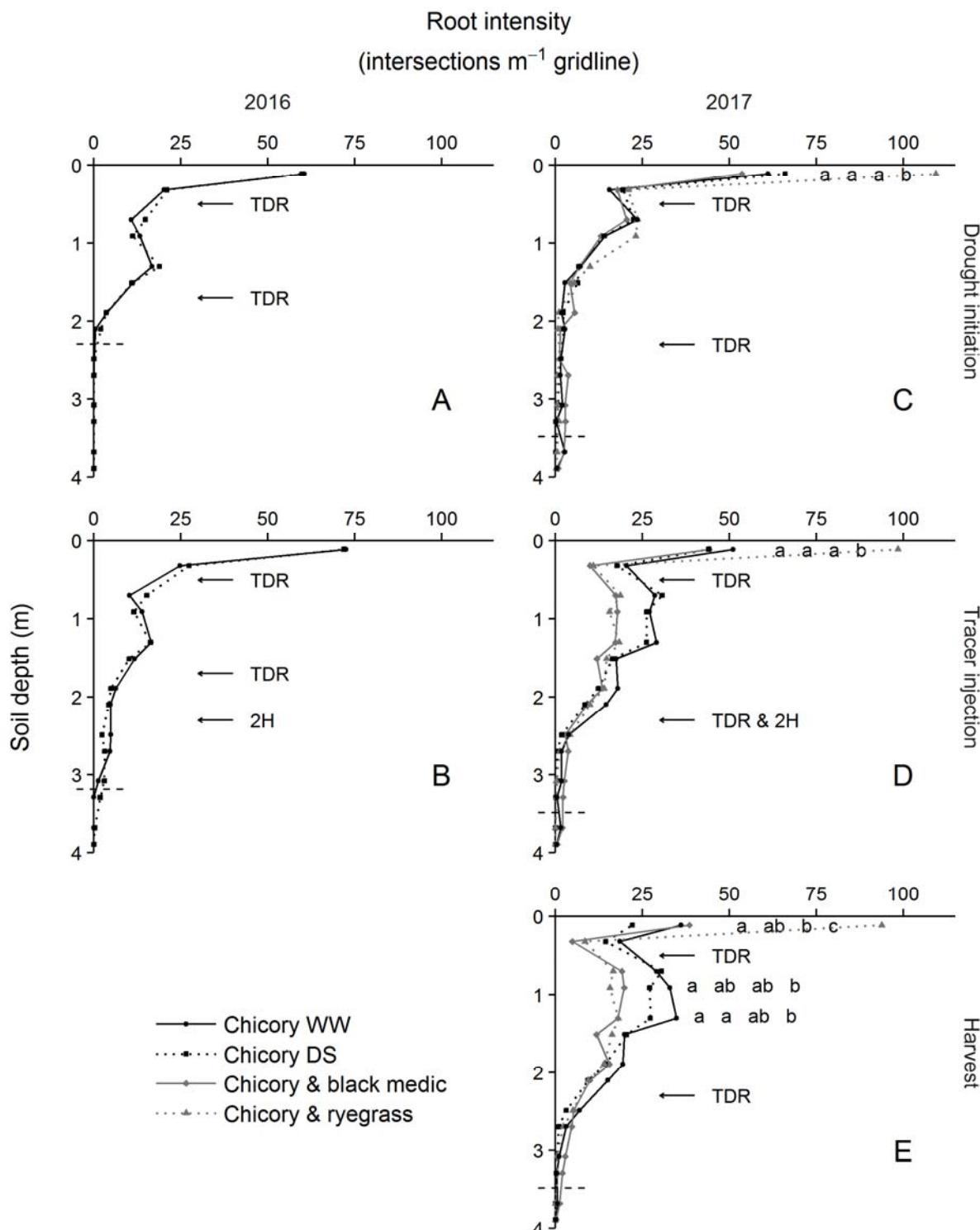
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750   Figure 4

751

752   Figure 5

753   2016 - 0.5 m   2016 - 1.7 m   2017 - 0.5 m   2017 - 2.3 m
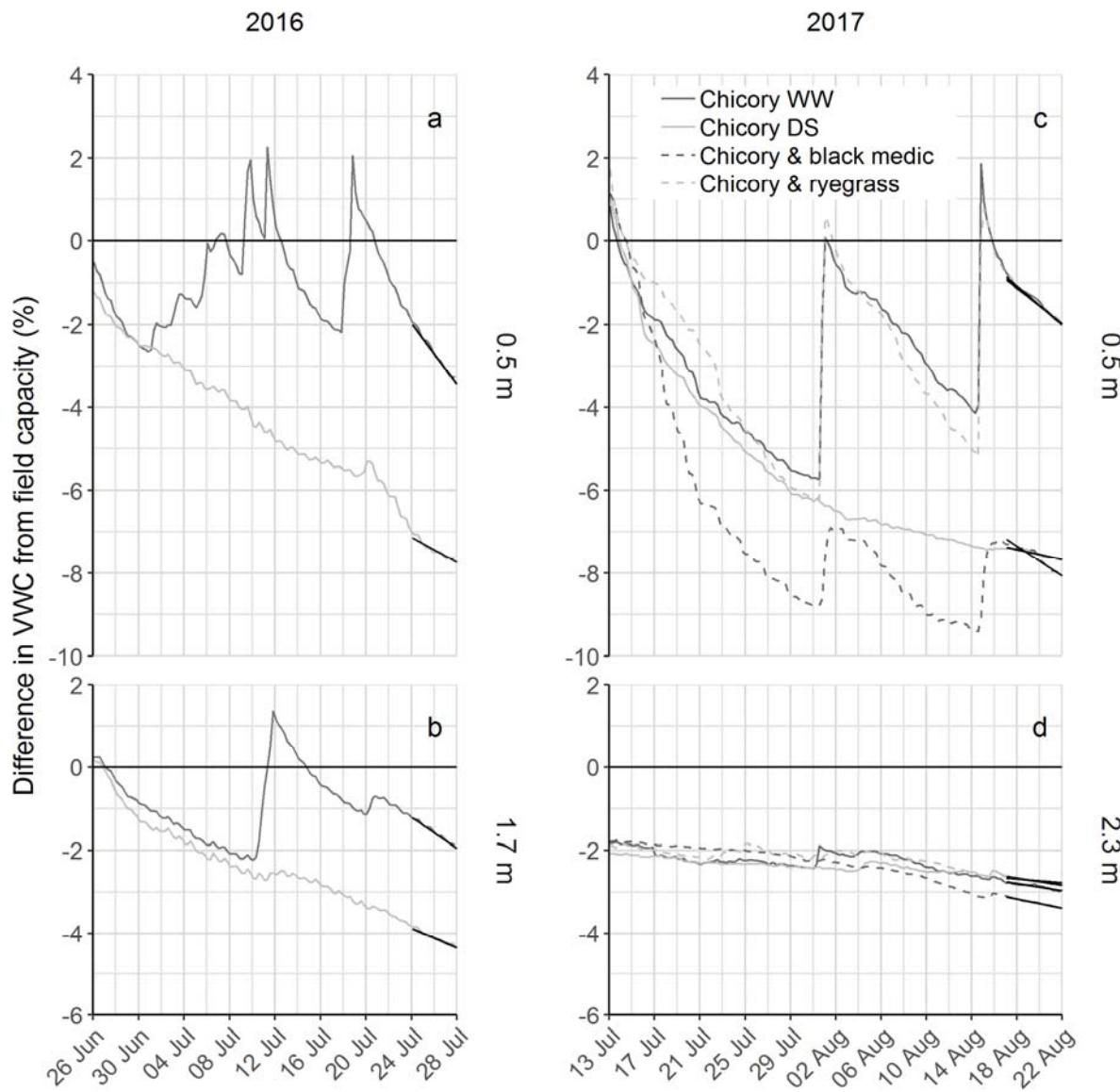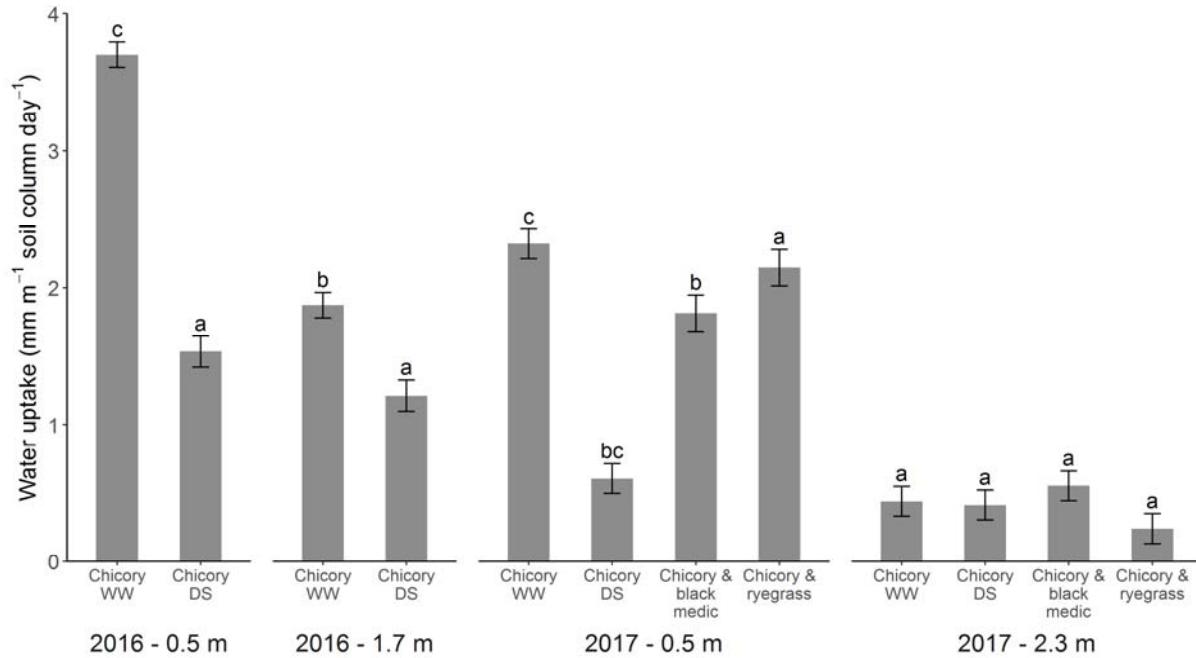
754   Figure 6

755

756   Figure 7

757

Legend (within figure):
Black medic
Ryegrass
Chicory

Y-axis: Aboveground biomass (ton ha^{-1})
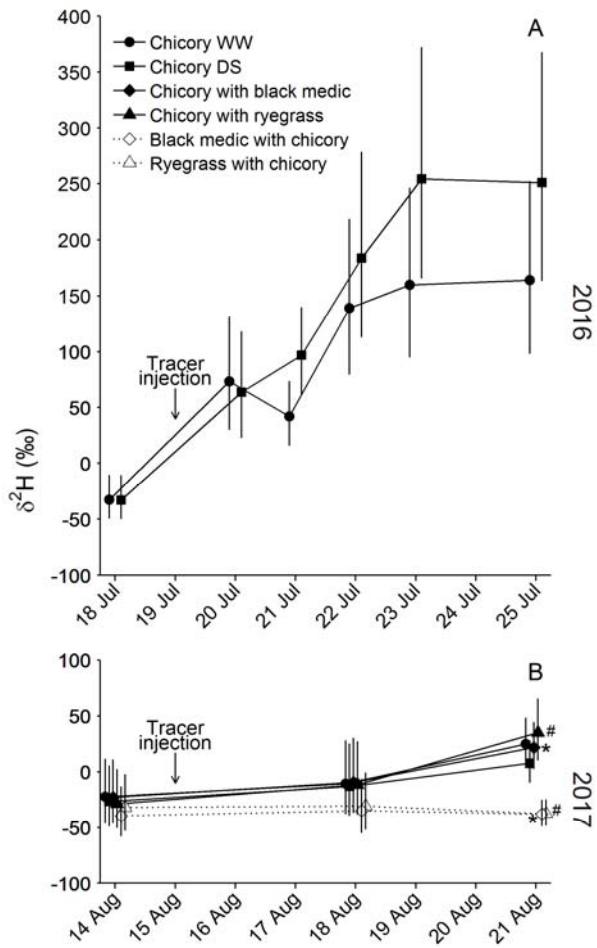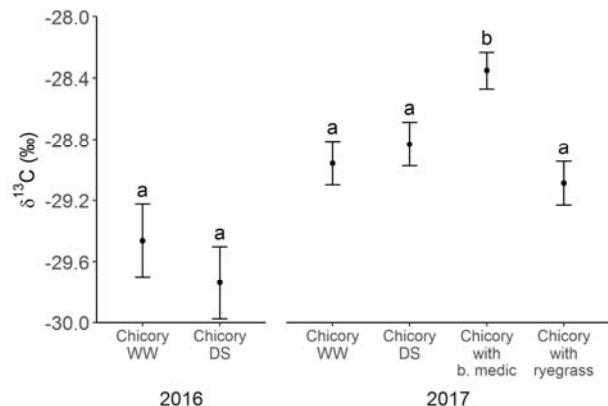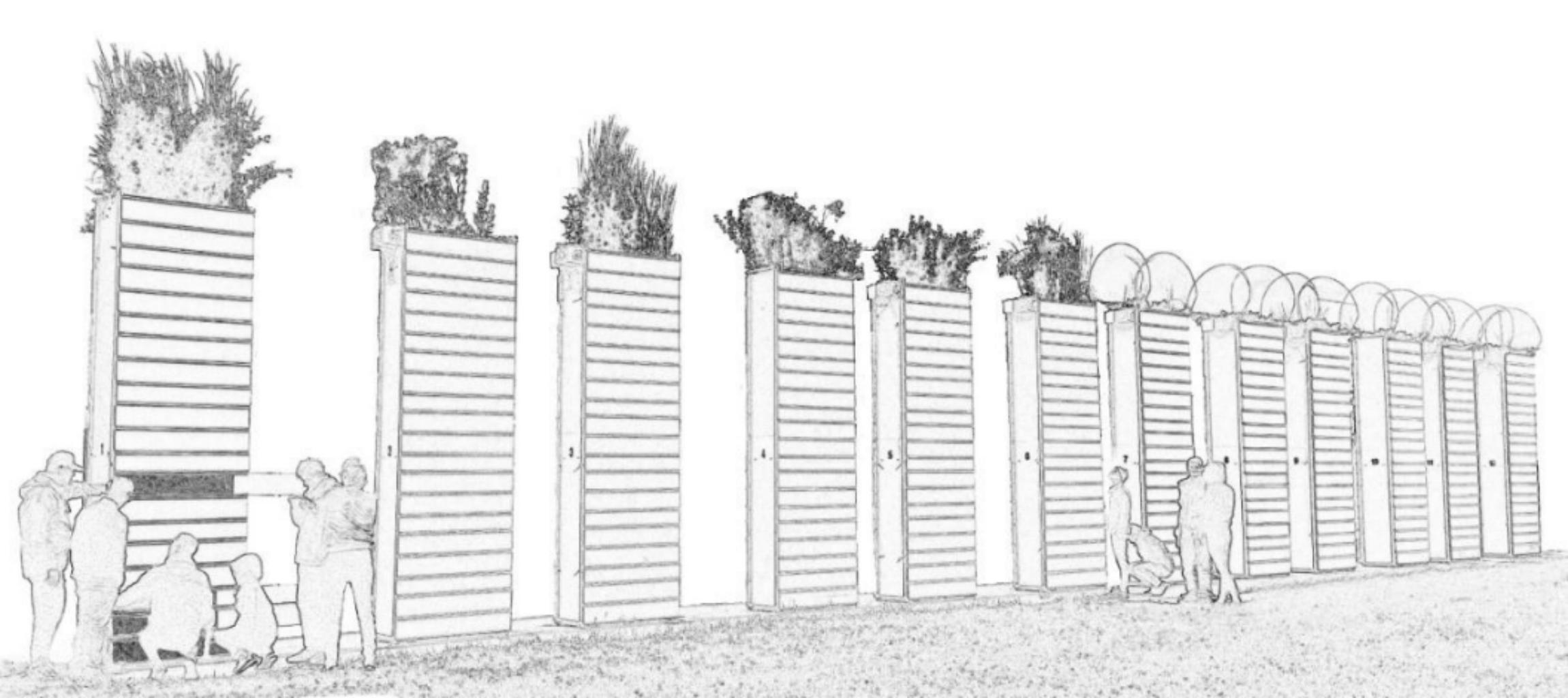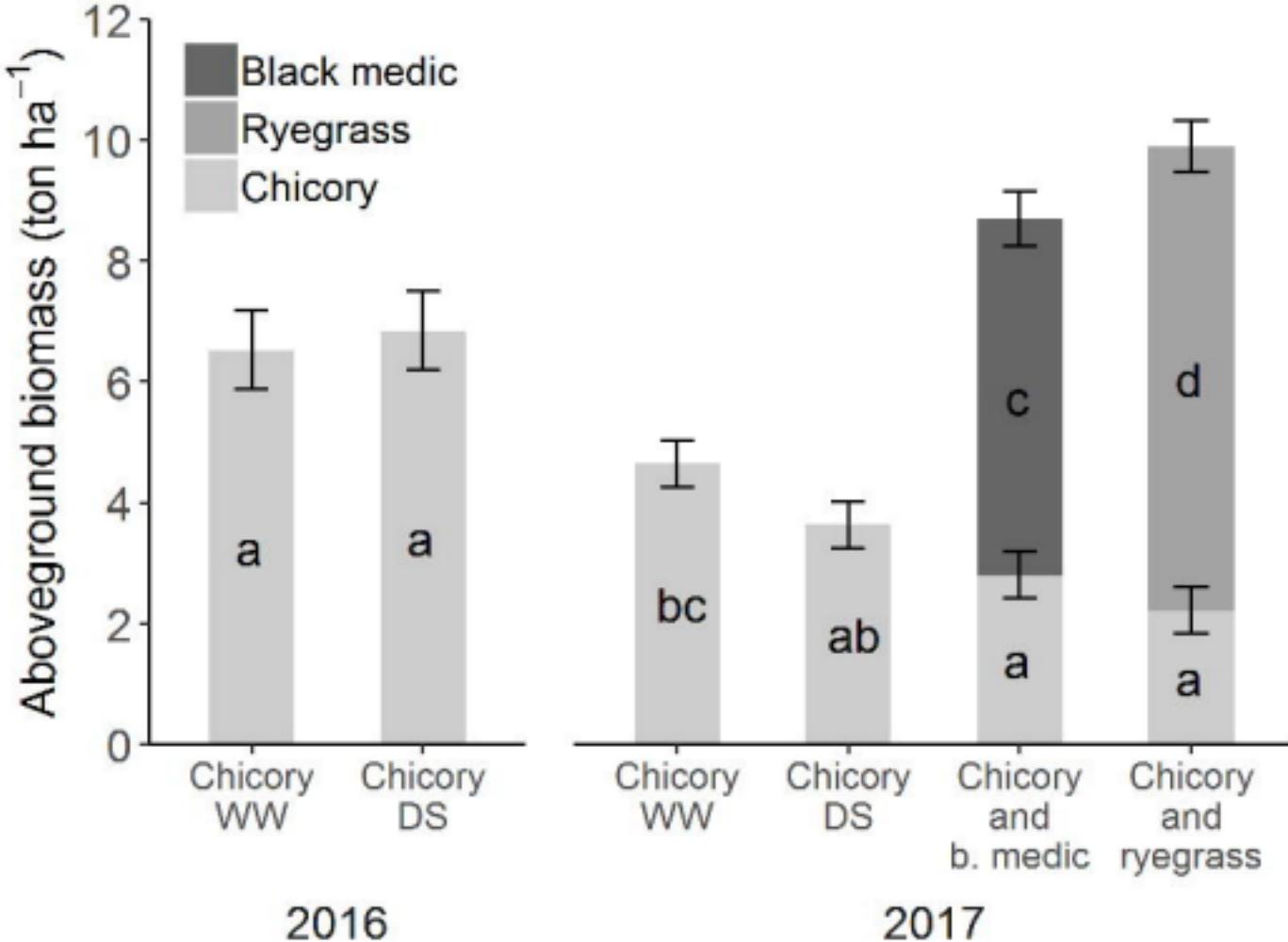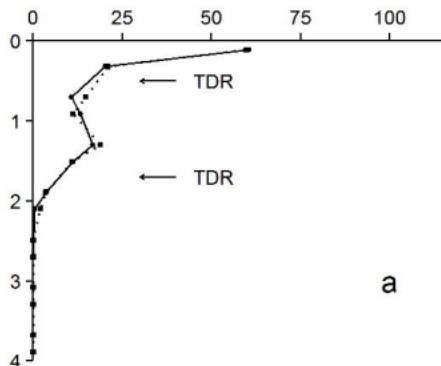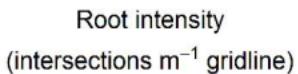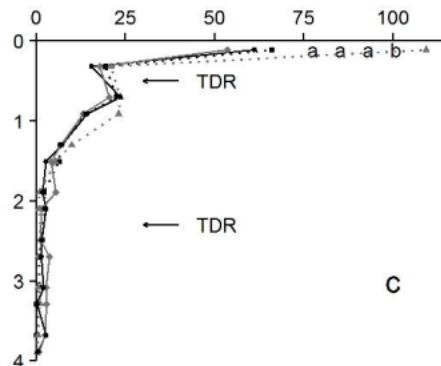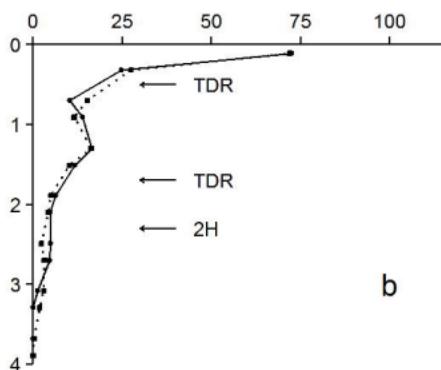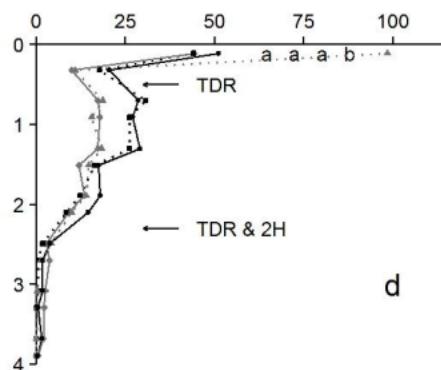
X-axis labels: Chicory WW, Chicory DS (2016); Chicory WW, Chicory DS, Chicory and b. medic, Chicory and ryegrass (2017)

Bar letters: a, a (2016); bc, ab, c, a, d, a (2017)

2016   2017

Root intensity
(intersections m⁻¹ gridline)