RESEARCH PAPER

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

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Highlight: Chicory water uptake from below 2 m contributes significantly to chicory water use. Still, chicory succumbs to topsoil drought-induced growth reduction.

Abstract

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 reached 3 m depth within a growing season and demonstrated significant water uptake from the deeper part of the root zone. We tested if chicory exposed to either topsoil drought or resource competition from shallow-rooted species 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 towards the end of the growing season. We found that water uptake from below 1.7 m in 2016 and 2.3 m in 2017 contributed significantly to chicory water use. However, neither drought nor intercropping increased the deep water uptake. We conclude that chicory benefits from being deep-rooted during drought events, yet it still succumbs to drought-induced growth reduction.

Key-words: Cichorium intybus L., Deep root growth, Deep water uptake, Drought resistance, Intercropping, Yield loss, Deuterium, hydrological tracer, ^{2}H, TDR sensor

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.

The potential influence of deep roots on water uptake has been highlighted numerous times (e.g. Canadell *et al.*, 1996; Lynch and Wojciechowski, 2015), still, information about the actual contribution of deep roots to water uptake remains scarce. Maeght et al. (2013) suggest that this is related to the absence of tools to measure deep root activity with sufficient throughput and standardization at affordable costs, and to the widespread assumption that as deep roots only represent a small fraction of the overall root system their contribution to root system function is marginal. It has also been questioned whether deep root growth under field conditions is too restricted by high soil strength, and unfavourable conditions such as e.g. hypoxia, acidity, and low nutrient availability, to substantially benefit the crop (Lynch and Wojciechowski, 2015; Gao *et al.*, 2016).

Whereas some soils definitely restrict deep root growth, other soils have shown to allow roots to grow in the deeper soil layers (Sponchiado *et al.*, 1989; Thorup-Kristensen and Rasmussen, 2015). In addition, even though the majority of the root biomass is found in the topsoil, deep roots can contribute significantly to water supply in crops, as there is little connection between root biomass and root activity (Mazzacavallo and Kulmatiski, 2015). Gregory et al. (1978) found that in the field, winter wheat had less than 5% of its root biomass below 1 m, and as long as the water supply was sufficient in the upper meter, the biomass reflected the water uptake well. However, when the topsoil dried, the roots between 1 and 2 m supplied the plants with up to 20% of the total water use. In a study conducted in an Amazonian tropical forest, Nepstad et al. (1994) found they would have underestimated the evapotranspiration by 60% in a dry year, had they not considered roots below 2 m.

Indirectly deeper root growth in crops has also been associated with deep-water uptake, as rooting depth has been shown to correlate positively with yield under drought in the field in e.g. wheat (Lopes and Reynolds, 2010), bean (Sponchiado *et al.*, 1989; Ho *et al.*, 2005), rice (Uga *et al.*, 2013) and maize (Zhu *et al.*, 2010). In addition, modeling studies indicate that selection for deeper roots in grain crops could significantly improve deep-water acquisition and thereby yield in water deficit seasons (Manschadi A *et al.*, 2006; Lilley and Kirkegaard, 2011). Common to most of these studies is that deep root growth is considered to be in the range of 0.5 to 1.5 m. But several

agricultural crops have the capability to grow roots below 2 m or even deeper within a season (Canadell *et al.*, 1996; Ward *et al.*, 2003; Thorup-Kristensen, 2006; Rasmussen *et al.*, 2015), and thereby get access to an extra pool of water originating from wet season surplus precipitation stored in the soil. E.g. has Lucerne (*Medicago sativa* L.) shown to decrease the soil water content at a 5 m depth (Fillery and Poulter, 2006).

Hydrological isotope tracer techniques have over the last two decades become an increasingly popular tool to acquire information on temporal and spatial water use patterns in plants (e.g. Bishop and Dambrine, 1995; Peñuelas and Filella, 2003; Beyer *et al.*, 2016). Injection of tracer into specific soil depths has proven to be a precise method to detect the relative water uptake from the chosen depth (Kulmatiski *et al.*, 2010; Kulmatiski and Beard, 2013; Bachmann *et al.*, 2015; Beyer *et al.*, 2016). The hydrological tracer techniques utilize the fact that no isotopic fractionation against isotope forms of hydrogen or oxygen occurs during soil water uptake by roots (Wershaw *et al.*, 1966; Dawson and Ehleringer, 1991; Bariac *et al.*, 1994; Mensforth and Walker, 1996).

The anthropocentric discussion of the importance of deep root growth in crop production is put in perspective by the fact that some plant species have evolved the potential to grow deep roots. Under what circumstances is that strategy beneficial? In this study, we hypothesize that deep root growth can help plants escape topsoil drought. More specifically, we aimed at testing the following hypotheses, using chicory (*Cichorium intybus L.*) as an example plant: 1) Chicory can grow roots below 3 m within a growing season. 2) Chicory has a significant water uptake from the deeper part of the root zone despite low root intensity. 3) When chicory is exposed to either topsoil drought or resource competition from shallow-rooted species, deep water uptake increases in compensation for the decreased topsoil water uptake.

Chicory is commonly grown in pasture mixtures for animal fodder or as a cash crop to produce inulin (Meijer *et al.*, 1993). It is known to be able to reach at least 2.5 m (Thorup-Kristensen and Rasmussen, 2015). And to be drought resistant (Monti *et al.*, 2005; Skinner, 2008; Vandoorne *et al.*, 2012*a*). To test the hypotheses we grew chicory as a sole crop and in an intercropping with the two shallow-rooted species ryegrass (*Lolium perenne L.*) and black medic (*Medicago lupulina L.*) in 4 m deep rhizotrons. We allowed extensive root development before

104 imposing a drought, as our focus is on the potential of deep roots to acquire water and not on
105 deep root growth during drought.
106
107 **Methods**
108 *Experimental facility*
109 We conducted the experiment in a semi-field facility at University of Copenhagen, Taastrup
110 (55°40'08.5"N 12°18'19.4"E), Denmark and repeated it for the two consecutive seasons, 2016 and
111 2017. We grew the crops in 4 m deep rhizotrons placed outside on a concrete foundation. The
112 rhizotrons where 1.2 x 0.6 m rectangular columns constructed of steel frames. A waterproof
113 plywood plate divided the rhizotrons lengthwise into an east- and a west-facing chamber with a
114 surface area of 1.2 x 0.3 m. The rhizotrons stood on a north-south axis, narrow side facing towards
115 one another (Figure 1). On the east- and the west facing fronts of the rhizotrons, 20 transparent
116 acrylic glass panels allowed inspection of root growth at the soil-rhizotron interface on the entire
117 surface. Each panel was 1.2 m wide and could be removed to allow direct access to the soil
118 column. Every third panel was 0.175 m tall, and the rest were 0.21 m tall. We used the narrow
119 panels for placement of equipment and soil sampling. The tall panels were used only for root
120 observations. To avoid disturbance of root growth we never removed these panels during the
121 experiment. All sides of the rhizotrons where covered in white plates of foamed PVC of 10 mm
122 thickness to avoid light exposure of soil and roots. On the fronts, the foamed PVC plates were also
123 divided into 20 panels. These were fixed in metal rails, allowing them to be slid off whenever we
124 had to observe the roots (Figure 1). A wick in the bottom of the rhizotrons allowed water to drain
125 out.
126 We used field soil as a growth medium. The bottom 3.75 m of the rhizotrons we filled with
127 subsoil taken from below the plough layer at Store Havelse, Denmark (

Table 1). We filled the upper 0.25 m with a topsoil mix of sandy loam and fine sandy soil, half of each, both from the University's experimental farm in Taastrup, Denmark. To reach a soil bulk density comparable to field conditions we filled the soil into the rhizotrons stepwise at portions of approximately 15 cm depth and used a steel piston to compact each portion by dropping it several times on the soil. We filled the rhizotrons in August 2015 and did not replace the soil during the two years. At the time of the experiment, average subsoil bulk density was 1.6 g m^{-3}, which is close to field conditions for this soil type.

We constructed rainout shelters to control water supply in the drought stress treatment. In 2016, we covered the soil with a transparent tarpaulin that had a hole for each plant stem. The tarpaulins were stretched out and fixed with a small inclination to let the water run off. It turned out that this design failed to keep out water during intense precipitation events, which happened twice during the season. Thus in 2017, we designed barrel roof rainout shelters instead, using the same clear tarpaulin and placed them on all rhizotrons. The rain-out shelters were open in the ends and on the sides to allow air circulation but were wider than the rhizotrons to minimize precipitation during windy conditions.

We installed a drip irrigation pipe (UniRam™ HCNL) with a separate tap in each chamber. The pipe supplied 5 l $hour^{-1}$, equivalent to 14 mm $hour^{-1}$ according to the surface area of the growth chambers.

Experimental design

We had two treatments in 2016 and four in 2017. In both years we grew chicory (*Cichorium intybus L.,* 2016: cv Spadona from Hunsballe frø. 2017: cv Chicoree Zoom F1 from Kiepenkerl) in monoculture under well-watered (WW) and drought stress (DS) conditions. In 2017, we also grew chicory intercropped with either ryegrass (*Lolium perenne L.*) or black medic (*Medicago lupulina L.*), both in a WW treatment. For chicory, we chose to work with a hybrid vegetable type cultivar in the second year to reduce variation among plants in size and development speed seen in the forage type used in the first year. In 2016, we transplanted four chicory plants into each chamber. In 2017, we increased to six plants per chamber. This was also an attempt to reduce within chamber variation. For the two intercropping treatments in 2017, we transplanted five ryegrass or black medic plants in between the six chicory plants.

158 For the 2016 season, chicory plants were sown in May 2015 in small pots in the greenhouse
159 and transplanted into the rhizotrons 30 September. Despite our attempt to compact the soil,
160 precipitation made the soil settle around 10% during the first winter. Therefore, 29 February 2016,
161 we carefully dug up the chicory plants, removed the topsoil, filled in more subsoil to reach 3.75 m
162 again before filling topsoil back in and replanting the chicory plants. A few chicory plants did not
163 survive the replanting and in March, we replaced them with spare plants sown at the same time as
164 the original ones and grown in smaller pots next to the rhizotrons. In 2017, we sowed chicory in
165 pots in the greenhouse 11 April and transplanted them to the rhizotron chambers 3 May (Table 2).
166 Chicory is perennial, it produces a rosette of leaves the first year and the second year it grows
167 stems and flowers.
168 We grew all treatments in three randomized replicates. The six chambers not used for the
169 experiment in 2016 but included in 2017 had also sunken during the 2015/2016 winter and the
170 same procedure was used to top up soil in these chambers before transplanting the chicory plants.
171 In 2016, we fertilized all chambers with NPK 5-1-4 fertilizer equivalent to 100 kg N ha^{-1}, half
172 on 1 April and the other half on 21 June. In 2017, we fertilized all chambers 3 May and 1 June
173 following the same procedure. Two chambers were accidentally over irrigated mid-June 2017 and
174 we re-fertilized these 16 June.
175 In 2016, we pruned the plants at 0.5 m height, several times between 24 May and 12 July to
176 postpone flowering and induce leaf and root growth.
177 We started drying out the DS treatments 26 June in 2016 and 13 July in 2017, where we
178 stopped irrigation and mounted the rainout shelters. In 2016, we kept irrigating the WW
179 treatments, whenever precipitation was considered insufficient to meet plant needs. In 2017,
180 where the rainout shelters excluded precipitation in all chambers we kept irrigating all treatments
181 apart from the DS to ensure sufficient water supply. However, we chose to supply the same
182 amount of water in all the irrigated chambers, which led to different levels of soil water content.
183
184 *Biomass and ^{13}C*
185 We harvested aboveground biomass 28 July in 2016 and 11 September in 2017. We dried the
186 biomass at 80°C for 48 hours. The biomass was analysed for $^{13}C/^{12}C$ ratio on an elemental analyser
187 interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) at the University of

California Stable Isotope Facility (Davis, California, USA). Isotope values are expressed in delta notation (δ) in per mill [‰] following the definition of Coplen (2011):

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

where R_{sample} is the ratio of the less abundant to the more abundant isotope ($^{13}C/^{12}C$) in the sample and $R_{standard}$ the ratio in a standard solution. For $\delta^{13}C$ the international standard Vienna PeeDee Belemnite ($R_{standard} = 11180.2 \times 10^{-6}$) was used. Analytical precision (σ) was 0.2‰.

The $^{13}C/^{12}C$ ratio in plants is directly related to the average stomatal conductance during growth, as discrimination between $^{12}CO_2$ and $^{13}CO_2$ during photosynthesis is greatest when stomatal conductance is high. When stomates are partially or completely closed, a greater part of the CO_2 inside the leaf is absorbed resulting in less fractionation and thereby higher $\delta^{13}C$ values of the plant tissue (Farquhar and Richards, 1984; Farquhar *et al.*, 1989).

Root measurements

We documented the development in root growth by taking photos of the soil-rhizotron interface through the transparent acrylic glass panels. For this purpose, we designed a "photo box" that could be slid on the metal rails in place of the foamed PVC panels, and thereby excluded the sunlight from the photographed area. We placed a light source consisting of two bands of LED's emitting light at 6000 K in the photo box. We used a compact camera (Olympus Tough TG 860). For each 1.2 m wide panel we took four photos to cover the full width of the panel. We photographed the roots on 21 June and 18 July 2016 and 6 July, 16 August and 12 September 2017, corresponding to the time of drought initiation in the DS treatment, ^{2}H tracer injection (see below) and for 2017, harvest. In 2017, harvest was postponed until 20 days after the ^{2}H tracer experiment, due to other tests running in the facility.

We recorded the roots using the line intersects method (Newman, 1966) modified to grid lines (Marsh, 1971; Tennant, 1975) to calculate root intensity, which is the number of root intersections m^{-1} grid line in each panel (Thorup-Kristensen, 2001). To make the counting process more effective we adjusted the grid size to the number of roots, i.e. we used coarser grids when more roots were present and finer grids for images with only a few roots. This is possible because

root intensity is independent of the length of gridline. We used four grid sizes: 10, 20, 40 and 80 mm. To minimize the variance of sampled data we used grid sizes that resulted in at least 50 intersections per panel (Ytting, 2015).

Soil water content

We installed time-domain reflectometry sensors (TDR-315/TDR-315L, Acclima Inc., Meridian, Idaho) at three depths to measure volumetric water content (VWC) in the soil. In 2016, the sensors were installed at 0.5 and 1.7 m depth. In 2017, the sensors were installed at 0.5 and 2.3 m depth. Soil water content was recorded every 5 min in 2016 and every 10 min in 2017 on a datalogger (CR6, Campbell Scientific Inc, Logan, Utah). Discrepancies in measured VWC among the sensors at field capacity (FC) let us conclude that the sensors were precise but not particularly accurate, meaning that the change over time in VWC was reliable but not the measured actual VWC. We have therefore estimated a sensor reading for each sensor at FC and reported changes in VWC from FC. We estimated FC as the mean VWC over a 48-hour interval. In 2017, the measurement was made in the autumn after excess water from a heavy rainfall had drained away. In the autumn, there is little evaporation and no plant transpiration to decrease VWC below FC, making it an optimal time to estimate FC. We did not have data from autumn 2016, so instead, we estimated FC in early spring.

Water uptake

We estimated water uptake from the VWC readings. We assume that water movement in the soil is negligible when VWC is below FC. Hence, the decrease in VWC can be interpreted as plant water uptake. Water uptake in mm m^{-1} soil column day^{-1} is therefore estimated as the mean decrease in VWC over a given time interval. We attempted to use intervals corresponding to the time of the ^{2}H tracer studies. In 2016, the interval was a postponed a few days (Figure 4, Table 2), and in 2017, the time interval did not cover the first two days of the tracer study (Figure 5).

For the period from onset of drought to harvest 2017, we tested whether the daily water uptake in 2.3 m was affected by daily mean VWC in 0.5 m across all treatments. For this period, the VWC in 2.3 m was close to FC in all treatments and therefore unlikely to affect the water uptake. As transpiration demand is high at this time of the year and plants are large, we assumed

that topsoil water limitations would limit total water uptake unless it is balanced by an increased water uptake lower in the profile. We excluded days in which the chambers were irrigated and one day after irrigation events to exclude periods with large soil water movement.

^{2}H tracer

We used ^{2}H labeled water injected into 2.3 m to trace water uptake from this depth. We mixed 90% $^{2}H_2O$ tracer with tap water 1:1, to achieve an enrichment of δ 5,665,651 ‰ and injected 100 ml per chamber. We removed one of the acrylic panels in each chamber temporarily to allow tracer injection and distributed it over 100 injection points in the soil. The injections were made at two horizontal rows of each 10 equally distributed holes 5 cm above and below 2.3 m respectively. In each of these 20 holes, we injected 5 ml tracer distributed between five points: 5, 10, 15, 20 and 25 cm from the horizontal soil surface. Tracer injection was made on 19 July 2016 and 15 August 2017.

We captured the tracer signal by collecting transpiration water using plastic bags. For studies using tracers, collecting transpiration water is considered valid, as the tracers increase the enrichment level several orders of magnitudes, which make the fractionation negligible (Thorburn and Mensforth, 1993; Beyer *et al.*, 2016). We sampled the transpiration water 1 day before tracer injection as a control and 1, 2, 3, 4 and 6 days after in 2016, and 3 and 6 days after in 2017. We fixed a plastic bag over each plant with an elastic cord that minimized air exchange with the surroundings. Transpiration water condensed on the inside of the plastic bag, which was folded inwards under the elastic cord to create a gutter for the water drops. Plastic bags were mounted on the plants two hours before noon and removed at noon.

We removed the plastic bags one by one, shook them to unite the drops, and transferred each sample to a closed plastic beaker. Later we filtered the samples through filter paper to remove soil and debris contamination and transferred the samples to glass vials.

We collected water from all plants and in most cases mixed the individual plant samples before analysis, taking equal amounts of water from each sample. Day 2 in 2016 and day 6 in 2017, we analysed the samples from each plant separately to get data on within chamber variation. For the control samples in 2017, we only collected water from two plants of each species per chamber. Single plant sample sizes varied from almost nothing to up to around 60 ml

277 in 2016 and 30 ml in 2017. The amount did not only reflect differences in transpiration rate, as it
278 was impossible to avoid spill when removing the plastic bags, and therefore we choose to use
279 equal amounts of water from each plant. For the control samples where variation was small, this is
280 of minor importance. The relatively large sample sizes for most samples limited the concerns of
281 fractionation due to evaporation during filtering and sample transfer.
282 The vials were shipped to Centre for Isotope Research, University of Groningen, The
283 Netherlands and analysed for ^{2}H on a continuous flow isotope ratio mass spectrometer (IRMS,
284 Isoprime 10) combined with a chromium reduction system (Europa PyrOH, Gehre *et al.*, 1996).
285 Isotope values are expressed in delta notation (δ) as given in equation 1. R_{sample} is the $^{2}H/^{1}H$
286 ratio in the sample and $R_{standard}$ for $\delta\,^{2}H$ is Vienna standard mean ocean water ($R_{standard} \approx$
287 $1/6412$). Analytical precision (σ) was 0.7‰.
288 In order to identify whether tracer was present in a sample, we adapted the criteria
289 proposed by Kulmatiski (2010). If a sample had a $\delta\,^{2}H$-value at least two standard deviations
290 higher than the control samples, tracer was assumed to be present.
291
292 *Statistics*
293 The effect of treatment on aboveground biomass of chicory, black medic and ryegrass was tested
294 in a mixed effects one-way ANOVA. Separate harvest of single plants allowed the inclusion of
295 chamber as random effect to account for the fact that the two intercropped species are not
296 independent.
297 The effect of soil depth and treatment on root intensity was tested in a mixed effects two-
298 way ANOVA. We included chamber as random effect to account for the fact that the different
299 depths are not independent. To meet assumptions of normality, depths where at least one of the
300 treatments had no roots in any of the replicates, were excluded from the model. Separate
301 analyses were made for each date.
302 The effect of soil depth and treatment on water uptake during a given time interval was
303 tested in a mixed effect three-way ANCOVA with time as covariate. In 2016, we excluded the
304 sensors from one replicate of the DS treatment because water reached it during a cloudburst. In
305 2017, we excluded two of the sensors in 0.5 m from the analysis, one in a chicory and ryegrass
306 intercropping treatment and one in a chicory and black medic intercropping treatment. The first

due to noise in the readings and the second due to readings showing a pattern in VWC that did not resemble the pattern of any of the other sensors.

The effect of treatment and time on ^{2}H concentration in transpiration water was tested in a mixed effects two-way ANOVA. We log-transformed the response variable to meet the assumptions of homoscedasticity. The first time point is from before tracer injection.

The effect of treatment on $\delta^{13}C$ was tested in a one-way ANOVA. For 2017, the model is a mixed effects model because samples for each plant were analysed separately.

In all cases, separate analyses were made for each year. All models used met the assumptions of normality and homoscedasticity. Differences were considered significant at $P < 0.05$. Data analyses were conducted in R version 3.4.4 (R Core team, 2018). Tukey test P-values for pairwise comparisons were adjusted for multiplicity, by single step correction to control the family-wise error rate, using the multcomp package (Hothorn *et al.*, 2008). For root intensity, we decided to control the family-wise error rate for each root depth. For the ^{2}H concentration, we only made pairwise comparisons for the last date.

Results

Plants grew well both years, and as hypothesised, roots were observed below 3 m by the end of the growing season. Both the uptake of ^{2}H tracer and sensor readings showed that chicory acquired water from 2.3 m. However, data does not suggest that a compensation takes place, i.e. deep water uptake was not increased to balance the decreased topsoil water uptake during drought.

Biomass

Plant development differed between the two experimental years. In 2016, the chicory plants were in their second growth year and went into the generative stage right from the start of the growing season. They started flowering ultimo May. Contrary in 2017, the chicory were in their first year of growth and stayed in the vegetative state. Aboveground biomass did not differ significantly between the two treatments in 2016 and was 6.52 and 6.85 ton/ha in the WW and DS treatment respectively. In 2017, chicory biomass was 4.65 and 3.64 ton/ha in the WW and DS treatment respectively, and 2.80 and 2.21 ton/ha when intercropped with either black medic or ryegrass.

337 Biomass of black medic and ryegrass was 5.89 and 7.68 ton/ha respectively. Both intercropping
338 treatments significantly reduced chicory biomass compared to the WW treatment. Ryegrass
339 produced significantly more biomass than black medic (Figure 2).
340
341 *Root growth*
342 Root growth was alike in all four treatments; however intercropping decreased total RI down to
343 around 2 m (only significant in few depths), except for 0.11 m depth, where the ryegrass
344 treatment had a significantly higher RI than the other treatments. The month-long summer
345 drought did not influence root intensity in any depths. In 2016, roots had reached 2 m at the time
346 of drought initiation, which was 3.5 months after transplanting. A month later, at the time of
347 tracer injection roots has reached below 3 m. In 2017, roots were observed almost to the bottom
348 of the rhizotrons already at drought initiation, 2 months after transplanting. However, only few
349 roots were present below 2 m. At the time of tracer injection, which was 3.5 months after
350 transplanting root intensity had started to increase down to 2.5 m, and at harvest, 4.5 months
351 after transplanting this was the case down to around 3 m (Figure 3).
352
353 *Soil moisture and water uptake*
354 During the drought, 135 and 97 mm of water was excluded from the DS treatment in 2016 and
355 2017 respectively. In 2016, the soil dried out gradually at both 0.5 m and 1.7 m depth in the DS
356 treatment and in the WW treatment between the precipitation and irrigation events. As a result,
357 the soil was drier in the DS than in the WW treatment in both of the recorded depths at time of
358 the tracer experiment (Figure 4).
359 Despite that, the WW and the two intercropping treatments in 2017 received the same
360 amount of water, less water reached the sensors at 0.5 m in the chicory and black medic
361 intercropping. This indicates a drier soil above the sensors withholding more water in this
362 treatment than in the other two irrigated treatments. At the time of the tracer experiment, soil
363 water content under the chicory and black medic intercropping was equal to the DS treatment,
364 whereas the two other treatments had a higher water content (Figure 5).
365 During the tracer rounds, chicory took up 3.7 and 2.3 mm water m^{-1} soil column day^{-1} from
366 0.5 m in 2016 and 2017 respectively in the WW treatment. Both years the uptake from 0.5 m was

reduced by more than 50% in the DS treatment. In the WW treatment chicory, took up 1.9 mm water m^{-1} from 1.7 m in 2016, whereas the uptake was only 0.44 mm water m^{-1} soil column day^{-1} from 2.3 m in 2017. In 2016, drought significantly reduced water uptake from 1.7 m, whereas no effect of drought was seen at 2.3 m in 2017. Common for both years was that the water uptake in the DS treatment was at the same level in the upper and lower depth.

Both intercropping treatments significantly reduced water uptake in 0.5 m, but no effect was seen at 2.3 m (Figure 6).

We did not find any effect of mean daily soil VWC in 0.5 m on water uptake in 2.3 m, giving no indication of a compensatory deep water uptake (Data not shown).

^{2}H enrichment

Chicory took up ^{2}H tracer from 2.3 m both years (Figure 7). Two days after tracer application in 2016, 21 out of 23 chicory plants demonstrated isotope ratios that were two standard deviations or more above controls. Six days after tracer application in 2017, it was 30 out of 64 chicory plants. No ryegrass or black medic plants indicated tracer uptake.

In 2016, the ^{2}H concentration in chicory plants in the DS treatment tended to be higher than in the WW treatment, but the difference was not significant. In 2017, no differences were seen in tracer uptake among chicory in the different treatments, but the ^{2}H enrichment of the black medic and ryegrass was significantly lower than that of the chicory plants they were intercropped with (Figure 7).

$\delta^{13}C$ enrichment

In 2016, there was no effect of drought on the ^{13}C concentration of the chicory biomass. Likewise, there was neither an effect of drought nor intercropping with ryegrass in 2017. However, intercropping with black medic increased the ^{13}C concentration in chicory indicating that chicory was more drought stressed in this treatment than in any of the other treatments.

Discussion

Deep root growth

In accordance with our hypothesis, chicory demonstrated its capability to grow roots below 3 m and did so within 4.5 months. However, root intensity decreased markedly below 2 m in 2016 and below 2.5 in 2017. The root intensity below 2 m at drought initiation, 2.5 m at tracer injection and 3.5 m at harvest in 2017 was very low and could be a result of roots from the 2016 crop still visible on the rhizotron surface. Studies covering a longer growing season have found extensive root growth in chicory down to 2.5 m, where equipment limitations prevented observations deeper down (Thorup-Kristensen, 2006; Thorup-Kristensen and Rasmussen, 2015). In the field, higher soil bulk density and other factors might restrict deep root growth, which is less likely in our semi-field facility with repacked soil (Gao *et al.*, 2016). However, we did use field soil with a soil bulk density of 1.6 g m^{-3}, which is comparable to field soils.

Apart from in the topsoil, both intercropping with ryegrass and black medic restricted total root intensity down to around 2 m. This has to be seen in the light of a total aboveground biomass that was twice as high as in the WW sole crop treatment. Observing that chicory biomass, on the other hand, was reduced to around one half when intercropped, suggests that both black medic and ryegrass had much lower root intensity below 0.3 m than sole cropped chicory and that the interspecific competition reduced both above- and belowground growth of chicory. Black medic and ryegrass are both shallow rooted and are unlikely to reach below 1 m (Thorup-Kristensen and Rasmussen, 2015), thus the deep roots observed in the intercropping treatments are assumed to be chicory roots.

Deep water uptake

The sensors documented water uptake in all treatments from 1.7 m in 2016 and 2.3 m in 2017. In fact, the sensors showed that in 2016, chicory water uptake at 1.7 m was c. 30% of its water uptake at 0.5 m even when well-watered. In 2017, chicory water uptake at 2.3 m was c. 10% of its uptake at 0.5 m. In absolute terms, water uptake from 1.7 m in 2016 was in the range of 1.5 mm m^{-1} soil column day^{-1} and from 2.3 m in 2017, it was 0.5 mm m^{-1} soil column day^{-1}. Due to the small plots placed at a windy position at 4 m height, evapotranspiration must have been substantially higher than the potential evapotranspiration measured nearby of 3.3 and 2.1 mm day^{-1} for the same periods in 2016 and 2017 respectively. Even though we did not estimate the total

426 evapotranspiration, it is clear that the water uptake from the deeper part of the root zone
427 substantially contributed to the total plant water balance.
428 The ^{2}H tracer uptake by chicory from 2.3 m both years support the sensor based water
429 uptake calculations. Furthermore, the tracer study confirmed that neither black medic nor
430 ryegrass had roots deep enough to acquire water from 2.3 m. This is a clear example of resource
431 complementarity in root competition in intercropping (Tilman *et al.*, 2001; Postma and Lynch,
432 2012).
433
434 *Response to water stress and intercropping*
435 Water uptake from 0.5 m was significantly reduced in the DS treatment compared to the WW
436 treatment indicating that the soil water potential was low enough to limit plant water uptake in
437 the DS treatment. Also, biomass was reduced in DS treatment, showing that plant growth was
438 restricted. Contrary to our expectations, we did not find a higher water uptake neither at 1.7 m in
439 2016 nor at 2.3 m in 2017 when plants were water limited in the topsoil. As biomass was only
440 reduced by 5 and 21% in 2016 and 2017 respectively, whereas water uptake was reduced by 59
441 and 74%, the reduction in water uptake cannot be explained by a reduced water need.
442 Although not significant, the ^{2}H tracer study indicated a higher ^{2}H concentration in the
443 transpiration water in the DS than in the WW treatment in 2016. This suggests a higher relative
444 water uptake from 1.7 m. A higher relative uptake from a certain depth can logically be explained
445 by an increase in water uptake from the given depth, a decrease in water uptake somewhere else
446 in the soil profile or a combination of both. As the water uptake based on the sensor calculations
447 show a significantly lower water uptake from 0.5 m in the DS than in the WW treatment in 2016, it
448 is likely that what we observed was the effect of decreased uptake in the topsoil.
449 We only observed a significant increase in ^{13}C concentration in chicory when intercropped
450 with black medic. Samples were taken from the total biomass, and not from plant parts developed
451 during the drought, which might explain why the treatment effects were only captured in the
452 black medic intercropping, where black medic appeared to have induced drought stress in chicory
453 even before the onset of the drought stress we induced.
454 Intercropping reduced total root intensity in 0.5 m by more than 40%. Still, water uptake
455 from this depth was only slightly decreased, indicating that the lower root intensity did not restrict

456 water uptake under well-watered conditions. Root density in upper soil layers of well-established
457 crops does not correlate well with water uptake (Anblin and Tennant, 1987; Katayama *et al.*,
458 2000), which can be explained by the high mobility of water in the soil, making a dense root
459 system superfluous. Following the logic behind Walter's two-layer hypothesis (Walter, 1939, 1971;
460 Walker and Noy-Meir, 1982), intercropping would lead to a vertical niche partitioning resulting in
461 an increased water uptake by the deep-rooted chicory when intercropped with a shallow-rooted
462 species. However, we did not observe an increase in deep water uptake.
463
464 *Absence of a deep water compensation effect*
465 We suggest three possible explanations for why we did not observe the hypothesised increase in
466 deep water uptake during drought or intercropping.
467 *1) The hydraulic resistance is too high to increase deep water uptake.* Theoretically, the
468 ability of root systems to extract water from deep roots depends not only on root system depth
469 but also on root system hydraulics (Javaux *et al.*, 2013). Root hydraulic conductivity limits the
470 potential water uptake, and differs among species, but also among different roots in a root system
471 (Ahmed *et al.*, 2018; Meunier *et al.*, 2018). The ability of a root system to compensate, i.e. extract
472 water where it is easily available, for instance from deeper soil depths, is, therefore, a function of
473 (1) the xylem conductance between the roots in the extraction zone and the root crown and (2)
474 the radial root conductance in the wet zone. Compensation has been observed in chicory below
475 0.6 m, but this was in a study allowing root growth down to only 1.5 m (Vandoorne *et al.*, 2012*a*).
476 In our experiment, the xylem conductance might simply have been too low in the deeper part of
477 the root zone to allow compensation, possibly because the deep soil measurements were made in
478 a zone with a low density of young roots (McCully, 1995; Meunier *et al.*, 2018). However, chicory
479 had 31% fewer roots in the black medic intercropping than in the WW treatment at 2.3 m, with no
480 reduction in water uptake, not supporting such a relationship between root density and water
481 uptake.
482 *2) Insufficient water supply in the topsoil induces root-to-shoot signalling causing stomatal*
483 *closure, despite sufficient water supply in deeper soil layers.* Signals by phytohormones like Abscisic
484 acid (ABA), produced when parts of the root system are under low water potential, might reduce
485 plant transpiration and consequently root water uptake also from deeper depths by triggering

stomatal closure (Zhang and Davies, 1990*a*,*b*; Tardieu *et al.*, 1992; Dodd *et al.*, 2008). Split-root experiments, where one side of the root system is under low water potential, have found reduced stomata conductance, despite sufficient water supply (Blackman and Davies, 1985; Zhang and Davies, 1990*b*). However, experiments with vertical heterogeneity in soil water content yield ambiguous results (Puértolas *et al.*, 2015; Saradadevi *et al.*, 2016). The hormonal signalling during topsoil drying has not been tested for chicory. But chicory does show an isohydric behaviour, decreasing stomatal conductance and maintaining leaf water potential during moderate drought stress (Vandoorne *et al.*, 2012*b*).

3) Deep water uptake compensation might have occurred, but was not captured in this experimental setup. Water uptake compensation could have happened between or below the depths covered by the sensors. In 2016, VWC was not just lower in 0.5 m in the DS treatment compared to the WW treatment but also in 1.7 m, which could have impaired the water uptake from this depth too. Water could also have been confounded with water redistribution in the soil column, leading underestimation of water uptake in depths where water is moving to an overestimation in depths where water is moving from.

In summary, chicory can grow roots down to 3 m depth within 4.5 months and benefit from a significant water uptake from below 2 m both during well-watered and drought conditions. Our study highlights the benefit of deep root growth for crop water uptake, but questions whether further compensation in deep water uptake takes place when water is limited in the topsoil. Drought decreased aboveground biomass, showing that chicory was not able to escape the drought. A compensation might however be pronounced for other crop species or for crops which have had more time to establish a deep root system.

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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

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

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

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

Figure 1: The rhizotron facility, consisting of 12 columns of 4 m height each divided into an east- and a west-facing chamber. See text for a detailed description.

Figure 2: Biomass harvested 28 July 2016 and 11 September 2017 in the well-watered (WW) and drought stressed (DS) chicory sole crop treatments, and the chicory intercropping treatments with ryegrass and black medic respectively. Error bars denote standard errors, and letters indicate significant differences in a mixed effects one-way ANOVA. Separate analyses were made for each year.

Figure 3: Root intensity in the well-watered (WW) and drought stressed (DS) chicory sole crop treatments and in the chicory intercropping treatment with ryegrass and black medic respectively in (A) 21 June 2016, (B) 18 July 2016, (C) 6 July 2017, (D) 16 August 2017 and (E) 12 September 2017, corresponding to the time of drought initiation in the DS treatment, 2H tracer injection and for 2017, harvest. Letters indicate significant differences among treatments in the given depth in a mixed effects two-way ANOVA (i.e. depth is included as a factor in the model, but pairwise comparisons are made depth wise). In depths without letters, none of the treatments differed significantly. To meet assumptions of normality, depths where at least one of the treatments had no roots in any of the replicates, were excluded from the model, indicated by the depths below the dashed lines. Separate analyses were made for each date. Arrows indicate the depth of TDR sensors and 2H tracer injection. SE are left out to ensure readability.

Figure 4: Difference in soil volumetric water content from field capacity in 0.5 and 1.7 m in 2016 in the well-watered (WW) and drought stressed (DS) chicory sole crop treatments. Line segments represent the outcome of a three-way ANCOVA on the time interval from 24 to 27 July. The slope of the segments gives the daily decrease in volumetric water content and is interpreted as daily plant water uptake. See also Figure 6.

Figure 5: Difference in soil volumetric water content from field capacity in 0.5 and 2.3 m in 2017 in the well-watered (WW) and drought stressed (DS) chicory sole crop treatments and in the chicory intercropping treatment with ryegrass and black medic respectively. Line segments represent the

outcome of a three-way ANCOVA on the time interval from 17 to 21 August. The slope of the segments gives the daily decrease in volumetric water content and is interpreted as daily plant water uptake. See also Figure 6.

Figure 6: Mean daily decrease in soil volumetric water content in 0.5 and 1.7 m 24 to 28 July 2016 and 0.5 and 2.3 m 17 to 21 August 2017 in the well-watered (WW) and drought stressed (DS) chicory sole crop treatments and in the chicory intercropping treatment with ryegrass and black medic respectively. All days included. The daily decrease in volumetric water content is interpreted as daily plant water uptake. Error bars denote standard errors, and letters indicate significant differences among treatments in a three-way ANCOVA, with depth and treatment as factors and time as covariate. Separate analyses were made for each year.

Figure 7: ^{2}H concentration in transpiration water before and after application of tracer in 2.3 m in (A) 2016 and (B) 2017 in the well-watered (WW) and drought stressed (DS) chicory sole crop treatments and in the chicory intercropping treatment with ryegrass and black medic respectively. We tested significant differences in a mixed effects two-way ANOVA. To meet the assumptions of homoscedasticity data was log-transformed. Separate analyses were made for each year and pairwise comparisons were only made for the last date. There was no effect of treatment in 2016. In 2017, the ^{2}H concentration in chicory and in black medic in the intercropping treatment differed. Likewise in the chicory and ryegrass intercropping. Differing treatments are marked with identical symbols.

Figure 8: ^{13}C concentration in chicory harvested 28 July 2016 and 11 September 2017 in the well-watered (WW) and drought stressed (DS) chicory sole crop treatments and in the chicory intercropping treatment with ryegrass and black medic respectively. Error bars denote standard errors, and letters indicate significant differences among treatments in a one-way ANOVA. Separate analyses were made for each year. For 2017, the model is a mixed effects model because samples for each plant in a chamber were analysed separately.

Figure 1

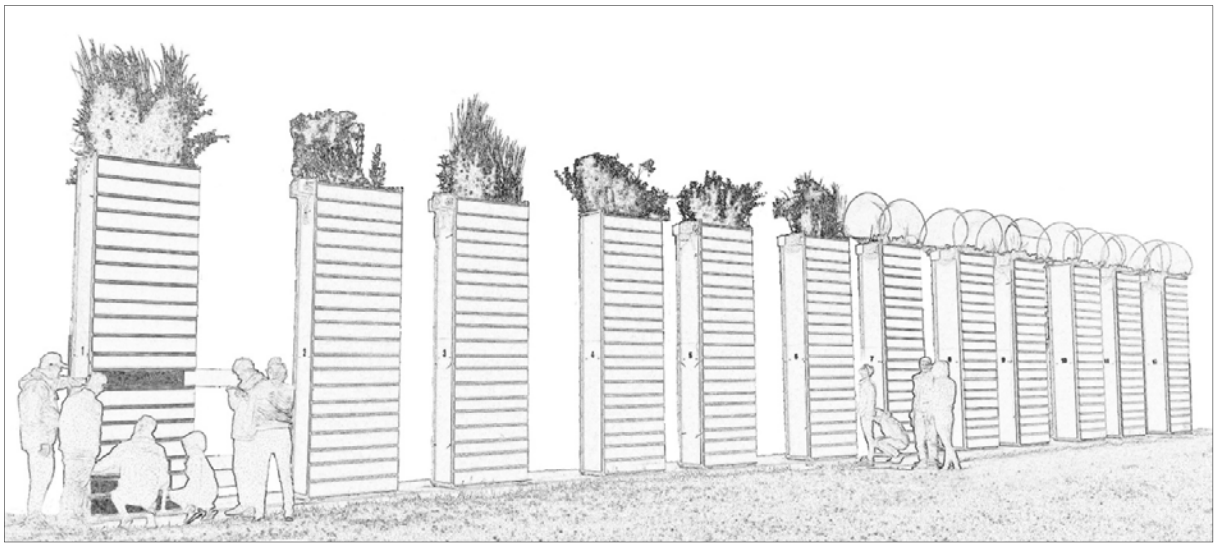


Figure 2

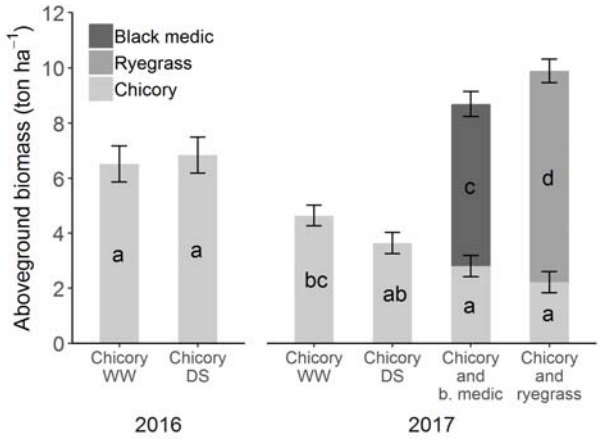


Figure 3

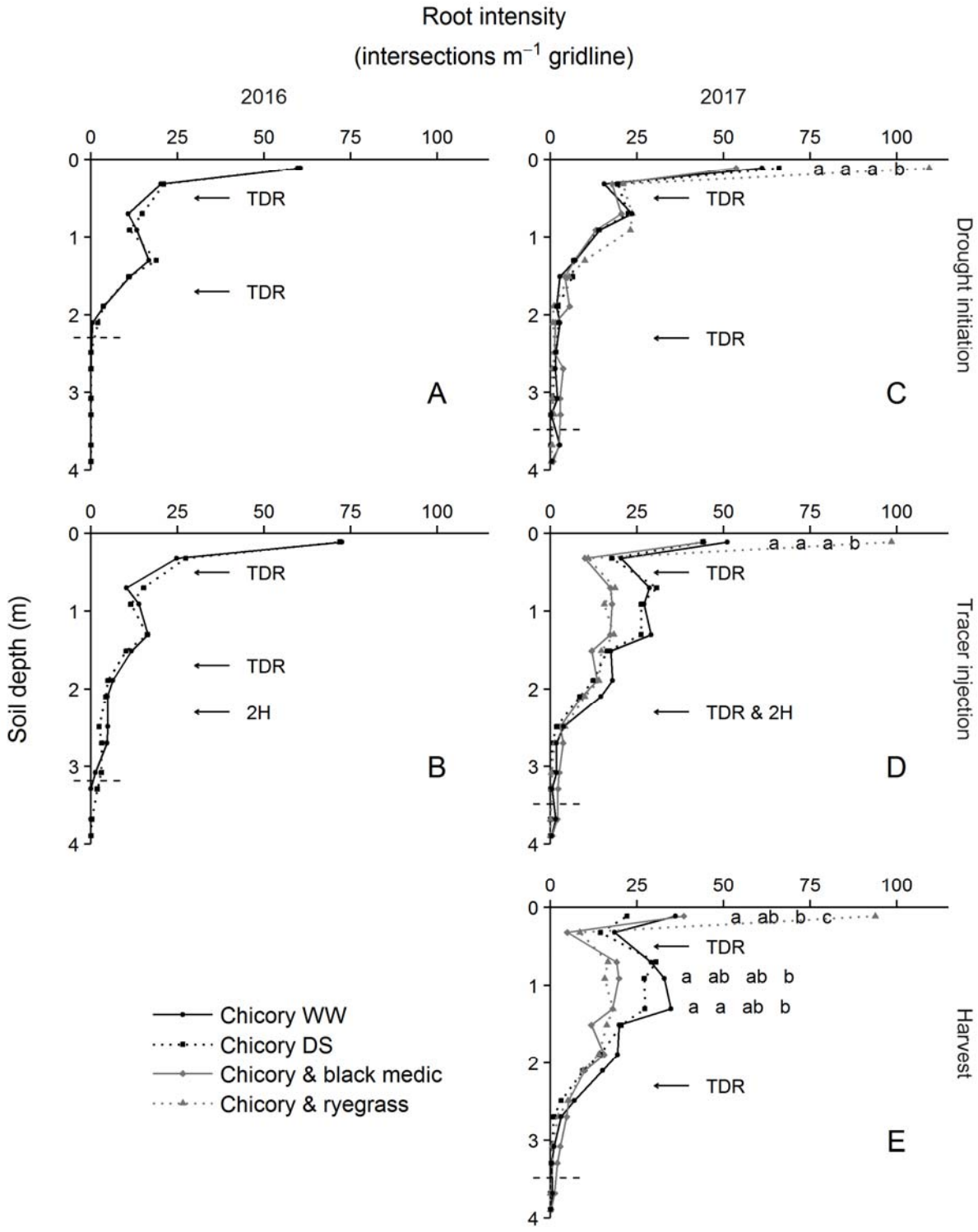


Figure 4

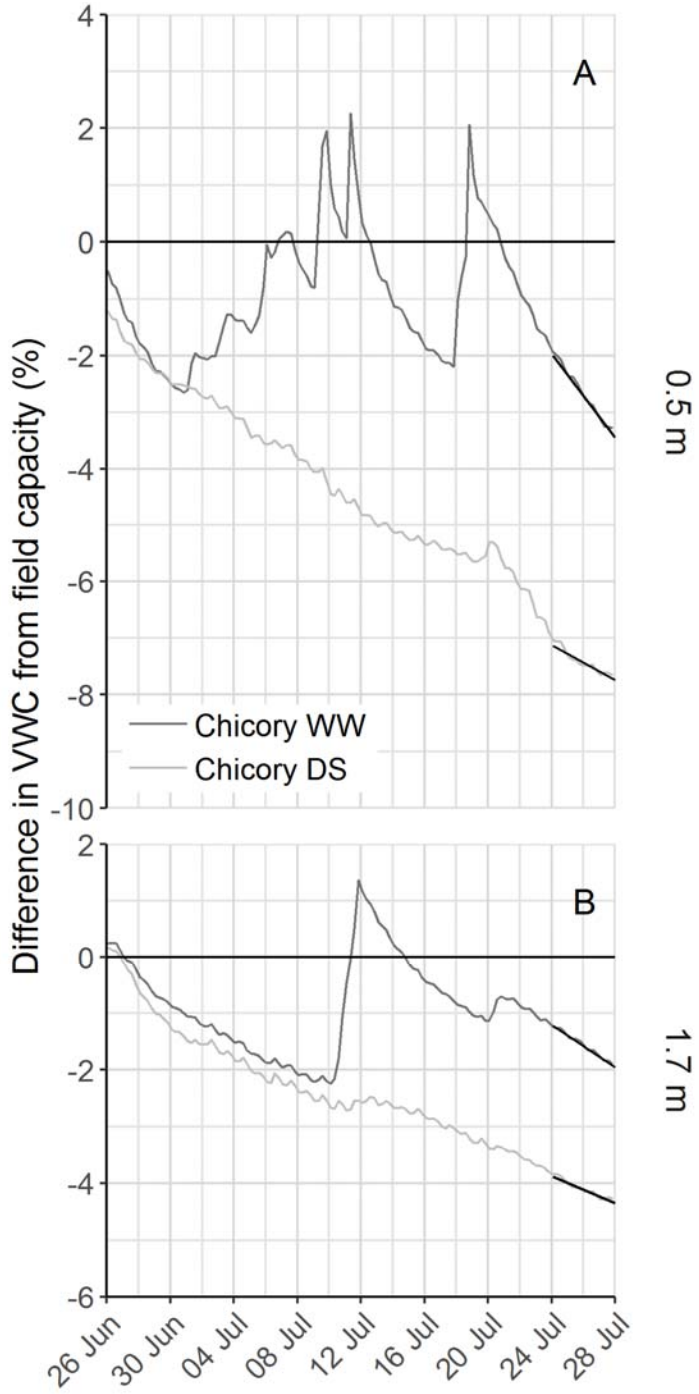


Figure 5

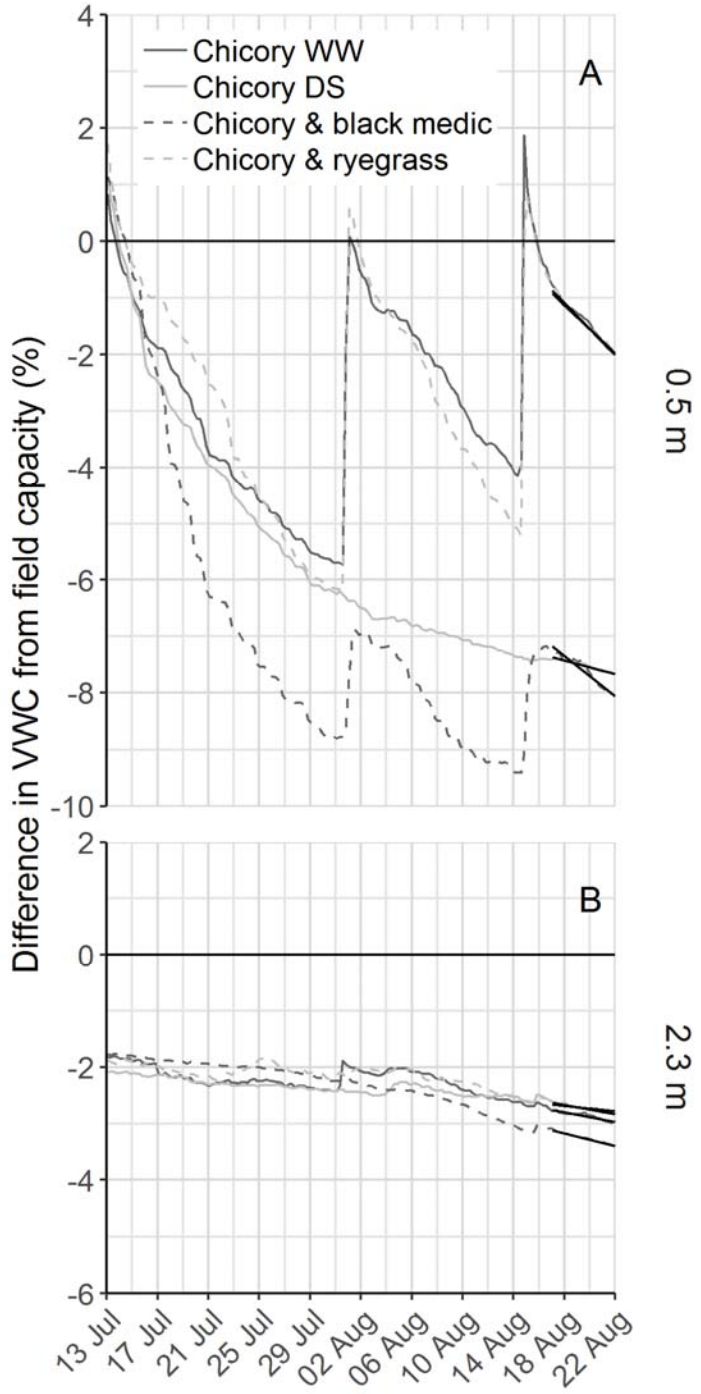


Figure 6

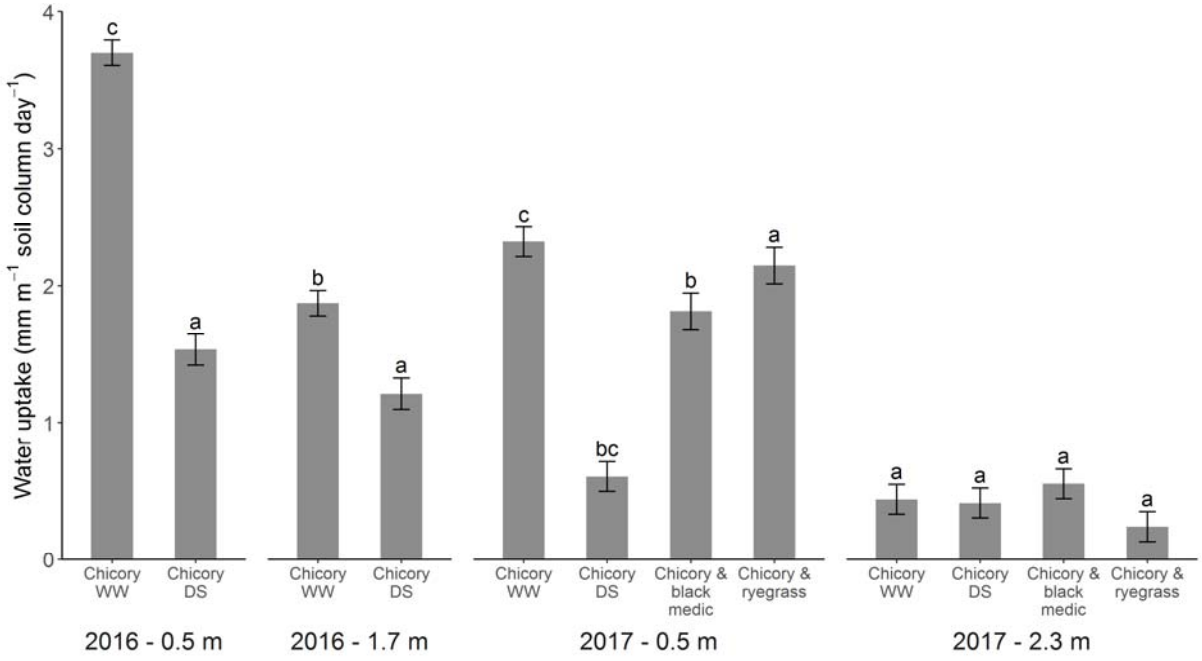


Figure 7

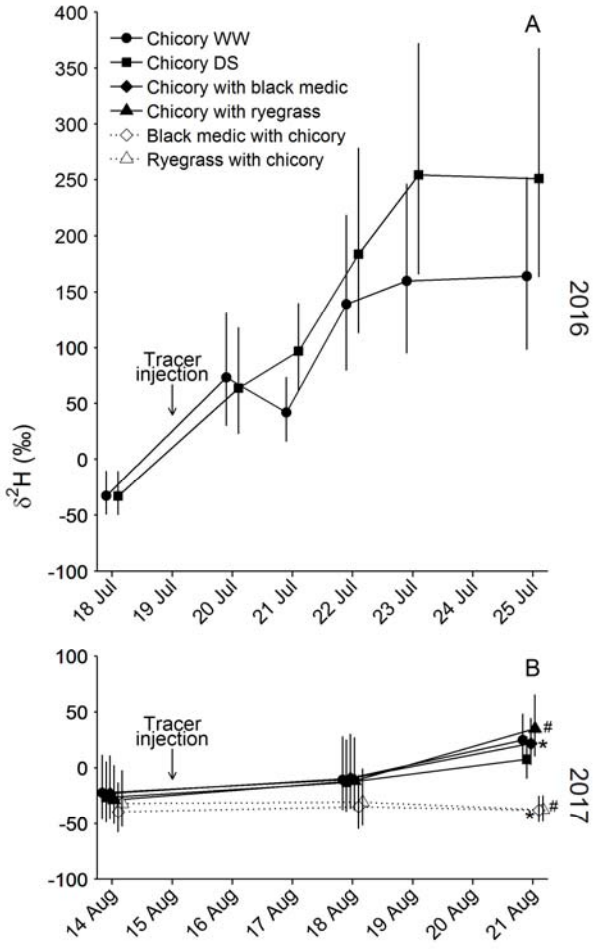
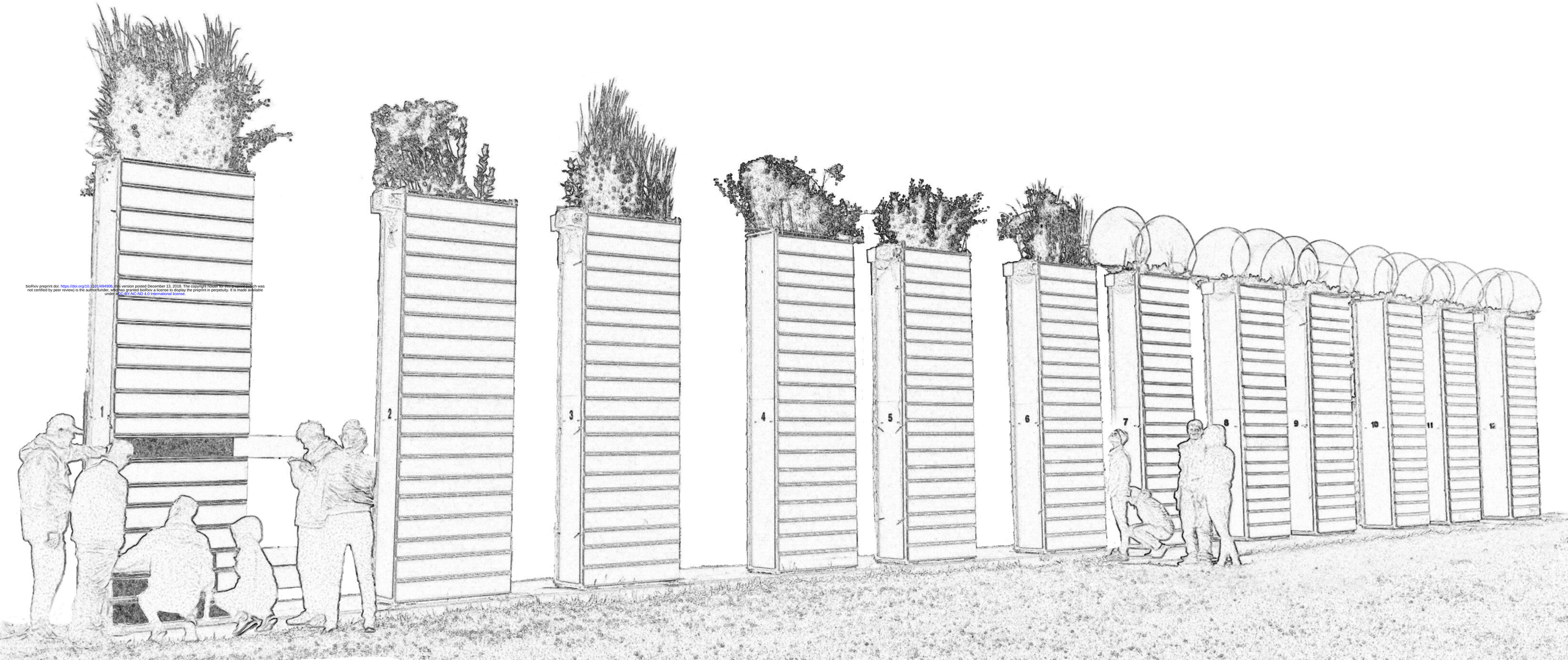
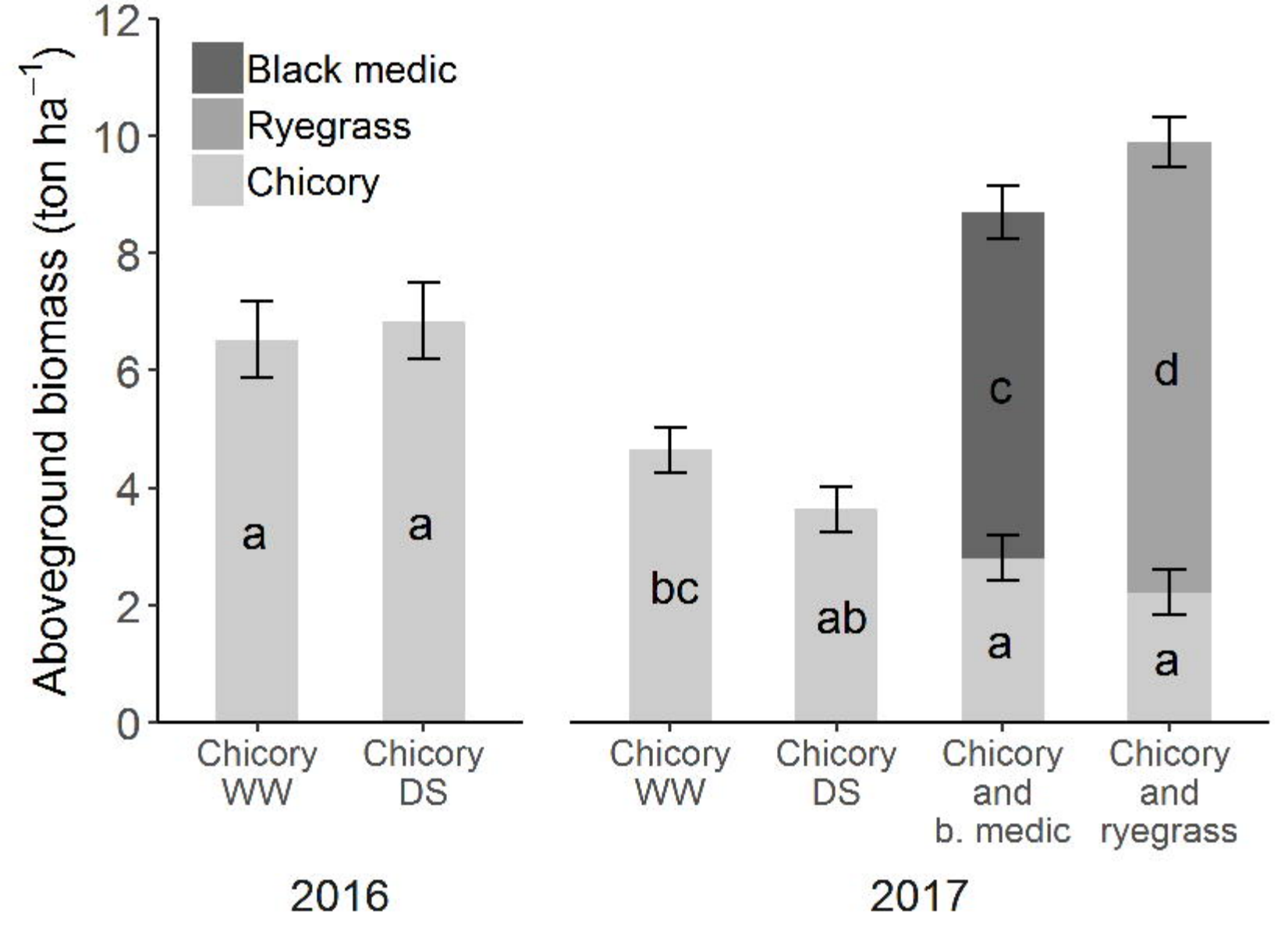
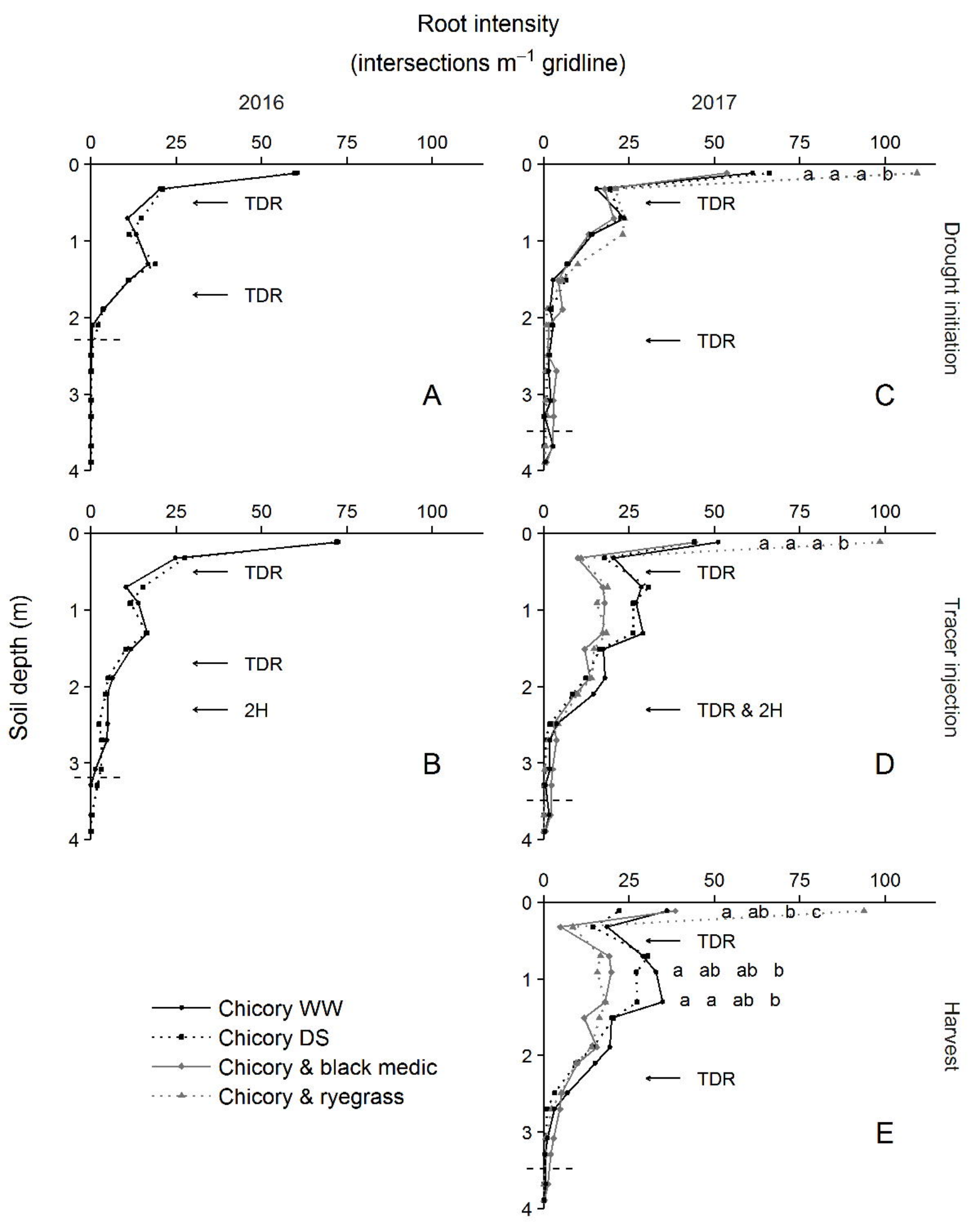
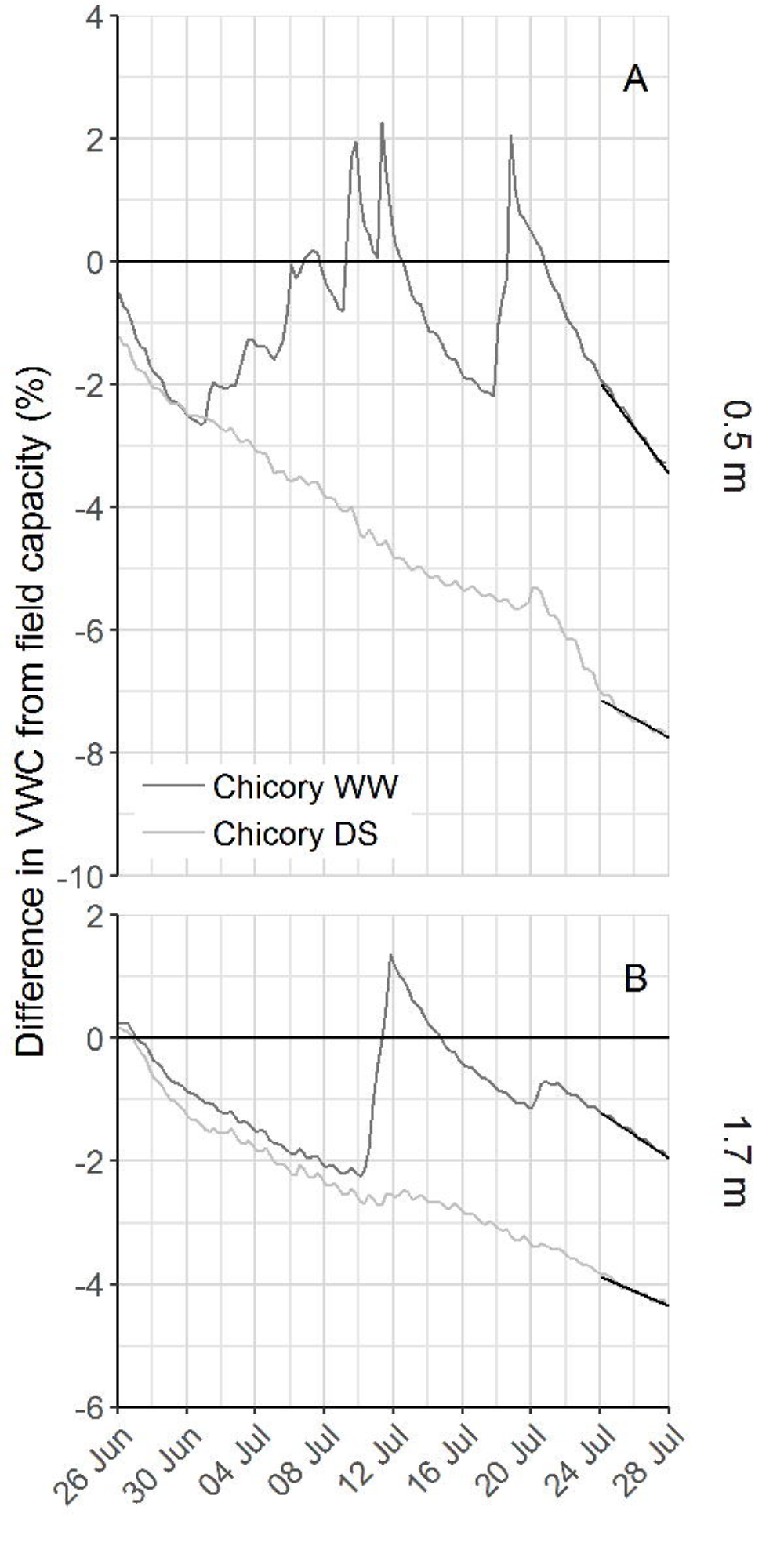


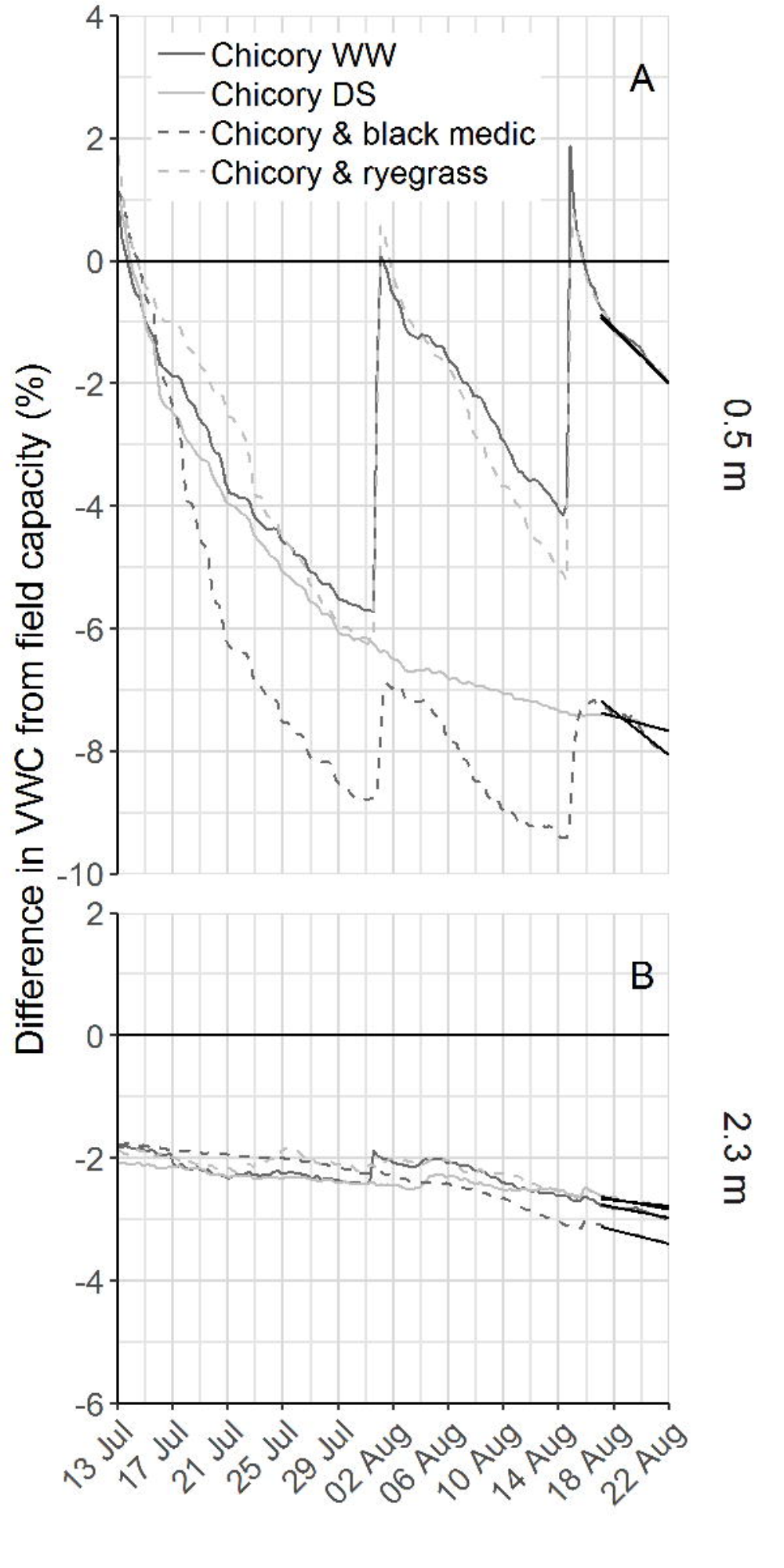
Figure 8

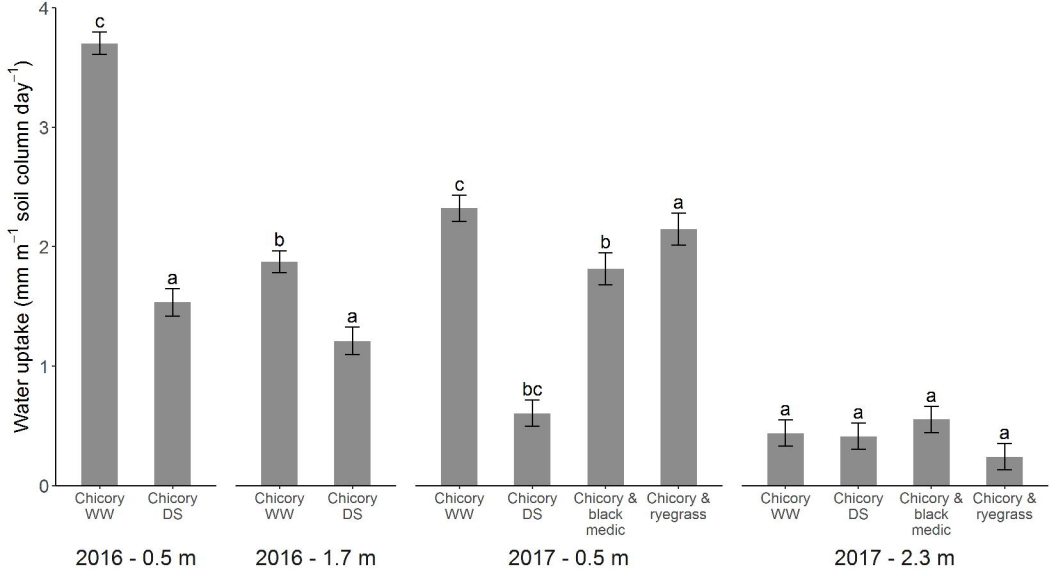


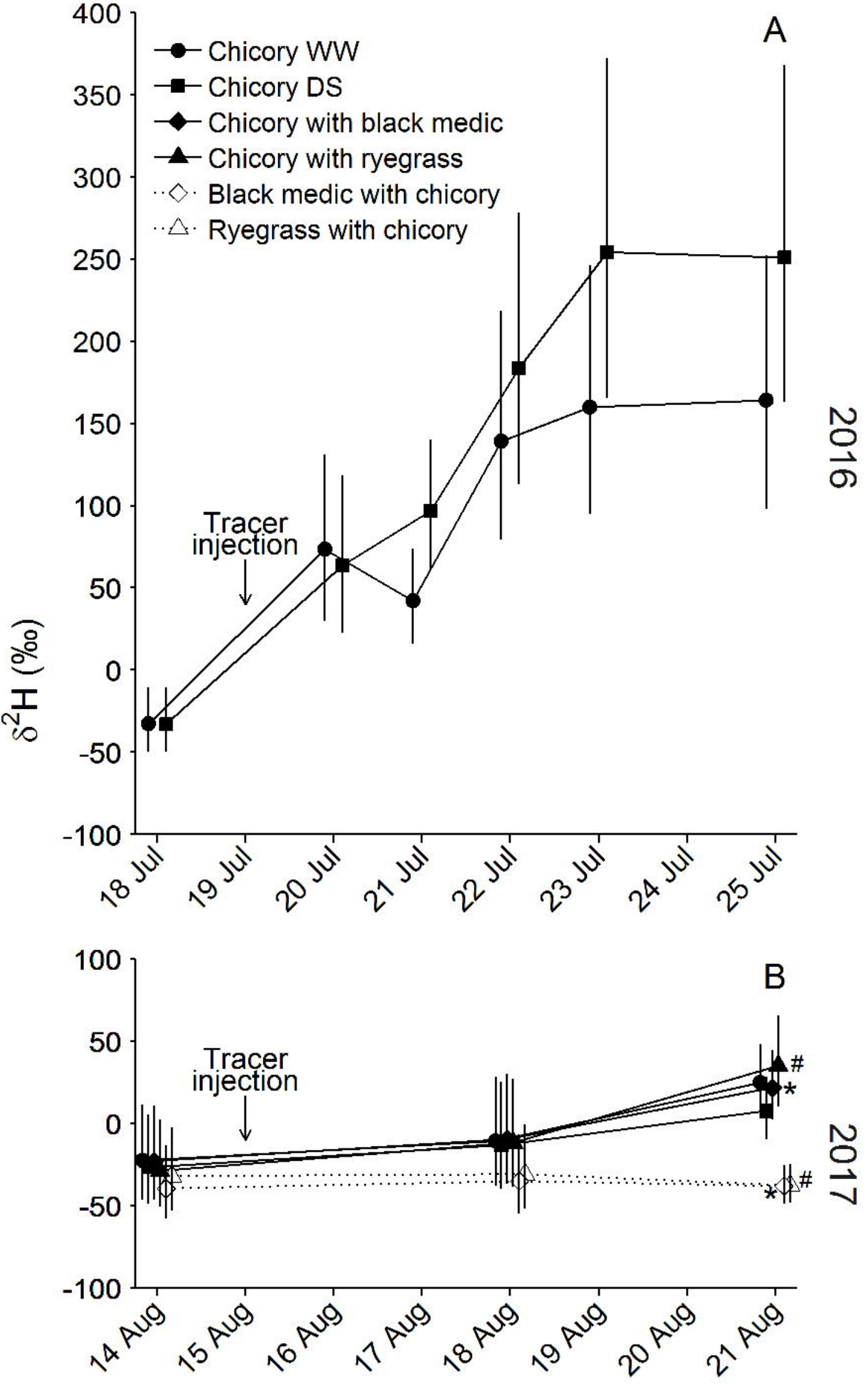

Aboveground biomass (ton ha^{-1})
12
10
8
6
4
2
0
Black medic
Ryegrass
Chicory
a
a
bc
ab
c
a
d
a
Chicory WW
Chicory DS
Chicory WW
Chicory DS
Chicory and b. medic
Chicory and ryegrass
2016
2017


Root intensity
(intersections m^{-1} gridline)
2016
2017
Soil depth (m)
Drought initiation
Tracer injection
Harvest
TDR
2H
TDR & 2H
A
B
C
D
E
a a a b
a ab b c
a ab ab b
a a ab b
Chicory WW
Chicory DS
Chicory & black medic
Chicory & ryegrass


A
B
0.5 m
1.7 m
Difference in VWC from field capacity (%)
Chicory WW
Chicory DS
4
2
0
-2
-4
-6
-8
-10
2
0
-2
-4
-6
26 Jun
30 Jun
04 Jul
08 Jul
12 Jul
16 Jul
20 Jul
24 Jul
28 Jul


Chicory WW
Chicory DS
Chicory & black medic
Chicory & ryegrass
A
B
0.5 m
2.3 m
Difference in VWC from field capacity (%)
4
2
0
-2
-4
-6
-8
-10
13 Jul
17 Jul
21 Jul
25 Jul
29 Jul
02 Aug
06 Aug
10 Aug
14 Aug
18 Aug
22 Aug


Water uptake (mm m^{-1} soil column day^{-1})
0
1
2
3
4
c
a
b
a
c
bc
b
a
a
a
a
a
Chicory WW
Chicory DS
Chicory & black medic
Chicory & ryegrass
2016 - 0.5 m
2016 - 1.7 m
2017 - 0.5 m
2017 - 2.3 m


A
Chicory WW
Chicory DS
Chicory with black medic
Chicory with ryegrass
Black medic with chicory
Ryegrass with chicory
Tracer injection
2016
δ2H (‰)
400
350
300
250
200
150
100
50
0
-50
-100
18 Jul
19 Jul
20 Jul
21 Jul
22 Jul
23 Jul
24 Jul
25 Jul
B
Tracer injection
2017
100
50
0
-50
-100
14 Aug
15 Aug
16 Aug
17 Aug
18 Aug
19 Aug
20 Aug
21 Aug
#
*
*
#


-28.0
-28.4
-28.8
-29.2
-29.6
-30.0
δ13C (‰)
a
a
a
a
b
a
Chicory WW
Chicory DS
Chicory WW
Chicory DS
Chicory with b. medic
Chicory with ryegrass
2016
2017