Rhizosphere 19 (2021) 100368

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Dual labelling by ^{2}H and ^{15}N revealed differences in uptake potential by deep roots of chicory

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ARTICLE INFO

Keywords:
Dual labelling
Dynamics
Nitrate uptake
Rhizotrons
Root intensity
Water uptake

ABSTRACT

Aims: Deep-rooted crops have been widely used in agricultural systems to access deep resources such as water and nitrogen (N). However, the potential of deep roots to take water and N at various depths have not been well studied. Here we used chicory (*Cichorium intybus* L.) to study the potential and dynamics of water and nitrogen uptake in deep soil layers (below 1 m).

Methods: Chicory plants grown in outdoor rhizotrons were labelled by injecting a $^{2}H_2O$ and $Ca(^{15}NO_3)_2$ mixture into the soil column at 1.1, 2.3 and 3.5 m depth. Five, ten and twenty days after injection, ^{2}H and ^{15}N were traced in transpiration water and leaves.

Results: We found enriched ^{2}H and ^{15}N in water and plant samples, and both water and N uptake were observed down to 3.5 m. The ^{2}H enrichment after injection at 1.1 m depth was 1552‰, almost 10 times higher than after injection at 2.3 m depth, which was 156‰. In contrast, injection at 1.1 and 2.3 m depth resulted in similar ^{15}N enrichment of leaf samples.

Conclusion: Deep water uptake was found to be more sensitive to increased depth and reduced root intensity than N uptake, and labelled N was used more rapidly than labelled water. We propose several possible explanations for the discrepancies between deep water and N uptake, and further discuss the challenges of using isotopes and models in deep root studies.

1. Introduction

Excessive application of nitrogen (N) leads to an accumulation of N in soils and a risk of leaching, which can cause subsequent pollution of groundwater (Cameron et al., 2013; Ju and Zhang 2017). Effective use of deep-stored water and nutrients in the soil profile by crops is therefore crucial to obtain high yields and minimize nutrient losses to the environment. Several strategies have been proposed to improve deep rooting and subsoil water and N use, in both genetic and agronomic ways (Gregory 2007; Kell 2011; Thorup-Kristensen and Kirkegaard, 2016).

Deep rooting has been highlighted for its potential use of unexploited soil water and nutrients (Thorup-Kristensen 2006a; White and Kirkegaard 2010; Lynch 2013), yet few studies have adequately investigated details of deep resource uptake. Thorup-Kristensen et al. (2020a) suggested that the main limitation of deep root research is that current methods for deep root research are costly and labour-consuming with insufficient throughput. Deep root growth is restricted in various ways such as soil acidity, soil compaction, hypoxia and suboptimal temperature (Lynch and Wojciechowski 2015), and the resource uptake is often constrained by plant demands as well as soil nutrient availability. These limitations make it even harder to isolate the value of deep roots in resource uptake.

Compared with topsoil, typically there are fewer unevenly distributed roots in deep soil layers (Fan et al., 2016). As crops usually do not reach maximum rooting depths until the end of their lifecycle, roots in deep soil exist for a shorter time. This leads to lower exploitation of deep soil resources. However, deep roots can contribute notably to crop water and nitrogen supply (Kristensen and Thorup-Kristensen, 2004; Lilley and Kirkegaard 2016). Nielsen and Vigil (2018) found that wheat and corn extracted water from 0 to 1.8 m in the soil profile, with more than 20% coming from the 0.9–1.8 m soil profile, despite the fact that more than 95% of the root biomass could be found within the top 1.04 m for wheat, and 0.9 m for corn. White cabbage, with a rooting depth of 2.5 m, remarkably reduced N_{inorg} by as much as 113 kg N ha^{-1} below 1 m soil depth (Thorup-Kristensen 2006a). Deep-stored water is important to dryland crops (e.g. wheat) as such water can be the only available source, and is particularly crucial during the grain filling period when water deficit may lead to great yield losses. During seasonal drought

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https://doi.org/10.1016/j.rhisph.2021.100368
Received 22 February 2021; Received in revised form 29 April 2021; Accepted 30 April 2021
Available online 12 May 2021

periods, a small number of deep roots that can take up the growth-limiting water could also be highly valuable to grain yield (Kirkegaard et al., 2007).

Water and N are the two resources with the greatest impact on crop productivity and have been widely studied. Despite the similarities, such as high mobility in soil, there are differences in transport and uptake processes of water and N. Water and dissolved nutrients are brought to the root surface from the soil by mass flow, while N also moves to the root surface independently of water movement via diffusion (Comerford 2005; Chapman et al., 2012). Given that water and N transport and uptake are interrelated (Plett et al., 2020), it is necessary to consider both when relevant studies are made. Being highly mobile, water and N can be acquired from the subsoil by deep roots. Unlike upper soil layers, where soil water and N availability are usually the limiting factors for root resource acquisition, the low root density and the short active period of the deep roots make root uptake potential and dynamics particularly important for efficient root uptake. Therefore, studies on deep root uptake capacity and dynamics are urgently needed.

Stable isotope labelling is a widely used tool in studying soil water and N uptake (Calder 1992; Kahmen et al., 2008; Rasmussen et al., 2020a). Dual labelling with ^{15}N and $^{2}H/^{18}O$ has been successfully used in water and N uptake studies in the top 1.5 m soil over 1–3 days (Bakhshandeh et al., 2016; Kulmatiski et al., 2017). These studies showed that water and nitrogen uptake occur in various depth of root zones, suggesting that root systems have independent uptake strategies for different resources. Similarly, we expect that water and N uptake from deep soil layers (>1.5 m) differ. In general, when there are fewer roots, water and N uptake decreases. However, with increased depth, deep water uptake occurs against gravity and hydraulic resistance (Lobet et al., 2014), while deep N uptake is not affected by these factors. As a result, root water uptake tends to be more influenced by increasing depth than nitrogen uptake. Further, water and N uptake are mainly driven by plant demand. Plant N uptake peaks during the early reproductive stage and then declines (Imsande and Touraine 1994), while plants maintain a high water demand also after the canopy has been built. Here, we may infer that deep water and N uptake will vary during the growing season, especially during the early reproductive stage, depending on the different plant demands.

Models provide us with an alternative way to study root water and N uptake (Wang and Smith 2004; Pedersen et al., 2010; Lilley and Kirkegaard 2016). Model simulations can be used to generalize the results and simulate the dynamics of uptake during the season, but experimental validation of such simulation results are required. Characteristics of the soil and root system, such as soil water and N availability, root distribution and soil and root hydraulic conductivities are used to evaluate water and N uptake (King et al., 2003; Pedersen et al., 2010). Although studies indicate that deep roots have great potential for water and N uptake (Kell 2011; Rosolem et al., 2017), to our knowledge, indirect comparisons against plant N uptake from subsoil have only been made down to 2 m (Pedersen et al., 2009). Further information on deep water and N uptake may provide inputs to evaluate and validate resource uptake modules in the model and get more detailed and precise predictions of root resource uptake.

In this study, we used ^{15}N and ^{2}H dual labelling to investigate temporal and spatial water and N uptake dynamics by deep roots. The following hypotheses were put forward 1) the uptake potential for water is more sensitive to increased depth and reduced root density than the uptake potential for N, 2) the dynamics of deep water and N uptake differ, labelled N being used more rapidly after injection than labelled water. With this study, it is also our aim to show that dual labelling with ^{15}N and ^{2}H labelled water can be used for the study of short term dynamics of water and N uptake from deep soil layers. Chicory (*Cichorium intybus* L.), which is known as a deep-rooted forb (Vandoorne et al., 2012; Thorup-Kristensen and Rasmussen, 2015), was used as a model plant.

2. Material and methods

2.1. Experimental site

The research was conducted using the rhizobox facility (Thorup-Kristensen et al., 2020b) in Taastrup, Denmark (55° 40′ 90.3$\tilde{5}$ N and 12° 18′ 24.8$\tilde{4}$ E, 23 m above sea level). The rhizobox facility is built for investigation of deep root growth and function. The experiment was performed in the spring/summer 2018 with chicory (cv. "Chicoree Zoom F1″) grown in the facility.

2.2. Experimental design

The rhizoboxes are 4 m tall, 1.2 m wide, 0.6 m thick, and fixed on the concrete ground. They are filled with subsoil to 0.25 m from the top taken from below the plough layer at Store Havelse, Denmark, while the top 0.25 m is topsoil collected from fields nearby the facility (Table 1). The average soil bulk density in the facility was 1.6 g cm^{-3}, with little variations among depths.

Each rhizobox is split into two 4 × 1.2 × 0.3 m chambers (Fig. 1), facing two opposite directions. The front of each chamber is divided into 20 panels being either 0.21 m or 0.17 m (every third) tall. Each panel is covered by an acrylic window, which is fixed by a metal frame. A white PVC board that can slide in the metal frame is placed outside the acrylic window to block solar radiation. The PVC boards can be removed to allow root imaging with a camera via the transparent acrylic windows. The acrylic windows can be removed temporarily for tracer injection. The rhizoboxes are outside and receive precipitation, with the option to supply additional with a drip irrigation system that is installed on top of the rhizoboxes with an irrigation rate of 14 mm h^{-1}.

Chicory plants were sown in a greenhouse on 11 April and transplanted to the rhizoboxes on May 3, 2017. Six chicory plants were planted in each chamber, corresponding to a density of 17 plants m^{-2}. All chambers were fertilized with a nutrient solution equivalent to 50 kg N ha^{-1}, 8 kg P ha^{-1}, 40 kg K ha^{-1} on April 12, 2018. On May 28, 2018, all chicory plants were cut down to 0.5 m, and on 14 June the plants started flowering. The main measurements of the experiment were initiated from 28 May to 29 June, after which the biomass was harvested. The weather data was obtained from a meteorological station on site. The mean temperature during this period was 18.0 °C and the total precipitation was 5.03 mm. To prevent drought stress, all chambers were irrigated for four, three, and 3 h on 4, 16 and 25 of June, respectively.

2.3. ^{2}H and ^{15}N labelling

Chicory's uptake of water and nitrate were studied by injecting an enriched $^{2}H_2O$ and $Ca(^{15}NO_3)_2$ solution into the soil volume at three different depths (1.1, 2.3 and 3.5 m), repeated in four chambers for each depth. 4.35 g $Ca(^{15}NO_3)_2$ (>98 at% ^{15}N) was mixed with 600 ml $^{2}H_2O$ (^{2}H content = 99.94%) and 600 ml distilled water. The following assumptions were made to determine the amount of tracer added: 1) the soil volumetric water content is no less than 15% and soil contains 50 kg N ha^{-1}; 2) the abundance of ^{2}H and ^{15}N in the pre-labelled soil pool is natural; 3) 10% of the ^{2}H and ^{15}N injected at a specific soil depth is taken

Table 1
Characteristics of soil used in the rhizoboxes (Rasmussen et al., 2020b).

Depth (m)	Organic matter (%)	Clay(%) <0.002 mm	Silt(%) 0.002–0.02 mm	Fine sand (%) 0.02–0.2 mm	Coarse sand (%) 0.2-2 mm	pH
0–0.25	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

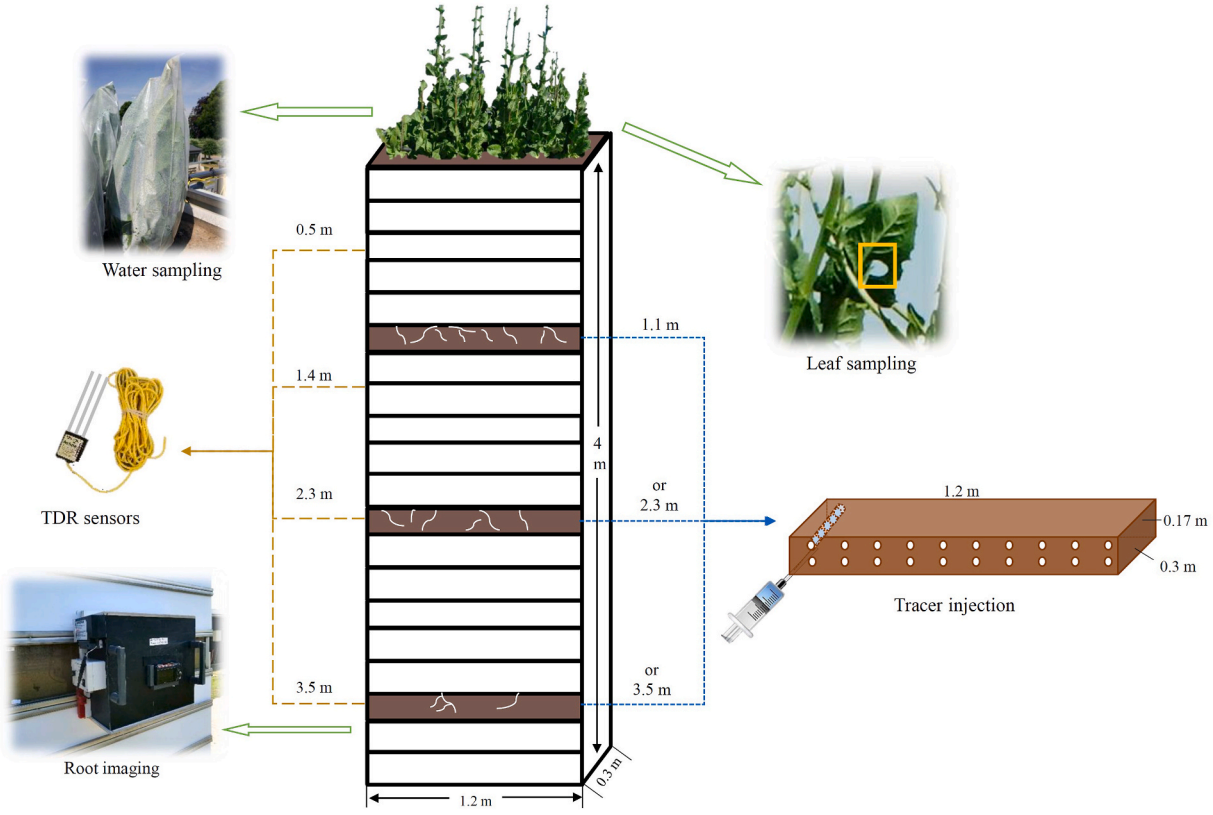

Fig. 1. Schematic drawing of a single chamber from the rhizobox facility and main activities conducted in this experiment. The yellow dashed lines indicated depths where the TDR sensors were installed. Tracer injection was conducted at 1.1, 2.3 or 3.5 m, as blue dashed lines indicated.

up, and 4) tracer distributes evenly at the targeted depth, with a labelled soil volume of 61.2 L. A 100 ml mixture of $^{2}H_2O$ and $Ca(^{15}NO_3)_2$ was injected per injection layer. At the selected soil layer, two parallel rows of ten injection points distributed 10 cm apart were made using a steel rod, resulting in a total of 20 holes. In each hole, the tracer solution was injected at five different points, giving an even distribution of the tracer mixture in 100 injection points in total for each injected depth (Fig. 1). Each point received 1 ml of the mixture. The injection was conducted between 13:00 and 16:00 on May 29, 2018. The injection of ^{15}N and ^{2}H was proven to significantly increase the fraction of ^{15}N and ^{2}H in the targeted depths (Table 2).

Collection of transpiration water and leaf samples to capture tracer uptake signals was conducted in the morning right before the injection as a control, and five, ten, twenty days after the injection. The transpiration water collection method has been validated previously (Calder 1992; Lambs and Saenger 2011; Beyer et al., 2016). From 9 to 11 a.m. on a sampling day, all plant biomass of each target plant was covered with a plastic bag and tightened by rubber bands at the bottom (Fig. 1). Transpiration water was collected 2 h later as droplets of condensed water gathered inside the bags. The water was transferred from bags to sealed plastic bottles. At the end of the experiment, all transpiration water samples were filtered with 2 μm filter paper to remove any leaf fractions, pollen and dust. Filtered water from all plants grown in the same chamber was pooled into one sample.

For ^{15}N analysis, leaf samples were collected by using a puncher with a diameter of 9 mm on the third to fifth leaves from the top on the same day as the transpiration water sample collections (Fig. 1). Two to three pieces of leaf samples were collected from each plant. Leaf samples from the same chamber were mixed and dried at 70 °C over 48 h to constant weight.

2.4. Soil water content and water uptake

Time-domain reflectometry sensors (TDR-315/TDR-315 L, Acclima Inc., Meridian, Idaho) were installed at four depths (0.5, 1.4, 2.3 and 3.5 m) and soil volumetric water content (VWC; %) was recorded every 10 min on a datalogger (CR6, Campbell Scientific Inc., Logan, Utah).

In November, the amount of precipitation fully saturated the soil column. Field capacity (FC) was estimated in each 1 m layer as the mean of VWC, three days after the highest VWC occurred. Assuming there is little water movement when the soil water content is below FC, the measured changes in VWC were used as an approximation of plant water uptake. As irrigation events triggered water movement in the soil (Fig. 2), only VWC data from the periods between irrigation were used,

Table 2
Soil volumetric water content, soil NO_3–N content, original and estimated δ^2H and $\delta^{15}N$ of targeted depths. Soil water content, NO_3–N content and $\delta^{15}N$ in non-enriched soil were measured right before injection.

Depth (m)	Soil volumetric water content (%)	Soil NO_3–N content (mg N kg^{-1} dry soil)	Estimated δ^2H in non-enriched soil water (‰)	Estimated δ^2H in enriched soil water (‰)	$\delta^{15}N$ in non-enriched soil (‰)	Estimated $\delta^{15}N$ in enriched soil (‰)
1.1	16.7	0.4	−36.9	31356.7	55.0	405548.8
2.3	16.5	0.4	−36.9	31737.2	137.4	405548.8
3.5	17.9	0.5	−36.9	29289.0	162.0	326425.6

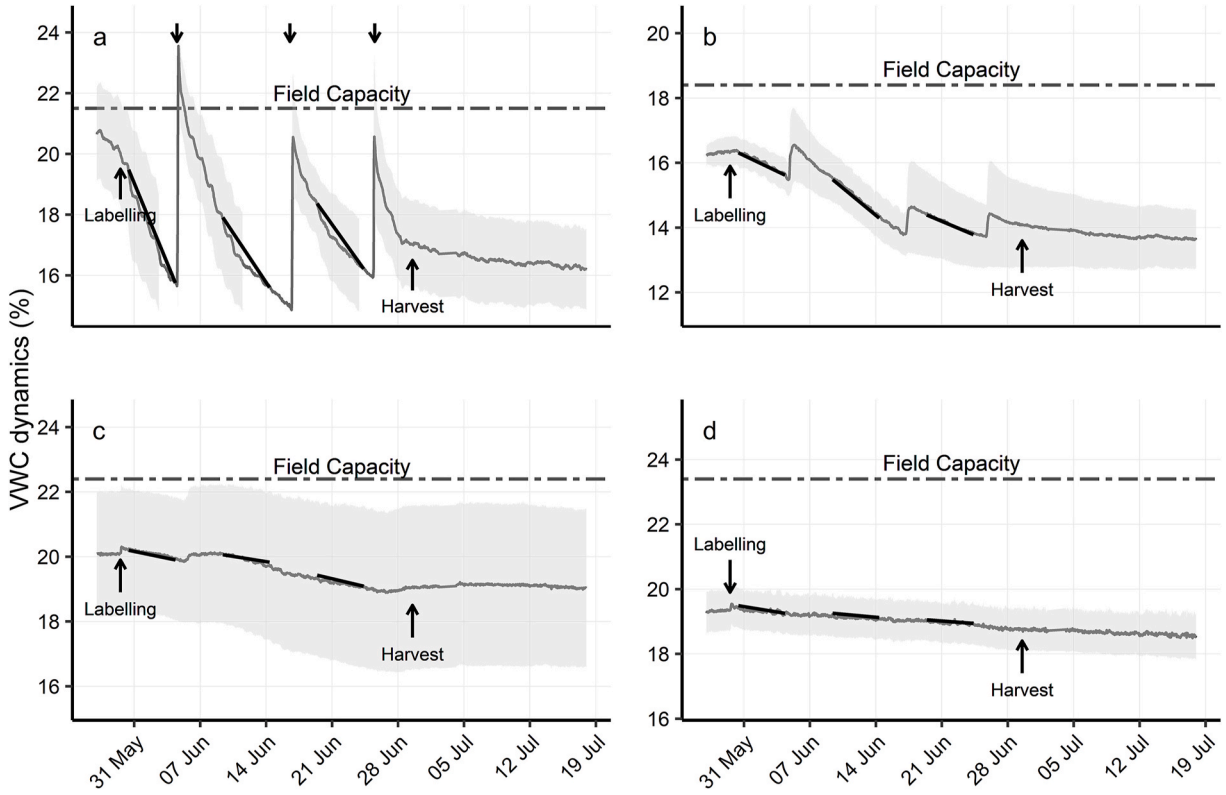

Fig. 2. Soil volumetric water content (VWC; %) dynamics at (a) 0.5, (b) 1.4, (c) 2.3 and (d) 3.5 m depths from 27 May to 18 July in 2018. Data was collected from TDR sensors at the corresponding depths. Data from 0.5 to 1.4 m depths were used to estimate VWC changes at 1.1 m depth. Irrigation events can be seen as peaks most clearly in (a). The segments represented periods that were selected to calculate daily water uptake in Fig. 6. Field capacity data were obtained subsequently, using data measured three days after soil columns were fully irrigated during November in the same year. Bands around the lines denote standard errors (n = 4).

avoiding any data in the first three days after irrigation and waiting until the sensor data indicated that irrigation triggered water movement had stopped. Three five-day intervals, 30 May to 4 June, 9 to 14 June, and 19 to 24 June, were chosen to estimate the average daily water uptake during the labelling period. For calculating daily plant water uptake estimates, each of the four sensors was taken to represent water content in a 1 m soil layer, thereby dividing the whole 4 m soil column into four 1 m sub-columns. Total water amount (W_a; mm m^{-1} soil column) in each sub-column was converted from VWC (Wv; %),

$$W_a = \frac{W_v \times V}{S} \times 1000 \quad (1)$$

where V and S are the volumes and bottom surface area of the sub-column. The daily average decrease of water in each sub-column in the five-day period was interpreted as daily water uptake (mm m^{-1} soil column day^{-1}). The simplification of getting water uptake from changes of VWC has been used in previous studies (Gaiser et al., 2012; Rasmussen et al., 2020a).

2.5. Root measurements

A digital camera (Olympus Tough TG 860) was used to record root growth on the surface of rhizoboxes via transparent acrylic windows. The camera was placed on a half-closed plywood box, with internal LED light strips as a light source (Fig. 1). It was designed to slide along the metal frames of each panel when the PVC boards were removed. With this camera box, four photos that covered the full area of the panel were taken on all 20 panels of each rhizobox chamber. During the experimental period, root imaging was done three times in total.

Root intensity (root intersections m^{-1} line) at each depth was calculated by using the line intersect method. The method was developed by Newman (1966), then modified by Marsh (1971) and Tennant (1975). It has been successfully used in minirhizotron and rhizobox studies previously (Kristensen and Thorup-Kristensen, 2004; Rasmussen et al., 2020b). In this experiment, the root images were covered from wide panels with 20 × 20 mm grids, and the total length of lines per panel was 3.97 m. Images from the first panels were excluded, as the upper part of the panels were exposed to sunlight due to the sinking of the soil, which gave us low-quality images. Narrow-panel images were also excluded as the soil there was disturbed a lot by injection, soil sampling, etc. In the rest panels, root intensity was recorded by counting the total number of roots intersecting the lines at each panel.

2.6. Soil and isotopic analyses

Soil samples from the injection layers were collected twice to compare soil mineral N and ^{15}N enrichment before tracer injection and at the end of the experiment. 20 g sub-samples of soil from each sample was mixed with 100 ml 2 M KCl solution. The solution was shaken for 1 h and filtered through 2 μm filter paper. After filtering, the samples were frozen.

At the end of the experiment, all collected biomass samples were weighed, milled, and encapsulated. ^{15}N concentration in solid and soil solution samples was analyzed using a continuous-flow isotope ratio mass spectrometer (IRMS). Mineral N content in the frozen soil samples was analyzed as well. δ^2H in transpiration water samples was analyzed using a Laser Water Isotope Analyzer V2 (Los Gatos Research, Inc., Mountain View, CA, USA). All analyses mentioned above were done at the UC Davis Stable Isotope Facility.

^2H and ^{15}N values were assumed to be present in samples with delta notation (δ). Definition of δ has been given by Coplen (2011):

$$\delta = \frac{R_{sample}}{R_{standard}} - 1 \quad (2)$$

In eq. (2), for δ^2H calculation, R_{sample} is $^2H/^1H$ ratio in samples and $R_{standard}$ here is Vienna standard mean ocean water (≈1/6412); for $\delta^{15}N$, R_{sample} is $^{15}N/^{14}N$ ratio in samples and $R_{standard}$ here is 0.003676467.

In this paper, ^2H and ^{15}N enrichment (‰) were calculated as the increase of $\delta\ ^2H$ and $\delta\ ^{15}N$ from pre-tracer sampling to post-tracer sampling.

2.7. Statistics

Data were collated and plotted using R (Version 3.5.3, R Core team 2019). The combined effect of sampling date and depth on root intensity was tested in a two-way ANOVA. To test the differences in root intensity among injected depths during the experimental period, a linear mixed model was used, where the depth was the fixed factor and the chamber was a random factor. Analyses of covariance (ANCOVA) was conducted to test depth and date mixed effects on ^{15}N enrichment in leaf and ^2H enrichment in transpiration water, with $\delta^{15}N/^2H$ in samples before labelling as a covariate.

Linear mixed models were used to test the main effects of soil mineral N concentration and ^{15}N and soil, with sampling date and injection depth as fixed effects, and the chamber was included as a random effect. One soil sample, which was sampled at 3.5 m depth on 16 July, was removed due to an unexpected high ^{15}N value compared to the others (atom% was 30% while other replicates were lower than 5%). The main effects of time and depth on daily water uptake was tested using a linear mixed model. Chamber was included as a random factor.

For ^2H enrichment and soil $\delta^{15}N$ analysis, data were log-transformed to fulfil assumptions of normality and homogeneity. Multiple comparisons (Tukey HSD; $P \leq 0.05$) were done based on values derived from linear mixed models, ANOVA or ANCOVA.

3. Results

3.1. Root intensity

Roots were present in the entire soil profile of the 4 m deep rhizoboxes under the one year old chicory plants before the time of injection (Fig. 3a). Root intensity declined with depth, with only few roots observed below 3.5 m. Additionally, there was a tendency towards a decline in root intensity during the experimental period in the upper 3 m of the soil (Fig. 3a), but the decline was not significant at any specific depth. To make sure labelling would not affect root growth, we tested differences of root intensity between labelled and non-labelled soil layers at the same depths, and no significant differences were found.

During the labelling period, the highest root density among all three injection depths was 3.9 intersections m^{-1} at 1.1 m depth, and the lowest was 0.3 intersections m^{-1} at 3.7 m depth (Fig. 3b), while root density was intermediate at 2.1 m depth with 1.7 intersections m^{-1}. Root intensity at 1.1 m was significantly higher than at the other two labelled depths.

3.2. ^2H and ^{15}N enrichment

On the first two sampling days, as well as five and ten days after injection, ^2H enrichment of transpiration water was significantly lower when the tracer was injected deeper (Fig. 4a). The enrichment after

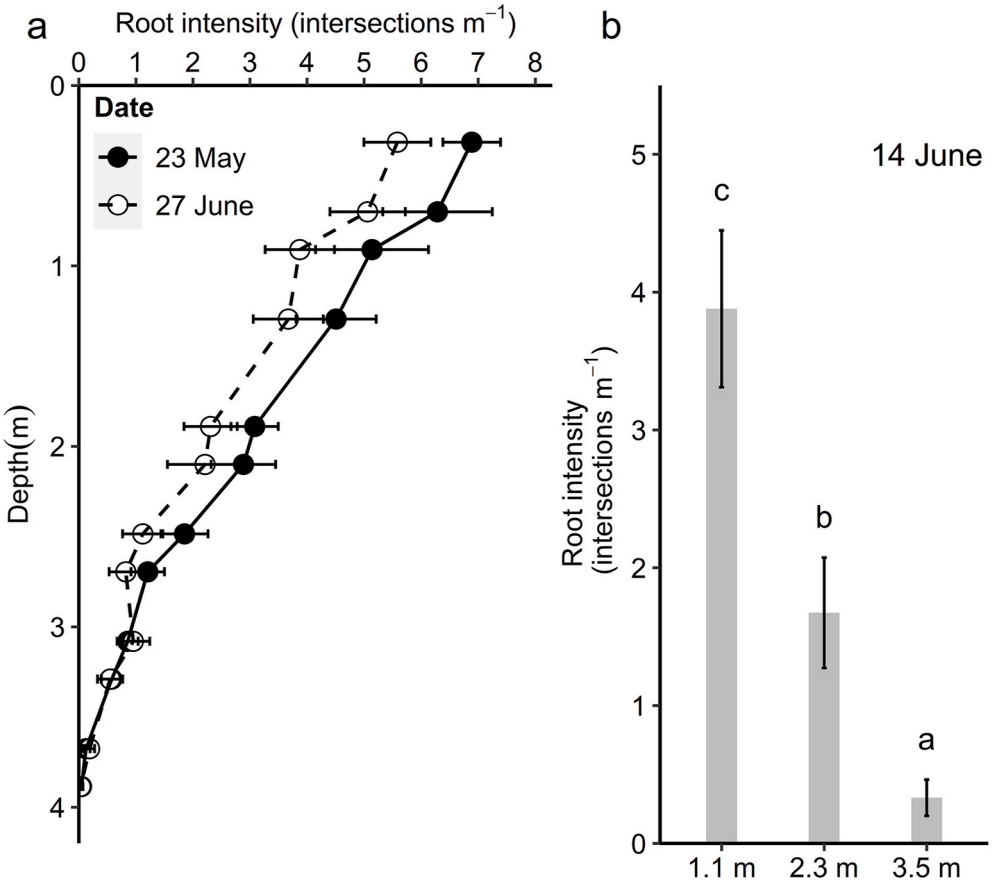

Fig. 3. (a) Root intensity measured on 23 May (six days before tracer injection) and 27 June (two days before harvest) in 2018. (b) Root intensity at three injected depths on 14 June. Root intensity at the injected depth was estimated based upon averages of root intensity at soil layers 0.2 m below and above injected depths. Error bars denote standard errors among all chambers where we injected tracers at different depths (n = 12). Mean values are shown here (±SE).

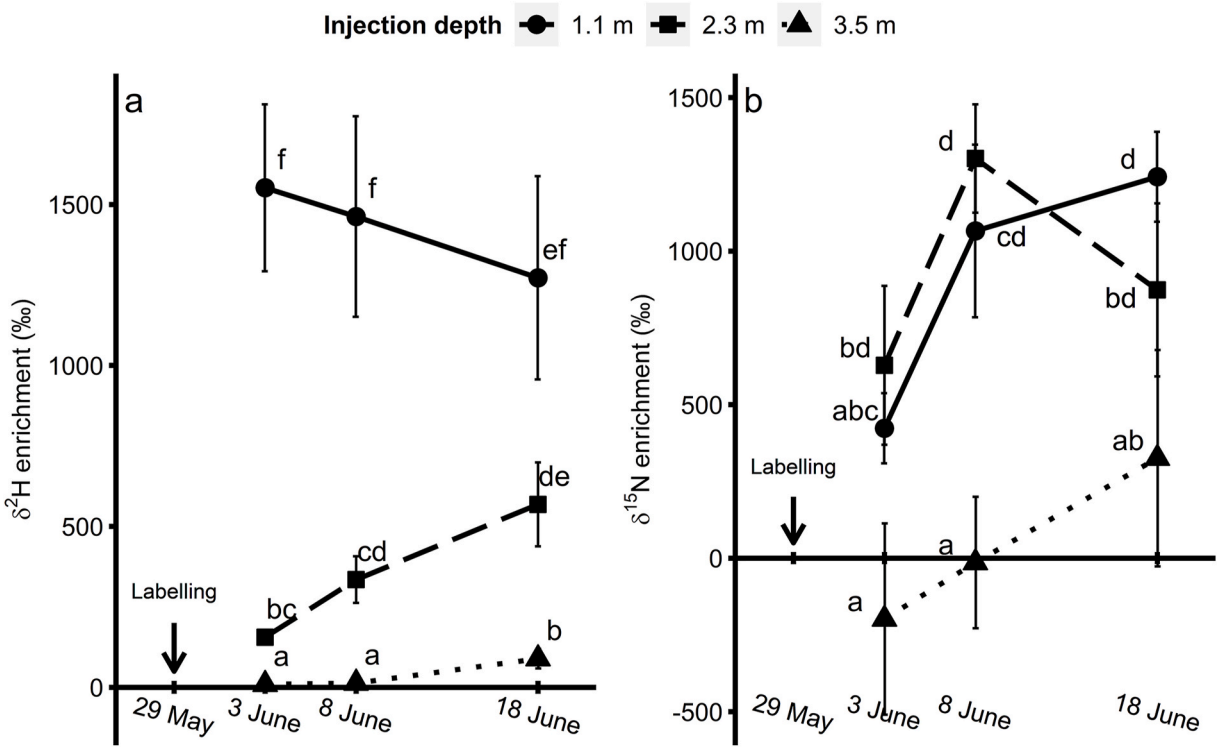

Fig. 4. (a) ^{2}H enrichment in transpiration water and (b) ^{15}N enrichment in leaf samples measured five, ten, twenty days after tracer injection at 1.1, 2.3, 3.5 m of soil depth. Mean values are shown here (±SE). Error bars denote standard errors (n = 4), and letters indicate significant differences across all the treatments ($p < 0.05$).

injection at 1.1 m depth was 1552‰, nearly 10 times higher than after injection at 2.3 m depth, which was 156‰. Almost no enrichment was observed after injection at 3.5 m depth. Furthermore, the time course of ^{2}H enrichment of transpiration water was affected by the injection depth, as the increase in enrichment was delayed by deeper injection. After injection at 1.1 m, maximum enrichment was observed already at the first sampling date, followed by a non-significant tendency to a decline later. After injection at 2.3 m, a significant increase over the sampling times was observed, and after injection at 3.5 m, no effect was observed until the last sampling date when a slight but significant

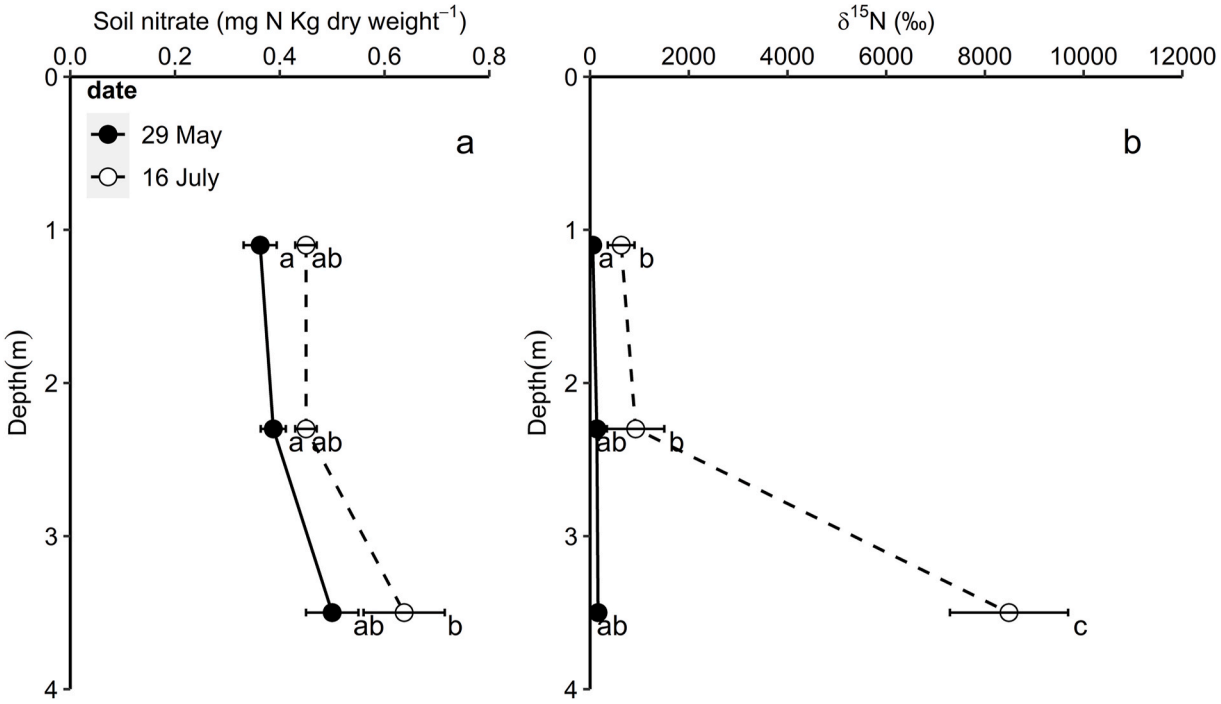

Fig. 5. (a) Soil nitrate concentration and (b) $\delta^{15}N$ at the three injection depths right before injection (29 May), and after final sampling (16 July). Mean values are shown here (±SE). Error bars denote standard errors (n = 4; in Fig. 5b and 3.5 m, 16 July, n = 3), letters indicate significant differences across all the treatments ($p < 0.05$).

increase in enrichment was seen (Fig. 4a).

The ^{15}N enrichment of leaf samples showed a somewhat different result, and the differences observed were smaller, leaving fewer significant differences (Fig. 4b). The ^{15}N enrichment of leaves after injection at the two upper layers showed similar results. Unlike the ^{2}H results, enrichment from 1.1 m to 2.3 m injections were high already at the first measurement date. As with ^{2}H, no enrichment was observed on the first two sampling dates from injection at 3.5 m depth, and only a non-significant increase was seen at the last date. Ten days after injection, ^{15}N enrichment from 1.1 to 2.3 m were significantly higher than from 3.5 m. However, twenty days after injection, there was no significant difference between ^{15}N enrichment from 2.3 m to 3.5 m.

3.3. Soil nitrate concentration and $\delta^{15}N$

As ^{15}N labelling provided an extra nitrate source, the soil nitrate concentrations tended to be higher at all depths after the experiment than before injection (Fig. 5a). Soil nitrate concentration measured before and after the injection showed smaller net increases of 0.09, 0.06 and 0.15 mg kg^{-1} at the three depths, respectively, but the effect was not significant.

The soil ^{15}N values also tended to be higher after ^{15}N injection at all depths (Fig. 5b), but the increase was much stronger at 3.5 m depth than at 1.1 and 2.3 m depth, and only significant there. Before the injection, soil δ ^{15}N values were relatively low and there were no differences among the depths.

3.4. Water uptake

The soil dried out gradually during the experiment at all four depths where water sensors were placed. While infiltrated water from irrigation events (Fig. 2) reached 2.3 m depth and caused an increase of soil water content right after irrigation events, soil water at all depths decreased continuously due to plant water uptake. At all depths, the water uptake after harvest was negligible, indicating that plants stopped extracting water from these depths.

During the selected five-day intervals, the volume of absorbed water decreased with increasing depth (Fig. 6). These intervals started at least two days after irrigation to avoid over-estimation of soil water content. Among all three periods, plants absorbed the most water from the uppermost 1 m of the soil. Within 1–6 days after labelling, the average daily water uptake by plants from 0 to 1 and 1–2 m soil column was 7.2 mm and 1.4 mm. At the same time, plants took less than 1 mm of water per day from the 2–3 and 3–4 m soil layers. Daily water uptake from 0 to 1 m decreased to 4.4 mm at 11–16 days after labelling, and after 21 days, plants still acquired more than 4 mm water from 0 to 1 m soil per day. Daily water uptake from 1 to 2 and 2–3 m tended to be higher in the middle of the labelling period and decreased thereafter, although none of these changes was significant.

4. Discussion

Fewer chicory roots were observed in deeper layers than were found in previous studies (Sapkota et al., 2012; Thorup-Kristensen and Rasmussen, 2015). However, despite the fewer roots, considerable water and N were taken from the soil below 1 m by chicory. Using a dual labelling technique, the work presented here successfully showed the short term uptake potential and dynamics of deep water and N. In this experiment, the root water uptake was found to be more reduced with increased depth and declined root density compared with N uptake. In addition, in the labelling period, ^{15}N tended to be exploited more rapidly than labelled water. The discrepancies between water and N uptake may be caused by various factors, which will be further discussed below.

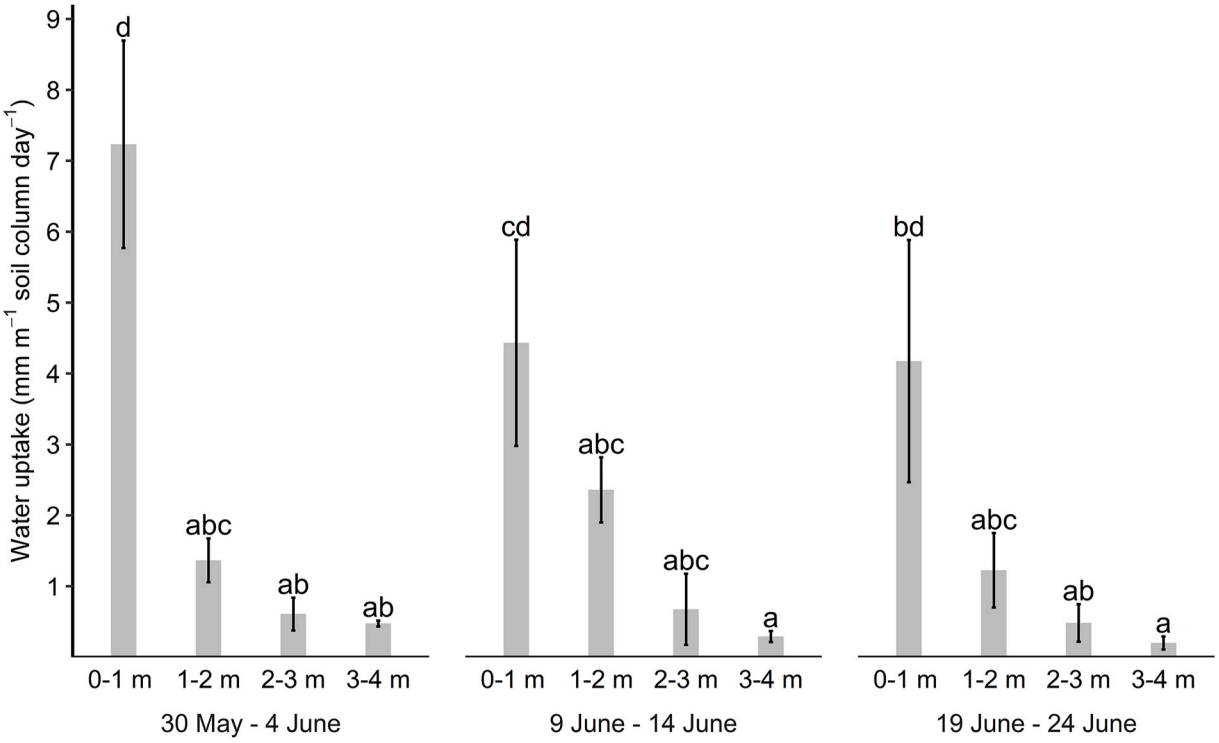

Fig. 6. Mean daily water uptake from 0 to 1, 1–2, 2–3 and 3–4 m depths after labelling. The daily decrease in soil volumetric water content per meter soil column was interpreted as daily water uptake. Soil volumetric water content per meter soil column was recorded by the TDR sensor located in the column. After isotopic labelling at given depths, the soil water content data of corresponded soil columns was collected. Data from 0 to 1 m and 1–2 m helped estimate water uptake from the depth where tracers were injected at 1.1 m. To avoid the effect of irrigation, daily water uptake from each depth was calculated as averages of three five-day periods (30 May to 4 June, 9 to 14 June, and 19 to 24 June), respectively. Error bars denote standard errors (n = 4), letters indicate significant differences across all the treatments ($p < 0.05$).

4.1. Deep water uptake

Water in soil moves with various processes, e.g. infiltration, redistribution, evaporation, plant uptake and drainage (Hillel 1980). Although the isolation of plant uptake from other processes is complicated, we are trying to simplify the processes with the current experimental setup to get an idea of deep water uptake. To avoid the effect of irrigation on water movement, we only selected the periods at least three days after irrigation for water use observations. Assuming there is little water movement caused by evaporation and drainage in wet, deep soil layers in rhizoboxes, details of deep water uptake from different depths can be obtained. ^{2}H enrichment of transpiration water decreased significantly with decreasing root intensity and increasing soil depth. Based on our estimated calculations, daily water uptake from the top 1 m soil reached 7 mm m^{-1} d^{-1} while less than 1 mm m^{-1} d^{-1} water was taken from 2 to 3 m soil during the experimental period. Although several studies have shown that deeper roots allow water acquisition from subsoil (White and Kirkegaard 2010; Gaiser et al., 2012; Cutforth et al., 2013), their limited ability to take up water can be a general feature, as indicated in the present results. Compared with topsoil, subsoil is hard for roots to penetrate, thus there are fewer roots in deep soil layers. The roots that can keep elongating in these conditions also prefer to grow in pores and cracks, which would lead to poor root-soil contact, making it harder to obtain water (White and Kirkegaard 2010). Further, due to higher proportions of immature young roots, roots in deep soil layers generally have higher axial resistance and therefore do not extract water as efficiently as old, shallower roots (Garrigues et al., 2006; Pierret et al., 2006).

4.2. Deep N uptake

^{15}N enrichment in leaves together with ^{15}N left in the soil after harvest indicated little N uptake from 3.5 m, probably as a consequence of the low root intensity. ^{15}N signals were seen in leaves five days after tracer was injected at 1.1 and 2.3 m, and no significant differences were seen for nitrate uptake from 1.1 to 2.3 m. Plant N uptake is affected by soil N availability (Kulmatiski et al., 2017) and plant uptake capacity (Robinson 1986). The plant uptake capacity is further determined by the interactions of plant N demand and root uptake capacity. At the two upper layers, where more roots were found than in the deepest layer, ^{15}N absorption occurred at high rates shortly after the injection. Even a relatively low root intensity at 2.3 m in our experiment was as efficient for N uptake as the higher root intensity at 1.1 m. Similarly, efficient deep N uptake was also found previously, e.g. Thorup-Kristensen (2006a, 2006b). Twenty days after injection, soil ^{15}N had been depleted in upper layers, leading to a decrease of ^{15}N in the young leaves sampled for ^{15}N analysis. We also noticed a gradual increment of ^{15}N enrichment in plants injected at 3.5 m at the same time. In the past few years, while root N uptake is often studied at the level of transporters and root systems (Rowe et al., 2001; Nacry et al., 2013; Kulmatiski et al., 2017), the intrinsic variation of N uptake among root segments has rarely been studied. Our results showed that N uptake may differ within a root system with time and N availability. When N is available in the soil, as it was in the first few days of labelling in our experiment, root length and uptake rate are limiting factors for N uptake. When N is gradually moved by plants from the soil, the availability rather than root length becomes the limiting factor. This explained the decreasing uptake from the top two layers and the lagging absorption from the deepest layer.

4.3. The disparity in the uptake of water and N

Due to the different sampling methods, the isotopic results of water and N were not directly comparable. By sampling young leaf material for ^{15}N analysis, the results include the effect of accumulation of ^{15}N in the plant material over time, contrary to the real-time isotope enrichment as ^{2}H enrichment in transpiration water. Also, the water and N content of injected depths are only partly comparable in the results, as there were no TDR sensors at 1.1 m. Nevertheless, we still conclude that there are discrepancies in water and nitrogen uptake. Soil $\delta^{15}N$ and plant enrichment indicated more rapid uptake of labelled nitrogen than water from the subsoil, especially from 2.3 m depth, which supported our hypothesis (2) that the dynamics of deep water and N differ. We observed low but increasing content of ^{2}H water in transpiration water from 2.3 to 3.5 m 20 days after injection, showing that labelled water remained in the soil, and was taken up at gradually increasing rates. ^{15}N enrichment in leaves showed insignificant changes 10 days after injection. Considering that little labelled N was left at 1.1 and 2.3 m, we concluded that a large proportion of labelled N was taken from these two depths during the first 10 days.

Our results are consistent with McCulley et al. (2004), who suggested nutrient uptake as a contributing explanation for the occurrence of deep roots. Instead of taking water directly from deep soil layers, deep roots played a more important role in altering water and nutrient distribution in the soil profile via hydraulic redistribution (McCulley et al., 2004). Here, we further examined the extent of water and N uptake at different depths and proposed several explanations on their uptake disparities. Firstly, the radial and axial resistances mentioned above may inhibit root water uptake from subsoil but no evidence has been shown for similar inhibition of nitrate uptake. Secondly, the water supply from the topsoil may have been sufficient to supply most of the water demand by the plants with repeated irrigation to the topsoil, while the N demand by the plants exceeded the topsoil supply, leading them to deplete all available soil layers. In deep soil layers where the transpirational force is absent, N may still move to the roots by diffusion (Comerford 2005; Plett et al., 2020). Moreover, from the molecular aspect, when plants are exposed to N limitation, the capacity of high-affinity transport systems (HATS) would be upregulated to improve the N uptake efficiency (Nacry et al., 2013). These mechanisms allow continuous N uptake from the deep soil layers, even when little water is taken up from there. Thirdly, within the same root system, the root water uptake potential of different segments are non-uniform. Upper roots near the soil surface were found to have higher radial and axial fluxes, which benefit both root water uptake and transport (Zarebanadkouki et al., 2013). Conversely, although nitrate uptake kinetics may also vary within root systems, the maximum influx rate of nitrate of root segments was most affected by plant age and nitrate deprivation time, rather than their position (York et al., 2016). This could explain why we observed less and slower water uptake from the lower layers, while the uptake rate of N in the top two layers did not differ significantly.

4.4. Methodological considerations in deep root studies

^{2}H and ^{15}N labelling is a promising way to study the dynamics of water and nitrate uptake (Calder 1992; Kahmen et al., 2008; Bakhshandeh et al., 2016; Kulmatiski et al., 2017). However, there are some inevitable problems when the technique is used in deep root studies. As ^{2}H and ^{15}N are highly mobile in the soil, they can move freely with water movement. In previous studies, ^{2}H moved 0.1 m up along the soil profile in a mesic savanna after one week of tracer injection at 1.2 m (Kulmatiski et al., 2010), while capillary rise transported ^{2}H at distances between +0.1 m and −0.05 m from 1 m in 35 days (Grunberger et al., 2011). Furthermore, there was a clear sign that a small amount of injected ^{2}H can move with the transport of water vapour in a longer period (Beyer et al., 2016). Thus, for labelling with these mobile resources, short time intervals between labelling and uptake measurements are preferable, to be certain that the tracer was taken up at approximately the same depth where it was injected. In addition to the inconsistencies in sampling methods mentioned above, the short active period of the deepest roots makes it even harder to choose the right labelling time. To study nitrate uptake from the deepest roots, ^{15}N labelling has to be done when the roots reach the deepest layers, which usually is at the late growth stages. Since nitrate uptake decreases after

flowering (Fischer 1993; Imsande and Touraine 1994), labelled ^{15}N accumulation in leaves can be relatively low if the isotope is applied after flowering. To obtain more precise results, destructive sampling is preferable, so actual ^{15}N uptake, rather than just ^{15}N enrichment can be determined, but this will require a higher number of treated plots, generally not possible in deep root studies.

Considering the complexities of root studies, several models have been developed and used in simulating resource uptake from soil (Ma et al., 2008; Kumar et al., 2015). However, deep root resource uptake has rarely been considered in soil-crop models. As we observed significant water and nitrogen uptake below 2 m, this should be included in future soil-crop models. Heterogeneous uptake among different parts of roots is often not well accounted for in the models (Rengel 1993; Javaux et al., 2013), and uniform estimations for the whole root system may lead to over- or under-estimation of uptake, especially for water uptake. We expect that our results can be used to better characterize the parameters in future simulations.

Previous studies already showed the substantial value of deep roots for resource uptake (Kristensen and Thorup-Kristensen, 2004; Rasmussen et al., 2020a). Our findings not only confirmed the contribution of deep roots to water uptake but further indicated their potential to uptake N is considerable as well. This potential can be valuable in maintaining crop productivity, especially under drought stress, where water and N uptake in topsoil can be both limited. However, we still lack the understanding of where and when the deep roots are active, and how efficient they can be. This is crucial information needed to improve deep resource use efficiency, as there are few roots in the deepest soil and they are only active within a short period. Here, with the help of isotope labelling, we have successfully looked into detailed uptake dynamics and proved that this method can be used in further studies.

In conclusion, we confirmed that deep-rooted chicory plants can take water and nitrate from the subsoil, and documented uptake to a depth of 3.5 m, but with different efficiency and dynamics. Compared with N, the root water uptake is prone to decrease with increased depth and fewer roots. These findings extend our previous observations on deep water and nitrogen uptake, and are meaningful for model calibration as well.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank statistician Signe Marie Jensen for advice regarding the statistical data analyses, technician Jason Allen Teem for his contribution to the experimental work and Madeline DuBois for English language editing. We acknowledge the Villum Foundation (DeepFrontier project, grant number VKR023338) for financial support for this study, and China Scholarship Council for the financial support of Guanying Chen for her PhD research. Special thanks to Prof. Sina Adl and two anonymous referees for their constructive remarks.

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